Online ISSN: 3009-7312
Print ISSN: 3009-6391

EJCBR

Egyptian Journal of Cancer and Biomedical Research

https://jcbr.journals.ekb.eg

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Since 2014

EGYPTIAN JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (EJCBR)
FORMARLY INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Exploring the diagnostic significance of astrocyte elevated gene 1 (AEG1) and Glypican 3 immunohistochemical expression in differentiating hepatocellular carcinoma and mimickers

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) ranks among the top causes of cancer-related deaths globally. Astrocyte elevated gene 1 (AEG-1) plays a crucial role in cancer development and progression by enhancing all hallmarks of cancer. Glypican3 (GPC3) is a proteoglycan found on the cell surface and shows significant overexpression in hepatocellular carcinoma. **Aim:** This study aimed to assess the diagnostic utility of AEG1 and Glypican3 in HCC with their different grades and precancerous lesions. **Material and Methods:** Cancer cases (n-60) were analyzed in this study, comprising 36 cases of HCC and 24 cases of precancerous lesions. The specimens underwent routine hematoxylin, eosin staining and immune-histochemical staining using AEG1 and GPC3 markers. **Results:** AEG1 was expressed in 94.4% of HCC cases and 12.5% of precancerous lesions, achieving a sensitivity of 94.4% and specificity of 87.5%. In comparison, GPC3 was expressed in 75% of HCC cases and 8.3% of precancerous lesions, with a sensitivity of 75% and specificity of 91.6%. GPC3 expression shows a statistically significant relation with high tumor grade, whereas AEG1 did not show a statistically significant relation with tumor grade. Combination of AEG1 and GPC3 demonstrated enhanced sensitivity (98.2%) and absolute specificity (100%), making the combined immunohistochemical panel highly effective for accurately diagnosing HCC and distinguishing it from precancerous lesions. **Conclusion**: AEG1 demonstrated higher sensitivity and diagnostic accuracy as compared to GPC3, which exhibited higher specificity. Further, combined AEG1 and GPC3 immunostaining can be used to achieve accurate diagnosis of HCC and to differentiate it from precancerous lesions.

Keywords: AEG1, Cirrhotic nodules, Dysplastic lesions, GPC3, HCC

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/jcbr.2024.305480.1362

ARTICLE INFO

Article history
Received: July 19, 2024
Revised: August 19, 2024
Accepted: September 13, 2024

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INTRODUCTION

Liver cancer is the sixth most common cancer and the fourth leading cause of cancer-related death worldwide (Singal et al., 2020). Globally, hepatocellular carcinoma is the most common type of liver cancer, accounting for 80% of all primary liver cancers (Bray et al., 2018). 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 (Samant et al., 2021). In Egypt, HCC represents the fourth most common cancer and the most common cause of mortality and morbidity-related cancer (Rashed et al., 2020). Moreover, Egypt ranks the third and 15th most popular country in Africa and worldwide, respectively (Algargni et al., 2022).

Most HCC patients have advanced stages at the time of diagnosis and have a poor prognosis. However, if identified at an 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 (Laube et al., 2021).

HCC is an extremely complex condition and there are multiple factors involved in its pathogenesis. Most HCC cases occur in the setting of chronic liver disease with cirrhosis being the fundamental risk factor and present in 80-90% of HCC patients (Suresh et al., 2020). It

is estimated 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 (McGlynn et al., 2021). However, Non-alcoholic fatty liver disease (NAFLD) is an emerging leading etiology as well (Sagnelli et al., 2020).

Cirrhosis is considered a precancerous lesion associated with a high probability of developing HCC (Desjonqueres et al., 2022). 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 (El Jabbour et al., 2019).

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 (Chartampilas et al., 2022).

The differential diagnosis between HGDN and early HCC is extremely challenging. Histological differentiation by morphology alone is not possible most of the time and definitive pathological criteria for differentiation between the two entities are currently lacking. In such cases, immunohistochemical study could be a potential diagnostic tool to identify and differentiate both lesions (Liao et al., 2023, Renne et al., 2021).

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 upregulated in HCC and its positive rate obviously increased following histological upgrading (Sun et al., 2017).

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 (Karadag Soylu, 2020).

Existing immunomarkers for differentiation between the HGDNs and early HCC are still of limited value (Li et al., 2020). So, an accurate diagnostic marker for detecting early HCC is fundamentally important, since early detection of HCC remarkably improves patient survival (Shen and Nam, 2018).

Astrocyte elevated gene-1 (AEG-1), also known as metadherin (MTDH), functions as a major oncogene for HCC and is highly overexpressed in patients with HCC of diverse aetiologies by multiple mechanisms including genomic amplification (Robertson et al., 2018). AEG-1 plays a vital role in promoting cancer development and progression by augmenting proliferation, invasion, metastasis, angiogenesis and chemoresistance, all hallmarks of aggressive cancer (Robertson et al., 2015).

However, it is important to note that further research and validation studies are still needed to establish the full diagnostic potential of AEG1 and its application in clinical settings. Nonetheless, the emerging evidence suggests that AEG1 holds promise as a valuable tool for distinguishing precancerous lesions from HCC, addressing the existing challenges in the literature (Banerjee et al., 2021, Srivastava et al., 2017).

The current work aimed to study the immunohistochemical expression of AEG1 and GPC3 in different grades of hepatocellular carcinoma cases and to evaluate the diagnostic significance of AEG1 and GPC3 in differentiating the cirrhotic nodules, dysplastic nodules, and hepatocellular carcinoma.

MATERIAL AND METHODS

Cases collection

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, 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 for our cases included the patient has primary hepatocellular carcinoma with no other malignancy, sufficient tissue specimens for immune staining and good quality of the blocks and Complete patient history and clinicopathological data (including age, sex, diagnosis, histological type, and tumor grade). Exclusion criteria included insufficient tissue for immunostaining or poor quality of the blocks, incomplete patient history and clinicopathological data and previous history of chemotherapy or radiotherapy.

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 ≥5cm) depending on the TNM staging system by the American Joint Committee on Cancer (AJCC) 8th edition (Chun et al., 2018).

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 (Nagtegaal et al., 2020). 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).

Tissue processing and staining

Hematoxylin and eosin were used to stain the paraffin blocks after they were serially sectioned (3-5 um 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.

Immunohistochemical procedure

The immunostaining was carried out for all cases utilizing: Astrocyte elevated gene 1 (AEG1) which is a rabbit polyclonal antibody against human MTDH (NP_848927.2) concentrated antibody (dilution 1:100). Also, Glypican3 antibody (GPC3) which is a mouse monoclonal antibody against human Glypican3 (Clone 1G12), concentrated antibody (dilution 1:50). Tumor slices, 5 µm in thickness, were placed on positively charged slides and dried at 37°C for 30 minutes. After deparaffinization, antigen retrieval was carried out with EnVision FLEX solutions at both high and low pH using a Dako PT Link unit at 97°C for 20 minutes. Immunostaining was performed using a Dako Autostainer Link 48. After applying a peroxidase blocking reagent, the slides were incubated with primary antibodies for 30 minutes. Diaminobenzidine (DAB) was then used as the chromogen, followed by the application of a horseradish peroxidase polymer for 20 minutes. The slides were counterstained with hematoxylin.

The stain was considered positive for AEG1 and GPC3 when showing membranous and/or cytoplasmic staining. The percentage of positive tumor cells (%) was considered regardless of the staining intensity. To determine the optimal cut-off, point for AEG1, Receiver Operating Characteristic (ROC) curve analysis was conducted. For GPC3, a stain was considered positive if at least 5% of the tumor cells showed positive staining (Guo et al., 2020).

Statistical analysis

The collected data were tabulated, and statistically analyzed using Statistical Package for the Social Sciences (SPSS) software version

25. Categorical variables were expressed as frequencies whereas numerical variables were expressed as mean ± SD. Accuracy, specificity, sensitivity, positive and negative predictive values were used to assess diagnostic values of the tested markers (Trevethan, 2017, Bartol, 2015). ROC curve to select the best cut-off point for AEG1 through assessing the diagnostic values of different percentages of AEG1 expression. 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 number of cases were correctly classified as hepatocellular carcinoma or precancerous lesions. Area under the ROC curves (AUC) of each marker were calculated which is a popular measure of the accuracy of a diagnostic test (Greiner et al., 2000). An effective way to improve the diagnostic accuracy is the combination of multiple markers: To combine the two markers (AEG1, 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 was used to analyze the relation between AEG1, GPC3 expression and the tumor grade, Significance was adopted at $p < 0.05$ (Moore, 1996).

RESULTS

This study was 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 of the studied cases are summarized in Table 1.

Expression of AEG1 and GPC3 in HCC specimens

AEG1 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 (Figure 1). The immunohistochemical results are summarized in Table 2.

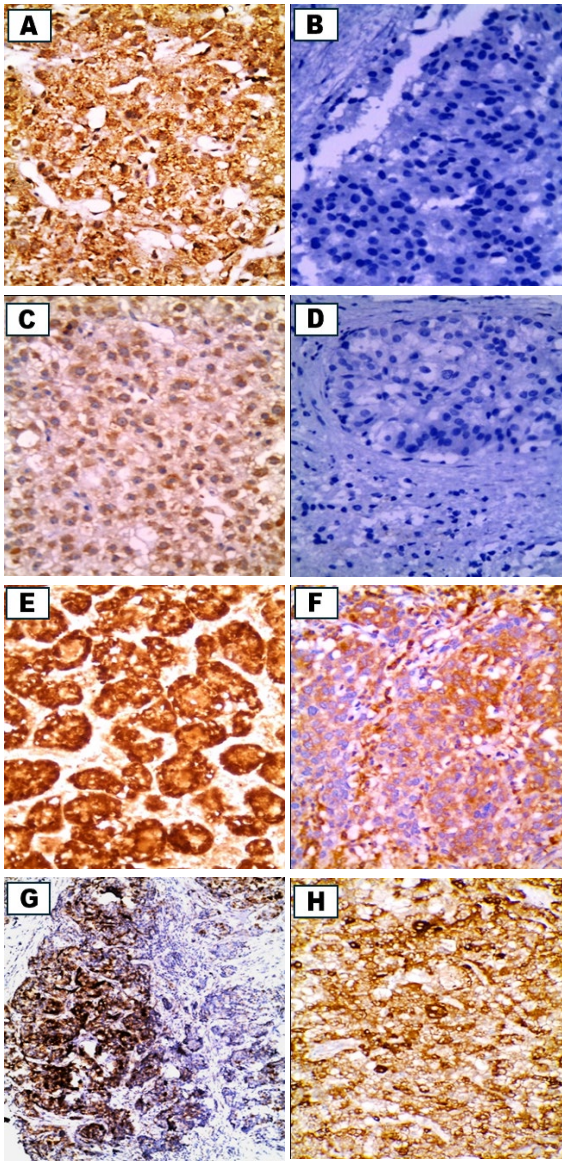

Figure 1. Immunohistochemical expression of AEG1 and GPC3 in HCC cases (x400). Well differentiated HCC cases (A), and (C) showed positive AEG1 expression. while (B) and (D) showed negative GPC3 expression. Moderately differentiated HCC (E) showed positive AEG1 expression and (F) showed positive GPC3 expression. Poorly differentiated HCC (G) showed positive AEG1 and (H) showed positive GPC3 as well.

Expression of AEG1 and GPC3 in hepatocellular carcinoma according to the tumor grade

As for AEG1 expression in different grades of HCC specimens, there is no statistically significant relation between AEG1 expression and tumor grade. Most well differentiated and moderately diff HCC cases, as well as all poorly differentiated cases showed positive AEG1 expression. Regarding GPC3 expression in different grades of HCC specimen, a statistically

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

Parameter	Number [%]
Sex	
Male	25 [69.4]
Female	11 [30.6]
Tumour focality	
solitary	26 [72.2]
Multifocal	10 [27.8]
Tumour 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.	%
AEG1	34	94.4	2	5.6
GPC3	27	75	9	25

AEG1 (astrocyte elevated gene 1), GPC3 (glypican3).

significant relation between positive GPC3 expression 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. Immunohistochemical results of AEG1 and GPC3 in different grades of HCC are summarized in Table 3.

Expression of AEG1 and GPC3 in precancerous lesions

Among the studied precancerous lesions, most cases (21 cases representing 87.5%) showed negative AEG1 expression while three cases (12.5%) displayed a brownish cytoplasmic and/or membranous staining distributed as all cases of cirrhotic nodules showed negative AEG1 expression while 21.4% of the dysplastic nodules (DN) were positive for AEG1. AEG1 expressions were detected in 33.3% of HGDNs and 12.5% of LGDNs (Figure 2 and Table 4).

Validity of AEG1 in the diagnosis of HCC

ROC curve was performed to identify the optimal cut-off value of AEG1 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%.

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 AEG1 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 AEG1 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 major contributor to the worldwide cancer burden. Incidence rates have increased in many countries in recent decades. Globally, HCC is the primary histologic type of liver cancer, accounting for about 80% of all primary liver cancer cases (El-Serag, 2020).

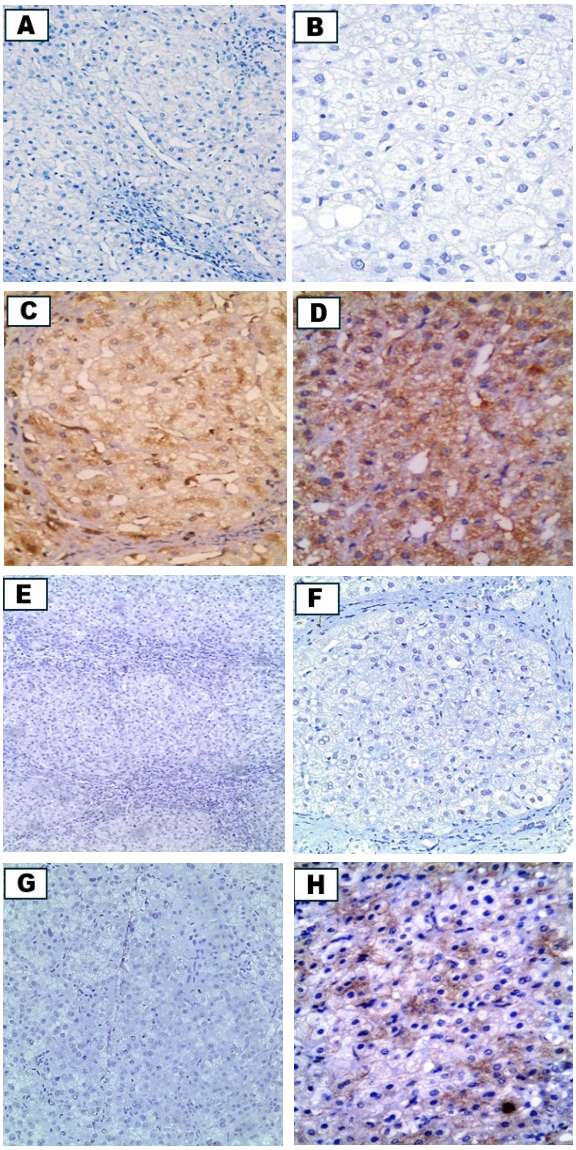

Figure 2. Immunohistochemical expression of AEG1 and GPC3 in precancerous lesions; (A) cirrhotic nodule showed negative AEG1. (B) LGDN showed negative AEG1 expression. (C) LGDN showed positive AEG1 expression. (D) HGDN showed positive AEG1 expression. (E) cirrhotic nodule showed negative GPC3. (F) LGDN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

Hepatocarcinogenesis is believed to be a multistep process from cirrhosis through dysplastic nodules including LGDNs and HGDNs to early HCC and finally advanced HCC (Jee et al., 2019). 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 is currently lacking (Quaglia, 2018).

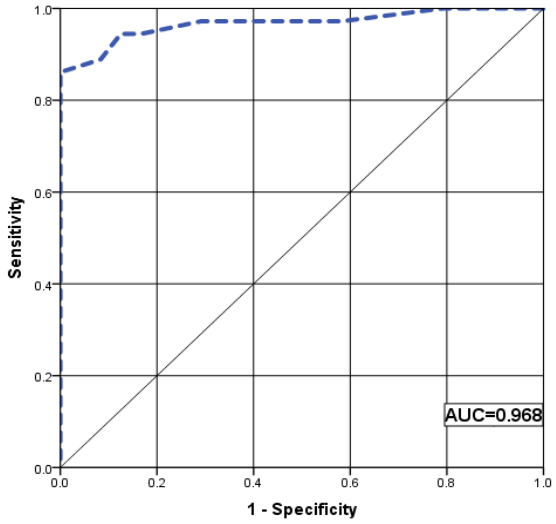

Figure 3. ROC curve for AEG1 expression. (AUC: area under the curve)

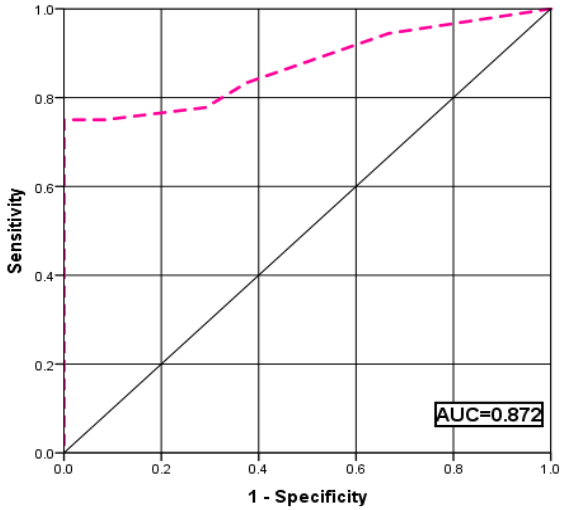

Figure 4. ROC curve for GPC3 expression. AUC (area under the curve)

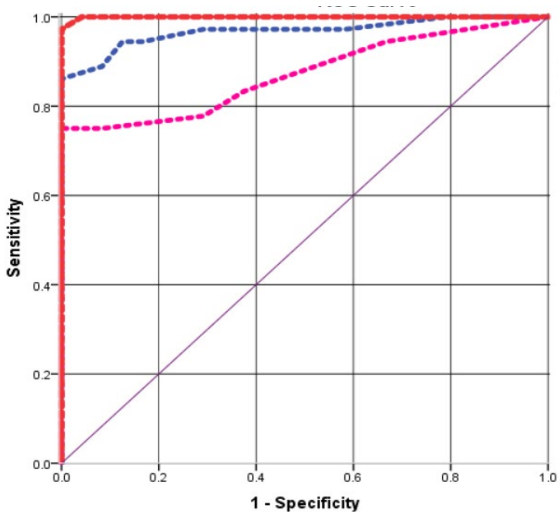

Figure 5. ROC curve for combination of AEG1 and GPC3 validity in the diagnosis of hepatocellular carcinoma.

Table 3. Expression of AEG1 and GPC3 in hepatocellular carcinoma according to the tumor grade.

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
χ2	24.083					35.106			
P-value	0.457					0.038*			

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

n=24 specimens	Cases	AEG1				GPC3			
		Positive		Negative		Positive		Negative	
	No.	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), AEG1 (astrocyte elevated gene1), GPC3 (glypican3), n (number).

Table 5. Measuring AEG1 expression cut-off point.

Test Result Variable(s): AEG1		
Positive if greater than or equal To[a]	Sensitivity	1 - Specificity
2.00	1.000	1.000
4.00	1.000	.958
5.50	1.000	.875
8.00	1.000	.792
11.00	.972	.583
13.50	.972	.417
15.50	.972	.333
18.00	.972	.292
22.50	.944	.167
40.00	.944	.125
57.50	.889	.083
62.50	.861	.000
66.50	.778	.000
69.00	.750	.000
71.00	.556	.000
73.50	.528	.000
77.50	.417	.000
82.50	.167	.000
85.50	.056	.000
88.00	.028	.000

Table 6. Combination of AEG1 and GPC3 in HCC diagnosis.

Marker	Sensitivity %	Specificity %	PPV %	NPV%	Diagnostic accuracy %	AUC
AEG1	94.4	87.5	91.8	91.3	91.7	0.968
GPC3	75	91.7	93.1	70.9	81.7	0.872
AEG1+ and/or GPC3+	97.2	100%	100	96	98.3	1.000

AEG1 (astrocyte elevated gene1), GPC3 (glypican3), PPV (positive predictive value), NPV (negative predictive value). AUC (area under the curve).

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 (Hytiroglou et al., 2022).

Astrocyte elevated gene 1 is considered a novel marker, that plays a critical role in the initiation and progression of cancer. AEG1 promotes cancer development and progression by augmenting proliferation, invasion, metastasis, angiogenesis and chemoresistance, all hallmarks of aggressive cancer (Sriramulu et al., 2021).

In normal liver tissue, AEG-1 expression is typically low or absent. AEG1 is downregulated in cirrhosis and dysplastic nodules suggesting a distinctive molecular signature that differentiates them from HCC. Conversely, the upregulation of AEG-1 expression in HCC indicates its involvement in hepatocarcino-genesis and makes it a valuable indicator of malignant transformation (Banerjee et al., 2021). It is worth mentioning that there is very limited literature data to evaluate the diagnostic role of AEG1 in hepatocellular carcinoma and precancerous lesions.

For such reasons, this study aimed to investigate the immunohistochemical expression of AEG1 and GPC3 in hepatocellular carcinoma and precancerous lesions including cirrhotic nodules and dysplastic nodules. The diagnostic value of AEG1 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 was the first to perform ROC curve analysis and setting a suggested optimal cut off value (40%) for expression of AEG1 that afforded the highest sensitivity and specificity for distinguishing hepatocellular carcinoma from precancerous lesions.

AEG1 provided 94.4% sensitivity, 87.5% specificity, and 0.968 AUC. AEG1 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., 2019 reported positive AEG1 expression in 91.8% of HCC specimens and 16.2% dysplastic nodules and adjacent non-tumorous tissue. 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., 2009 who studied the expression of AEG1 in HCC cases only and reported expression in 93.6%.

On the other hand, Zhu et al., 2011 and Jung et al., 2015 recorded lower values of positive AEG1 expression was reported in 54.2%, 67% respectively of their HCC studied cases. These discrepancies in the results can be attributed to the different methodology, tissue processing and different antibodies used.

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 (Guo et al., 2020).

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 who noticed nearly the same results; Li et al., 2023 reported GPC3 sensitivity of 73.6%, 96.2% specificity, 95.12% positive predictive value and 78.13% negative predictive value. Although, Mohamed et al., 2022 reported GPC3 sensitivity of 80%, 82.5% specificity, 83.3% positive predictive value and 79.2 % negative predictive value.

However, There is wide variability in GPC3 sensitivity ranging from 54.1% up to 86.4% and specificity ranging from 79% to 100% (Zhang et al., 2016, D'Errico, Coral et al., 2021, Ren et al., 2021, Mohamed and Eldowik, 2022, Hui et al., 2023, Wang et al., 2020, and Li, 2023). 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 (Zhang et al., 2012) to 11% (Shafizadeh et al., 2008, Wasfy and Eldeen, 2015). LGDNs showed positive staining in 8% (Wang et al., 2006) while

HGDNs showed positive staining in 7% (Di Tommaso et al., 2007) up to 22% (Wang et al., 2006).

On the other side Yamauchi et al., 2005 reported positivity in 33% of cirrhotic nodules, 25% of LGDNs and 75% of HGDNs. Also, Gong et al., 2014 found that 5.5% LGDN and 50% HGDN were positive for GPC3. This variability could be explained by differences in the used antiGPC3 antibody and immunohistochemical technique, this forms a likely explanation for why they could not discriminate well differentiated HCCs from LGDNs and HGDNs.

The present study investigated the expression of AEG1 and GPC3 in different grades of HCC. AEG1 expression was observed to increase progressively with tumor grade; however, no statistically significant relation was found between AEG1 expression and high tumor grade [p value = 0.457]. In contrast, GPC3 expression showed a significant increase with higher tumor grades, with a statistically significant relation [p-value = 0.038]. This association may be attributed to GPC3 acting as an oncofetal protein that promotes cell growth, differentiation, and tumor formation.

In agreement with this finding, Wasfy and Eldeen, 2015 and Elzeftawy et al., 2022 reported a statistically significant relation between GPC3 expression and high tumor grade. However many studies showed that the expression of GPC3 was lower in well-differentiated HCC (50%-72.7%) than in moderately or poorly differentiated HCC (83%-89%) but with no statistically significant relation (Hegazy, 2016, Shafizadeh et al., 2008, Di Tommaso et al., 2007).

In contrast, Wang et al., 2020 who reported opposing results that GPC3 showed no obvious difference in its 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., (2005) suggested that GPC3 is a good marker for the identification of well-differentiated HCC hence he reported expression in 78% of cases.

Analysing these findings, the current study highlighted that AEG1 was more sensitive than GPC3 in the detection of HCC, suggesting its potential as a diagnostic marker for HCC. However, GPC3 was more specific than AEG1 in the exclusion of precancerous lesions. It should be mentioned that the combination of different markers could improve their validity in the identification of HCC. Therefore, this work was extended to investigate the validity of AEG1 in combination with GPC3 in the diagnosis of HCC. Double combination of AEG1 and GPC3 in the current study provided better sensitivity (98.2%), specificity (100%) and a larger AUC (1.000) compared to AEG1 and GPC3 alone.

In comparison to other commonly used combinations, according to Hegazy, et al., (2016) combining GPC3, Arginase and HepPar-1 provide 87.5% sensitivity, 78.1% specificity, 80% PPV and 86.2% NPV. While combining Arginase with GPC3 provides 87.5% sensitivity, 87.5% specificity, 87.5% PPV and 87.5% NPV. In addition, Li, et al., 2023 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 AEG1 and GPC3 was the promising combination serving the highest sensitivity and specificity in the detection of HCC cases and differentiating it from precancerous lesions.

CONCLUSIONS

AEG1 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 AEG1 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 AEG1 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 AEG1 that showed no statistically significant relation with the tumor grade. AEG1 and GPC3 are useful combination offering higher sensitivity and specificity so the application of both markers in clinical practice will achieve better results

RECOMMENDATIONS AND LIMITATIONS

This study is constrained by small sample size and limited diversity in tumor subtypes, as well as a scarcity of studies examining AEG1 expression across different cancers and metastasizing hepatocellular carcinoma (HCC). Therefore, we recommend further research into the expression of AEG1 and GPC3 using a broader range of cases, encompassing various histopathological subtypes of HCC. In addition, it is crucial to focus specifically on dysplastic lesions and early HCC. Additionally, investigating AEG1 expression in different cancer types and metastasizing hepatocellular carcinoma is recommended.

FUNDING

The authors funded this research independently without external financial support.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ROLE OF AUTHORS

All authors of this research paper have directly participated in the planning, execution, or analysis of this study. All authors of this paper have read and approved the final version submitted.

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

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