RESEARCH ARTICLE

Diagnostic accuracy of glypican-3 in differentiating hepatocellular carcinoma from metastatic liver tumours

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Abstract

Objective: To determine the diagnostic accuracy of Glypican-3 in differentiating hepatocellular carcinoma from metastatic liver tumours while taking histopathology as the gold standard.

Methods: The cross-sectional study was conducted at Shaukat Khanum Memorial Cancer Hospital, Lahore, Pakistan, from January 1 to June 30, 2016, and comprised cases of malignant liver tumours. Samples were collected from the pathology department. Glypican-3was applied on them. Tumours were classified as positive when they showed >5% positivity and negative when showing ≤5% positivity. Frequencies and percentages of cases showing GPC3 positivity and negativity along with frequency and percentages of hepatocellular carcinoma and metastatic tumours were calculated.

Results: Of the 240 patients, 143(59.58%) were males and 97 (40.42%) were females. Overall mean age was 54.65 ± 13.46 years. On histopathology, 134 cases were hepatocellular carcinoma (55.83%) and 106 (44. 17%) cases turned out to be metastatic carcinoma. Glypican-3staining was positive in 116 (48.33%) cases and negative in 124(51.67%). Sensitivity was 82%, Specificity 94.33%, positive predictive value 94.82% and negative predictive value 80.64%. Diagnostic accuracy was 87.5%.

Conclusion: Glypican-3 was found to be a highly sensitive and specific Immunohistochemistry stain distinguishing hepatocellular carcinoma from the clear majority of metastatic carcinomas of the liver.

Keywords: Diagnostic accuracy, Hepatocellular carcinoma, Glypican 3, Metastatic carcinoma. (JPMA 68: 1029; 2018)

Introduction

Hepatocellular carcinoma (HCC) is the most common malignant tumour of liver and accounts for 70-85%[1] of primary liver malignancies. HCC incidence is increasing with time and this tumour is the third most common cause of cancer-related deaths worldwide.[2] Major risk factors causing HCC are chronic Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) infection and chronic alcohol intake.[3] It is very important to diagnose and distinguish HCC from other primary liver and metastatic tumours for the most effective treatment of disease and assessment of its prognosis. Recent advances in imaging technology have facilitated early detection of small tumours in the liver, but frequently the findings are non-specific and histopathological diagnosis is the ultimate choice.[4,5]

Current diagnostic methods for HCC include serum markers, imaging techniques and pathological studies including immunohistochemistry (IHC).[6] The morphological diagnosis of HCC may be straightforward in many cases. However, poorly-differentiated HCC and metastatic tumour pose diagnostic difficulties. For such cases, IHC markers are needed to facilitate correct diagnosis. The IHC stains which are useful for diagnosis of HCC include HepPar-1, Polyclonal carcinoembryonic antigen (pCEA), CD10 and Glypican-3 (GPC3).[7] HepPar 1 and pCEA have low sensitivity (50%) for the diagnosis of poorly-differentiated HCC.[8,9]

GPC3, (a member of heparansulfate proteoglycan family, is an oncofetal protein expressed in the embryo and involved in morphogenesis and growth control during development. GPC3 induces apoptosis in certain cells lines via the anchoring of the protein to the cell membrane, indicating that GPC3 functions as an inhibitor of cell proliferation.[10,11] GPC3 is inactive in the normal adult liver, and is reactivated in HCC. This reactivation in HCC has led to significant attention in GPC3 as a diagnostic IHC marker.[12]

GPC3 as a potential tumour marker for HCC was first reported in a study which said GPC3 messenger ribonucleic acid (m-RNA) was preferentially expressed in HCC.[10] Different studies show that GPC3 has different sensitivity and specificity values in HCC. These studies were performed in other countries, including USA, Egypt and China).[13-15] Tothe best of our knowledge no such study has been done in Pakistan previously and predictive values will be different in Pakistan depending on the prevalence of disease. The current study was planned to determine the diagnostic accuracy of GPC3 in differentiating HCC from

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metastatic liver tumours, and to determine the exact sensitivity and specificity of GPC3 in HCC.

Material and Methods

The cross-sectional study was conducted at Shaukat Khanum Memorial Cancer Hospital, Lahore, Pakistan, from January 1 to June 30, 2016, and comprised cases of malignant liver tumours. Samples were collected from the pathology department. GPC3 stain was performed on all cases. Monoclonal antibody to GPC3 (Gc33, Ultra view dab detection, Ventana kit) was used. IHC-stained slides were then evaluated. Tumours were classified as GPC3 positive when tumour cells showed >5% (cytoplasmic and membranous) positivity and GPC3 negative when tumour cells showed >5% (cytoplasmic and membranous) positivity. Diagnostic accuracy of GPC3 was noted. Frequencies and percentages of cases showing GPC3 positivity and negativity along with frequency and percentages of HCC and metastatic tumours were calculated.

Results

Of the 240 cases, 143 (59.58%) were males and 97 (40.42%) were females. Overall mean age was 54.65 ± 13.46 years (range: 14-75 years) (Table-1). On histopathology, 134(55.83%) cases were HCC and 106(44. 17%) turned out to be metastatic carcinoma. GPC3 staining was positive (Figure-1) in 116 (48.33%) cases and negative in 124 (51.67%) (Figure-2). Besides, 24(18%) HCC cases showed negative GPC3 staining and 110(82%) HCC cases showed positive GC3 staining. Overall, 106(44.17%) cases were metastatic carcinoma on histopathology, and, of them, 6(5.7%) showed positive GPC3 expression, and 100(94.3%) metastatic carcinoma cases showed negative

Table-1: Frequency distribution of cases according to age.

Age	Frequency	Percentage (%)
Less than 20 years	1	0.4
21-40 years	34	14.2
41-60 years	147	61.3
More than 60 years	58	24.2
Total	240	100.0
Mean ± Standard deviation (SD)	54.65 ± 13.46	

Table-2: Frequency distribution 2x2 table showing true positive (TP), true negative (TN), false positive (FP) and false negative (FN) cases.

Diagnosis	Results (Total cases)	Glypican-3 Positive cases	Glypican-3 Negative cases
Hepatocellular carcinoma	134	110 (TP)	24 (FN)
Metastatic tumours	106	6(FP)	100(TN)

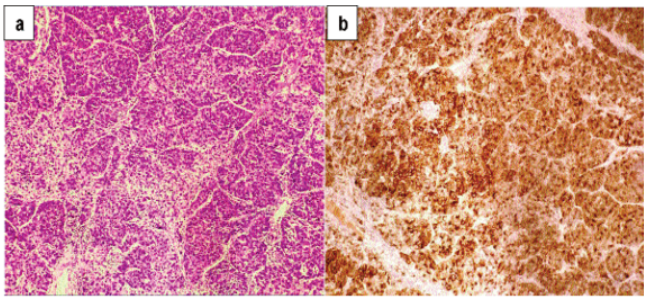

Figure-1: Demonstrating a) Hepatocellular carcinoma (100X). b) Cytplasmic and membranous Glypican-3 positive stain (100X).

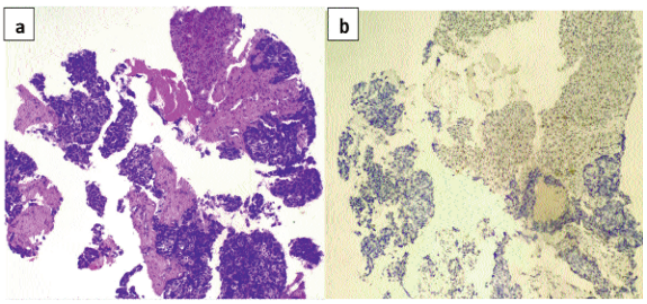

Figure-2: Demonstrating a) Metastatic carcinoma (100X). b) Negative Glypican-3 stain (100X).

GPC3 staining. The true positive (TP), true negative (TN), false positive (FP) and false negative (FN) cases were identified (Table 2). Sensitivity of GPC3 in the diagnosis of HCC was 82%, specificity was 94.33%, positive predictive value (PPV) 94.82%, and negative predictive value (NPV) was 80.64%. Diagnostic accuracy of GPC3 in HCC was 87.5%.

Discussion

GPC3 is a member of glypican family of heparansulphate proteoglycans. GPC3 shares similar expression pattern to that of a-fetoprotein (AFP), repressed prenatally, postnatally and reactivated in HCC. The current study was aimed at assessing the role of GPC3 IHC in differentiating HCC from metastatic carcinoma of the liver, in terms of sensitivity, specificity, predictive values, and diagnostic accuracy.

In our study, most of the patients were between 41-60 years (n=147). Mean age of patients was 54.65±13.46 years. Out of 240 cases, 143 were males and 97 were females. These results are comparable with different studies.[13] In one study performed in China, mean age was 54 years, most patients were males. This shows that HCC is more common in middle aged males.[14]

In our study, out of 240 cases, 116 cases showed GPC-3 positive staining, while GPC-3 negative cases were 124.

Sensitivity was 82%, specificity was 94.33%, PPV was 94.82%, and NPV was 80.64%. Diagnostic accuracy was 87.5%. One study showed similar results in terms of sensitivity (95.2%), specificity (83.3%), PPV (93%), NPV (88.2%) and total accuracy (91.7%).[13] In another study performed in China, sensitivity of GPC3 was 83.4% and specificity was 100%.[14] In one American study, GPC3 was found to be expressed in the majority of cases of HCC with a sensitivity of 83.3%, specificity of 96%, PPV of 95% and NPV of 85.7%.[15]

So, we found GPC3 to be a sensitive and specific marker for diagnosing HCC. But it was also noted that a small percentage of HCCs (18%) were negative for this marker. In addition, we must realise that GPC3 is not entirely specific for HCC, as 5.7% of metastatic tumours also showed GPC3 positivity. One must correlate the GPC 3 staining with tumour morphology to render a diagnosis of HCC.

Conclusion

GPC3 is highly sensitive and specific IHC marker for distinguishing HCC from the clear majority of metastatic carcinomas of liver. Positive GPC3 staining along with tumour morphology is a tool in the diagnosis of HCC.

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Conflict of Interest: None.

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