IMMUNOHISTOCHEMICAL EXPRESSION OF GLYPICAN-3 IN HEPATOCELLULAR CARCINOMA

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ABSTRACT

Objective: To determine the frequency of immunohistochemically expression of Glypican-3 in hepatocellular carcinoma on liver biopsies.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from Jun 2015 to Jun 2016.

Material and Methods: A total of 55 liver biopsies from the patients of hepatocellular carcinoma were included in the study. Age, gender, tumor grade and immunohistochemically expression of glypican-3 were noted. The data were analyzed by SPSS version 21. Mean and SD were calculated for numerical variables such as age. Percentages and frequencies were calculated for gender, tumor grade and immunohistochemically expression of glypican-3. The data collected for study were statistically analyzed using chi-square test.

Results: Out of 55 patients of hepatocellular carcinoma, 44 (80%) were males and 11 (20%) were females. The age of the patients was between 41 and 80 years with an average age of 59.7 years and standard deviation of ± 8.8. Out of 55 cases, 32 cases (58.2%) were well differentiated, 18 cases (32.7%) were moderately differentiated and 5 cases (9.1%) were poorly differentiated. Forty-eight cases of hepatocellular carcinoma (87.3%) showed positivity for glypican-3 while 7 cases (12.7%) were negative. A significant statistical association was not seen among age, gender, tumor grade and glypican-3 expression, *p*-value being >0.05.

Conclusion: The high expression of glypican-3 in hepatocellular carcinoma suggests its diagnostic utility and also its value in distinguishing hepatocellular carcinoma from other hepatic lesions. As antiglypican-3 therapy is currently under clinical evaluation, it might be useful as a therapeutic target in glypican-3 positive hepatocellular carcinoma cases.

Keywords: Glypican-3, Hepatocellular carcinoma, Immunohistochemical expression.

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for about 80% of all primary liver cancers[1]. It is the 5th most common cancer in the world[2], and the 3rd most common cause of cancer-related death globally[3]. HCC represents about 6% of all the newly diagnosed cancers and around 1% of all deaths, having one of the highest mortality rates worldwide[4]. A large burden of HCC occurs in Eastern Asia[5]. Around 80% of HCC cases occur in South-East Asia and Africa[2]. Around 55% of all HCC cases worldwide are reported from China[6]. The epidemiology of HCC in Asia is changing due to modification of the risk factors[7]. Its incidence in Pakistan is 23.4%, making it the commonest malignancy diagnosed on liver biopsies[8]. The leading cause of HCC in Pakistan is hepatitis C followed by hepatitis B[2].

It is the fifth most common cancer in men and seventh most common cancer in women[9], with the male to female ratio ranging between 2:1 and 4:1, depending upon the area of occurrence. In Asian and Western countries it occurs beyond the age of 75 years, whereas in African countries it tends to peak in the 60 to 65 age group for men and 65 to 70 age group for women[10].

Glypican-3 (GPC3) is the most novel marker of HCC. Its has membranous, canalicular, cytoplasmic or mixed positivity. It is expressed in the

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majority of hepatocellular carcinomas but not in the normal liver or in benign hepato-cellular lesions[3]. GPC3 is a 60 kDa cell-surface protein which is one of the 6 members of glypican family[11]. Glypicans are a family of heparan sulfate proteoglycans that are bound to cell surface by a glycosyl-phosphatidyl-inositol (GPI) anchor. Glypicans regulate the signaling activity of several growth factors including Wnts, Hedgehogs, bone morphogenetic proteins and fibroblast growth factors. GPC3 encoding gene is located on chromosome Xq26. GPC3 has a vital role in regulating cell growth, differentiation and migration[12]. GPC3 promotes the tumor growth by stimulating Wnt signaling by forming a complex with Wnt and its receptor, Frizzled, resulting in increased transcriptional activity of B-catenin[3].

GPC3 is an emerging therapeutic target against HCC, as two immunotherapeutic approaches are in phase II clinical trials. One of them is a monoclonal antibody that induces antibody-dependent cellular cytotoxicity and the other one is a vaccine that induces cytotoxic T lymphocytes[3]. Anti-GPC3 monoclonal antibodies (MDX-1414 and HN3) are currently undergoing clinical evaluation in patients with HCC. HN3 blocks the binding of growth factors and triggers intracellular signaling, leading to inactivation of yes-associated protein (YAP) and inhibition of cell proliferation in hepatocellular carcinoma. In the absence of HN3, growth factors bind to GPC3 and promote cell proliferation[13].

The studies carried out in China in 2010 and 2011 showed that 83.4% and 65% of HCC cases expressed GPC3[12,14] respectively. This expressionis 49% and 75.7% in the American studies conducted in 2008[15,16]. However, there is no local study available in this regard.

GPC3 expression has also been correlated with poor prognosis in HCC, as GPC3-positive HCC patients have a lower 5-year survival rate than GPC3-negative HCC patients[17].

MATERIAL AND METHODS

This descriptive cross-sectional study was carried out at Armed Forces Institute of Pathology, Rawalpindi from 15th June 2015 to 14th June 2016, after the approval of ethical committee. A total of 55 patients of HCC were included in the study, irrespective of the age and clinical presentation of the patients by non probability, consecutive sampling technique. Sample size was calculated using WHO sample size calculator according to following parameters:

- Confidence level (1–α) = 95%
- Anticipated population proportion (P) = 83.4%[14]
- Absolute precision required (d) = 10%
- Minimum sample size (n) = 55

Table: Stratification of GPC3 expression according to Age groups, gender and tumour grade.

Clinicopathological variable	Cases (n)	Percentage (%)	GPC3 Expression		*p*-value
			Positive	Negative	
Age groups (years) (n=55)					
41-50	12	21.8	10 (83.3%)	2 (16.7%)	NA
51-60	17	30.9	16 (94.1%)	1 (5.9%)	
61-70	20	36.4	17 (85%)	3 (15%)	
71-80	6	10.9	5 (83.3%)	1 (16.7%)	
Gender (n=55)					
Male	44	80	40 (90.9%)	4 (9.1%)	0.13
Female	11	20	8 (72.7%)	3 (27.3%)	
Tumour grade (n=55)					
Well differentiated	32	58.2	27 (84.4%)	5 (15.6%)	NA
Moderately differentiated	18	32.7	17 (94.4%)	1 (5.6%)	
Poorly differentiated	5	9.1	4 (80%)	1 (20%)	

The formalin fixed paraffin embedded (FFPE) liver biopsies of HCC were selected. Poorly fixed and inadequate liver biopsies were excluded from the study. Immunohistochemical assay for GPC3 was done by using Bio SB kit as per the manufacturer's guidelines as follows: The FFPE tissue sections were cut at 3 µm thickness and placed on clean glass slide with pre-attached adhesive on its surface. They were incubated at 58 degrees Celsius for 4 hours. The sections were deparaffinized with xylene 1 and 2, for 3 minutes each. They were rehydrated in decreasing concentrations of alcohol, 90%, 80% and 70% for 3 minutes each, followed by running tap water for 5 seconds. The slides were placed in coplin jar with 0.01 M Tris-EDTA buffer at pH of 9.0. 750 W domestic microwave was used to treat the slides for 20-30 minutes for heat mediated antigen retrieval. Slides were washed with distilled water for 20-40 minutes. After cooling down the sections, they were brought to phosphate buffered saline (PBS) at pH 7.3 for 5 minutes. PBS was washed and excess was wiped off the sections. Endogenous peroxidase activity blocked by incubating in 0.5% hydrogen peroxide in methanol for 5 minutes. The slides were washed in three series of PBS, 2 minutes each. 100 uL of primary antibody of GPC3 was instilled on the sections and incubated for 60 minutes. The slides were again washed in three series of PBS, for 60 minutes. The slides were then incubated in avidin-biotin complex for 10 minutes. They were rinsed with distilled water. They were incubated in DAB (diaminobenzidine) substrate solution for 5 minutes. Then the slides were washed with water and counter stained with haematoxylin for 40 seconds. The slides were dehydrated by placing them in increasing concentrations of alcohol, 70%, 80%, 90% and 100% alcohol for 3 minutes each. Clearing was done by placing slides in xylene for 3 minutes. The slides were mounted with Canada balsam.

Immunohistochemistry (IHC) results were interpreted independently by two experienced histopathologists. FFPE samples of HCC and normal liver without the primary antibody served as positive and negative controls, respectively. GPC3 staining intensity was recorded as: no cell staining (negative), <10% of tumour cells stained (1+), 10-25% of tumour cells stained (2+), >25% of tumour cells stained (3+). However, ≥10% of tumor cells stained was considered positive.

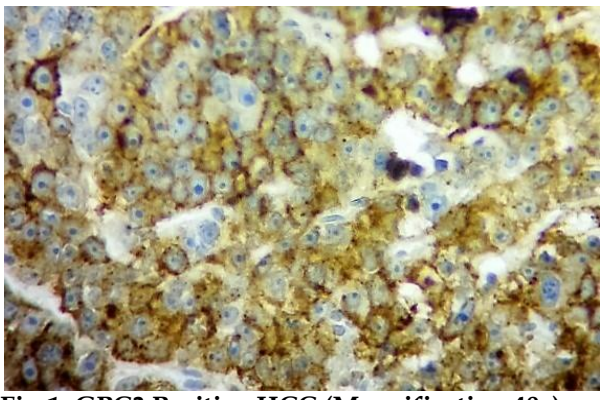

Fig-1: GPC3 Positive HCC (Magnification 40x).

Age, gender, tumor grade and immunohistochemical expression of GPC3 were noted. The data were analyzed by SPSS version 21. Mean and SD were calculated for numerical variables such as age. Percentages and frequencies were calculated for gender, tumor grade and immunohistochemical expression of GPC3. Statistical analysis between age, gender, tumor grade and GPC3 expression were done using Chi-square test. A *p*-value less than 0.05 was considered as a significant value.

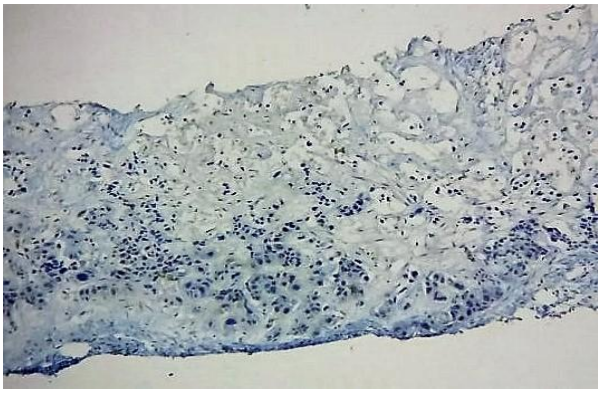

Fig-2: GPC3 Negative HCC (Magnification 20x).

RESULTS

During the study, a total of 55 cases of hepatocellular carcinoma were included. The

distribution of cases according to different age groups, gender, tumor grade and immuno-histochemical expression of GPC3 were summarized in table. A significant statistical association was not seen among age, gender, tumour grade and GCP3 expression, *p*-value being >0.05.

The ages of patients ranged from 41 to 80 years with a mean age of 59.7 years and standard deviation of ± 8.8. In our study, most of the patients belonged to 7th decade (36.4%, n=20) of life. There were 44 (80%) males and 11 (20%) females with a male to female ratio of 4:1.

Out of 55 cases, 32 cases (58.2%) were well differentiated, 18 cases (32.7%) were moderately differentiated and 5 cases (9.1%) were poorly differentiated.

Forty eight cases of hepatocellular carcinoma (87.3%) showed positivity for GPC3 (fig-1) while 7 cases (12.7%) were negative (fig-2). Twenty seven cases (84.4%) of the well differentiated, 17 cases (94.4%) of the moderately differentiated and 4 cases (80%) of the poorly differentiated tumors showed positivity for GPC3.

DISCUSSION

The age range in our study was 41-80 years. It was comparable to the studies carried out by Anatelli *et al*[15] in Chicago, United States and Yan *et al*[12] in China, the age ranges in these studies being 36-87 years and 31-76 years, respectively.

The mean age in our study was 59.7 years, which is in concordance with a study conducted by Wang *et al*[16] in Washington, United States (60.1 years). Our mean age was 2.7 years younger compared to the mean age in the study conducted by Anatelli *et al*[15] in Chicago, United States. It was 7.7 and 5.7 years older than the studies conducted in China by Yan *et al*[12] and Wang *et al*[14], respectively.

Our study revealed male predominance in HCC, with a male to female ratio of 4:1, however it was 2.5:1, 1.66:1, 1.96:1 and 1.71:1 in the studies conducted by Yan *et al*[12], Wang *et al*[14], Anatelli *et al*[15] and Wang *et al*[16], respectively.

In our study, 58.2% tumours were well differentiated, 32.7% were moderately differentiated and 9.1% were poorly differentiated. However, it was different in the studies conducted by Yan *et al*[12] (24%, 59%, 17%), Wang *et al*[14] (35.3%, 57.1%, 5.7%), Anatelli *et al*[15] (46%, 43%, 8%) and Wang *et al*[16] (46%, 40.5%, 13.5%).

The expression of GPC3 observed in our study (87.3%) was comparable to the study conducted by Wang *et al*[14] in China (83.4%). However, it was 65%, 49% and 75.7% in the Chinese and American studies conducted by Yan *et al*[12], Anatelli *et al*[15] and Wang *et al*[16], respectively. The difference in percentage expression of GPC3 might be either due to the regional variation depending upon different genetic makeup of the population or might be due to the difference in sample size.

In our study, 84% of the well differentiated, 94% of the moderately differentiated and 80% of the poorly differentiated tumours showed positivity for GPC3. However, it was different in the studies conducted by Yan *et al*[12] (41.9%, 74.43%, 64.39%), Wang *et al*[14] (73.7%, 93.5%, 67%), Anatelli *et al*[15] (38%, 59%, 50%) and Wang *et al*[16] (66.7%, 80%, 93.3%).

Age, gender and tumour grade were not significantly correlated with GPC3 expression (*p*-value >0.05), which was comparable to the Chinese and American studies conducted by *Yan et al*[12], and *Anatelli et al*[15] and *Wang et al*[16], respectively.

CONCLUSION

The high expression of GPC3 in HCC in this study suggests its diagnostic utility and also its value in distinguishing HCC from other hepatic lesions. As Anti-GPC3 therapy is currently under clinical evaluation, it may be useful as a therapeutic target in GPC3 positive HCC cases.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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