DOI: https://doi.org/10.53350/pjmhs2023176202

ORIGINAL ARTICLE

Frequency of Immunohistochemical Marker GPC-3 Expression in Malignant Tumors

NOSHABA RAHAT[1], HUMERA SHAHZAD[2]
[1]Associate Professor Pathology BMSI JPMC.
[2]Assistant Professor pathology BMSI JPMC
Correspondence to: Dr. Noshaba Rahat, Email: Dr Noshaba12@gmail.com, Contact: 03355463823

ABSTRACT

Objective: To determine the frequency of immunohistochemical marker GPC-3 expression in malignant tumours in a tertiary care hospital.

Material & Methods: This cross sectional study was conducted at the department of Histopathology, Dr. Ziauddin Medical University and Hospital, north campus, Karachi, a total of 114 cases of malignant tumours were enrolled using consecutive sampling technique in a period of six months from 1st April, 2012 to 30th September, 2012. Patients of both genders and all age groups of histologically diagnosed case of hepatocellular carcinoma (HCC) metastatic tumours, germ cell tumours (GCT) and squamous Cell Carcinoma (SCC) were all included in the study. The patients with benign tumours, malignant lymphoma, brain tumours, breast tumours, colon carcinoma and malignant melanoma were excluded from the study. The study outcome was determined as frequency of positive GPC-3 expression.

Results: A total of 114 cases were enrolled. Average age was 48.8 years with majority of cases (70.4%) were 40 years or above age. Male gender was slight dominance (53.5%). Squamous cell carcinoma was the most common (78.9%) followed by, germ cell tumours (10.5%) including (6.1%) yolk sac tumour and 4.3% Germinoma. Hepatocellular carcinoma was found in (6.1%) study cases. Most of the cases (45.6%) were moderately differentiated (SCC and HCC),followed by poorly (42.1%) and well differentiated (12.3%) grading. The frequency of positive GPC-3 expression was 25.4% in all malignant tumour in the current study. It is diagnostic and accuracy is more than other markers for definitive diagnosis and tp differenciate between primary and metastatic tumors.

Conclusion: In conclusion out of 114 cases 25.4% of total cases show GCP-3 positively, among which GCT were 24.1% and HCC were 85.7% which is quite similar to other reported studies. Type of the tumour was found to be significantly associated with the age group above 40 years (P=0.000). There is significant association between type of tumour and GCP-3 expression (P=0.000).

Keywords: Malignant Tumours, SCC, HCC GCT, GPC, GPC-3 expression

INTRODUCTION

Glypicans are heparan sulfate proteoglycans that are bound to the external surface of the plasma membrane by a glycosyl-phosphatidly linositol (GPI) linkage[1]. There are six glypican family members in the human genome (GPC-1 to GPC-6) [1,3 9].It is normally expressed in foetal liver and placenta but not in adult liver[3]. Experimental evidence using cell cultures suggests that silencing occurs in tumours derived from the adult tissues which may normally express GCP-3, and function as a tumour suppressor in these tissues. In tissues with no adult expression, GCP-3 may act as an oncofoetal protein [3,1o,11]

It is important to determine the type and/ or site of origin of metastatic tumours for optional clinical management. In the absence of a clinically known or suspected primary site, morphologics and immunohistochemical evaluation are key to determining the tumour lineage and origin. Routine microscopy may reveal characteristic that are diagnostic or suggestive of lineage (e.g mucin, melanin and keratin). Absence of morphologically distinctive features often necessities the use of immunohistochemistary particularly in poorly differentiated malignancies. It is an ancillary technique for evaluation of metastatic tumours and should be used in the context of routine morphology and clinical information. Even if a basic tumour lineage is apparent on routine haematoxylin eosin stain, confirmation of site of origin [e.g, lung, colon] may be clinically important. Immunohistochemistary is widely used in diagnosis of metastatic tumours both in the context of confirming a clinically suspected site of origin as well as a tumour of unknown origin. Most tumour specific or organ-specific makers may variably react with other tumour type,. also hence for metastatic tumour of unknown origin.The use of a panel of markers is strongly encouraged. Characterization of type of origin of metastatic tumours requires judicious use of linage and organ specific tissue markers[5].

Hepatocellular carcinoma (HCC) is one of the most common malignancy worldwide[6], The distinction among different hepatic nodular lesions,HCC and liver metastasis is often elusive and challenging for pathologist[7]. With the global pandemic of hepatitis B and C infections, the incidence of HCC is rapidly increasing worldwide[8].

The diagnosis of HCC often requires the use of immunohistochemistry especially in small biopsy specimes. Hep Par I and CEA have low sensitivity for poorly differentiated HCC. Hence, they may not be helpful in the setting for poorly differentiated hepatic neoplasm in distinguishing HCC and metastatic adenocarcinoma. Studies showed that GCP-3 has a high sensitivity for HCC and especially useful in identification of HCC as it has higher sensitivity compared to hep Par[3].

Studies have found the presence of GPC-3 in malignant tumour i.e, 79% in HCC, 2 to 54% in squamous carcinoma, 52% in germ cell tumours and 80% in placental trophoblastic tumour.[1,4,9]

HCC rapidly metastasizers and frequently recurs after treatment and there is no effective systematic therapy due to its high chemo-resistance. Antigens expressed by tumours cells are highly specific and may serve as potential targets for immunotherapy. GPC-3 is proved to be high immunogenic without autoimmunity in animal model. Studies on mouse model showed that GCP-3 is useful not only for the diagnosis of HCC but also for immunotherapy of HCC. So it can be a good candidate for an ideal tumour antigen for HCC immunotherapy[6,10]. Therefore, many studies from all over the world are currently underway to determine the significance of GCP-3 expression as diagnostic maker in malignant tumours.

The experience with the antibody is still limited and its expression in various tumours has not been widely studied. There is no local literature available for the GCP-3 expression in malignant tumour so far. Hence we conducted the current study to determine the frequency of immunohistochemical expression GCP-3 in malignant tumour commonly seen in our practice. The results of this study would be useful in establishing the magnitude of its presence in cancer cases, If its magnitude is high then further studies will be carried out for its diagnostic accuracy. So it could be utilized as diagnostic or panel marker.

MATERIAL AND METHOD

The descriptive, cross sectional study was conducted at Dr. Ziauddin Medical University and Hospital, North Campus, Karachi from 1st April, 2012 to 30th September, 2012. Patients of both genders and all age groups of histologically diagnosed case of hepatocellular carcinoma (HCC) metastatic tumours, germ cell tumours (GCT) and squamous Cell Carcinoma (SCC) were all included in the study by Non-probability consecutive type sampling technique. All benign tumours,Malignant Lymphoma, brain tumours, Breast tumours, Colon Carcinoma and Malignant Melanoma were excluded.

The sample size was estimated by using the World Health Organization (WHO) sample size calculations. The confidence interval level was considered at 95% and participating population was taken as 40%[9] . By considering the absolute precision at 9%, the required sample size was 114 patients with malignant tumour.

All resected specimens and biopsy material were grossed according to guidelines and sections were taken. These sections were processed overnight. Then the sections of formalin fixed paraffin embedded tissue were cut at 4 µm and stained by Haematoxylin and Eosin (H & E) for morphologic evaluation.

Immunohistochemical staining for GPC-3 was performed according to the standard protocol. Mouse monoclonal antibody specific for GPC-3 (clone IGI2 cell Marque), with a dilution of 1:400 for 1 hour at 25^0C was used. Sections were mounted on silence coated slides. Antigen retrieval was done according to antibody pretreatment charts, tissue was quenched of endogenous peroxidise, slides were stained with antibodies and then these slides were placed in chromogen.

Specific positive control (in case of larger tissue) were placed on same slides for quality control. Negative control were run on different slides. All cases were evaluated by senior pathologist with five years experience in the histopathology reporting. GPC-3 was labelled as positive in the presence of ≥ 5% of cells staine

Data was entered and analyzed by using SPSS version 20 USA. The descriptive analysis was carried out and reported as mean with standard deviation, median and range (min and max) for continuous variables such as age of patients. For categorical variables such as gender, type of tumours, site and histological grade, presence of GPC-3 expression and its categories (negative and positive), frequencies and %ages were calculated and reported. Further, the data was stratified according to age, sex, tumour type, site and histological grade to minimize the impact of effect modifiers. By using chi-square test, p-value less than 0.05 was considered as significant.

RESULTS

In the current study we enrolled a total of 114 cases of malignant tumours i.e. squamous cell carcinoma, germ cell tumours and liver tumors in adult and paediatric population in both genders.

Out of 114 cases, most of the cases i.e. 90 (78.9%) were squamous cell carcinoma. Twelve (10.5%) were germ cell tumour. Among which 4 (33.3%) were Germinoma and 8 (66.7%) were yolk sac tumour. In liver tumours, 7 (6.1%) were hepatocellular carcinoma. Out of the remaining five (4.2%), 2 cases were metastatic carcinoma, while one case was non-small cell neuroendocrine carcinoma, neuroendocrine tumor and Wilms tumor each.

The mean (standard deviation) age of all the enrolled cases in this study was 48.8 (±18.6) years and median age was 50.5 years.(table 3)Majority of cases (26.3%) were biopsies of patients aged between 51-60 years old.

The male to female ratio in our study was 1: 0.9. Out of 114 cases, 61 (53.5%) were biopsies of male while 53 (46.5%) were biopsies of female cases

The findings of immunohistochemical expression (glypican 3) sows 29 (25.4%) cases were positive GPC-3 whereas 85 (74.6%) showed negative GPC-3 staining.

The comparison of histological grading of HCC with GPC3 positive expression is presented in 7 cases, 6 (85.7%) were poorly differentiated and 01 (14.28%) was moderately differentiated HCC. Other tumors i.e. neuroendocrine tumour, Metastatic tumours, non-small cell Carcinoma and Wilm's tumour were negative.

GPC-3 positivity was present in 10 (62.5%) cases of poorly differentiated squamous cell carcinoma and 6 (37.5%) of moderately differentiated SCC . In GPC-3. Negative Squamous Cell Carcinoma 46 (56%) were moderately differentiated, 37 (41.1%), 1 (1.1%) was poorly and well differentiated respectively.

Among GCT, 4 (33.3%) cases of Germinoma were negative, whereas all 8 (66.7%) cases of yolk sac tumour were positive 85 caseswere GPC-3 negative out of all 74 (87.1%) were SCC, 5 (5.9%) were GCT and 4 (4.6%) were others type of liver tumours respectively. There is significant association between type of tumour and GPC-3 expression (P=0.000).

Table 1: Distribution of Cases According to Age (n-14)

Type of tumor	Age group (Year)				P- value
	1-20	21-40	41-60	61 and Above	
Squamous cell carcinoma	2 (1.7%)	12 (10.5%)	49 (42.9%)	27 (23.7%)	0.000
Germ cell tumour	7 (6.14%)	4 (3.5%)	1 (0.88%)	0	
Hepatocellular carcinoma	1 (0.88%)	0	5 (4.4%)	1 (0.88%)	
Other	1 (0.88%)	1 (0.88%)	1 (0.88%)	2 (1.7%)	

Table 2: Comparison of Findings of GPC-3 Expression According to Type of Tumour (n = 114)

	GPC-3 expression		P value
Type of tumour	Positive (n=29)	Negative (n=85)	
Squamous cell carcinoma	16 (55.2%)	74 (87.1%)	0.000
Germ cell tumour	7 (24.1%)	5 (5.9%)	
Hepatocellular	7 (17.2%)	0 (0%)	
Other*	0 (0%)	5 (4.38%)	

*included metastatic carcinoma, non small cell carcinoma, neuro endocrine carcinoma and Wilms tumor.

Table 3: Comparison of Findings of GPC-3 Expression in GCT According to the site and histological subtype (n = 12)

SITE	Histological subtype	No. of cases	GPC-3	
			Positive	Negative
Gonadal				
Ovary	Dysgerminoma	02	-	2 (16.6%)
Testis	Seminoma	02	-	2 (16.6%)
	Yolk sac tumour	03	3(25%)	-
Extragonadal				
Sacrococcygeal	Yolk sac tumour	02	02 (16.6%)	-
Pelvic	Yolk sac tumour	01	01 (8.3%)	-
Abdominal	Yolk sac tumour	01	01 (8.3%)	-
Terminal ileum	Yolk sac tumour	01	01 (8.3%)	-

DISCUSSION

Many immunohistochemical antibodies are available for finding the type, origin and site of tumours including Hep Par I and AFP in HCC, however, these are not very sensitive in poorly differentiated HCC. Shafizadeh et al. Reported 89% poorly differentiated HCC showing GPC-3 staining compared to 63% showing Hep par I positivit[3,9]. Another study by Yamauchi et al. Reported 100% poorly differentiated HCC showing GPC-3 positivity compared to 67% cases positivity showing with Hep par [4,13]

The purpose of any diagnostic method is to detect the tumour and its staging early so that it can be managed effectively. A study conducted by Ali et al. reported 87.3% GPC-3 positivity in HCC, he concluded that The high expression of GPC3 in HCC in their study suggests its diagnostic utility and also its value in

distinguishing HCC from other hepatic lesions and Anti-GPC-3 may be useful as therapeutic target in GPC-3 positive HCC.[6,16]

Due to focal nature, multiple growth patterns and close association of yolk sac tumour with embryonal carcinoma, former can be confused with the latter. Identifying and distinguishing these 2 types has clinical significance, as presence or increased percentage of embryonal carcinoma is associated with initial metastasis as well as recurrences and lack of yolk sac tumours correlates with more relapse. AFP is positive in 70 to 100% of yolk sac tumors and 0-33% of embryonal carcinomas. GPC-3 immunostaining makes it easy to distinguish between these 2 subtypes as all yolk sac tumours show GPC-3 immunopositivity. Whereas more than 90% of embryonal carcinoma were negative (Zynger et al.)[16].

In the current study, the frequency of GPC-3 expression in GCT and HCC was 58.3% and 71.4% respectively which are quite comparable with other studies around the world. Several previous studies have found high frequency (49%) of glypican-3 expression in HCC and cirrhotic liver in USA[17]. Yamauchi et al reported diffuse GPC-3 staining in (84%) of HCC cases[13].Majeed et al.reported 82% of HCC showed positive GPC-3 expression while 94% of metastatic liver tumor showed negative GPC-3 expression,their study revealed Glypican-3 is a highly sensitive and specific marker for distinguishing hepatocellular carcinoma from the metastatic carcinomas of the liver.[11] In another study by Di Tommaso et al, GPC-3 expression was detected in (74%) cases of HCC[18], which is quite similar to our findings. In our study lung squamous cell carcinoma showed 100% GPC-3 positivity. Baumhoer et al reported 54% GPC-3 expression in lung SCC[15]. This difference in results could be due to limited number of cases in our study as compared to larger number of cases in the latter.

In HCC, GPC-3 expression was more i.e 6 (42.9%) in HCC poorly differentiated compared to moderately differentiated 1 (7.1%). A study from USA reported more expression of GPC-3 in moderately and poorly differentiated tumors[15]. Another study by Capurro et al[17,18], Juanping et al[17] and Wang et al[18] who reported GPC-3 positivity in 70%, 72% and 85% respectively in poorly differentiated HCC. These results are similar to our findings. In accordance with Yamauchi et al[13] who reported 100% cases of poorly differentiated hepatocellular carcinoma showing GPC-3 staining with membranous, canicular and cytoplasmic staining patterns. Our results also showed 100% cases of poorly differentiated HCC showing GPC-3 positivity. In our study both cytoplasmic and membranous staining patterns were observed.

Current study revealed high frequency of GPC-3 expression in yolk sac tumor and HCC than other tumors. Zynger et al demonstrated GPC-3 expression in 100% of yolk sac tumours while seminoma were consistently negative which is in concordance with our results.[17]

Local data on GPC-3 immuno staining is not available. The current study is one of the very few clinical studies conducted on immunohistochemical stain Glypican-3. Our data showed GPC-3 positivity in poorly differentiated HCC in accordance with Shafizaadeh et al[8] who reported 89% positivity in their study. Shafizadeh and coworkers concluded that glypican-3 is superior to Hep par-1 for identification of poorly differentiated HCC.

Finally more local studies on larger number of tumors needed to observe the sensitivity and specificity of GPC-3 in HCC and to distinguish yolk sac tumour from seminoma, embryonal carcinoma and other tumours.

The overall frequency of glypican-3 expression in malignant tumours in our study was 29 (25.4%) which is quite low compared to reports from baumhoer et al[15]. Our study showed quite low frequency of GPC-3 immunoreactivity in tumors other than HCC and yolk sac tumors. This could be due to inclusion of different types of tumours in our study compared to analysis on single tumour in other studies. Our results revealed GPC-3 positivity in 17.8% cases of SCC which is lower than reports from Baumhoer et al[15] which is 84%. This difference may be due to larger sample size in their study.

CONCLUSION

It is concluded from current study that there is low frequency of GPC-3 positivity in tumours other than yolk sac tumors and HCC. Combined use of GPC-3 and AFP in cases of YST could increase the sensitivity of detection of these entities compared with use of a single antibody. On the other hand, combined use of GPC-3 and Hep par I may be more helpful in identifying poorly differentiated HCC.

Recommendations:

Further studies in a wide variety of tumors are necessary before specificity of GPC-3 for HCC and YST can be firmly established.

In distinguishing poorly differentiated HCC from metastatic carcinoma GPC-3 shows higher sensitivity than Hep par I. Further studies recommended to evaluate its specificity in poorly differentiated HCC.

Expression of GPC-3 by most HCC had a significant impact in diagnostic Practice. Ongoing clinical trials will establish in the future, whether the i mpact of initial discovery will also be extended to the therapy of HCC.

REFERENCES

1. Amjad I,Mushtaq J,Altaf K etal;frequency of new hepatocellular carcinoma in patients suffering from hepatitis c virus infection related cirrhosis exposed to direct acting antiviral bases therapy .pjmhs vol16n003 march 2022
2. Ali SS,Shaukat J,Ahmed R etall;immunihistochemical expression of glypican 3 in hepatocellular carcinoma .pafmjvol68 no4 2018 august
3. MajeedS, Mushtaq S, Azam M etal; (2018). Diagnostic accuracy of glypican-3 in differentiating hepatocellular carcinoma from metastatic liver tumours. *J Pak Med Assoc*, *68*(7), 1029-1031.
4. Sun B,HaungZ,WangBI etal;significance of glypican 3 expression in hepatocellular cancer diagnosis .med science 2017;23,850-955.pmcid :pmc 53250411.
5. Yukihiro H,Kataoka H;Glypican3 is aprognostic factor and immunotherapeutic target in hepatocellular carcinoma .ward j gastrorntrol2016 jan 722 (1)27-s
6. Zhou F ,glypican3 Apromoising biomarker for hepatocellular carcinoma diagnosis and treatment .med res rev 2018 pmid 28021802
7. Ou-Yang RJ, Hui P, Yang X J, Zynger D L; (2010). Expression of glypican 3 in placental site trophoblastic tumor. *Diagnostic pathology*, *5*(1), 1-6.
8. Filmus J, Capurro M, Rast J;Glipicans. Genome Biology. 2008; 9: 224.
9. Shafizadeh N, Ferrell L D, Kakar, S; (2008). Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Modern Pathology*, *21*(8), 1011-1018.
10. Zynger D L, Everton M. J, Dimov N D ,Chou P M ,Yang, X. J. (2008). Expression of glypican 3 in ovarian and extragonadal germ cell tumors. *American journal of clinical pathology*, *130*(2), 224-230.
11. Krishna M; (2010). Diagnosis of metastatic neoplasms: an immunohistochemical approach. *Archives of pathology & laboratory medicine*, *134*(2), 207-215.
12. Eun S ,Lee K B, Park S Y etal; (2008). Glypican-3 and MAGE-1 are predictors of tumor invasiveness of hepatocellular carcinoma. *Basic and Applied Pathology*, *1*(4), 174-181.
13. Yamauchi N, Watanabe A, Hishinuma M etal ;(2005). The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Modern pathology*, *18*(12), 1591-1598.
14. Nakatsura T ,Yoshitake Y ,Senju S etal; (2003). Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochemical and biophysical research communications*, *306*(1), 16-25.
15, Baumhoer D,Tornillo L, Stadlmann S; (2008). Glypican 3 expression in human nonneoplastic, preneoplastic, and neoplastic tissues: a tissue microarray analysis of 4,387 tissue samples. *American journal of clinical pathology*, *129*(6), 899-906.
16. Zynger D L, Dimov N D, Luan C etal; (2006). Glypican 3: a novel marker in testicular germ cell tumors. *The American journal of surgical pathology*, *30*(12), 1570-1575.
17. Anatelli F, Chuang S T, Yang X J., & Wang, H. L. (2008). Value of glypican 3 immunostaining in the diagnosis of hepatocellular carcinoma on needle biopsy. *American journal of clinical pathology*, *130*(2), 219-223.
18. Di Tommaso L,Franchi G, Park Y N etal; (2007). Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. *Hepatology*, *45*(3), 725-734.