IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 21, Issue 3 Ser.9 (March. 2022), PP 11-16
www.iosrjournals.org

Diagnostic utility of Arginase-1 in differentiating hepatocellular from nonhepatocellular carcinoma

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Abstract:
Background*: Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver and sixth most common cancer in the world. The morphological features of hepatocellular carcinoma often overlap with those of metastatic tumors and intrahepatic cholangiocarcinoma. There is a limited number of immunohistochemical biomarkers used for distinguishing HCCs from non HCCs. Arginase-1 has been described in literatures as a potential immunohistochemical marker of hepatocellular differentiation. Only few studies have evaluated Arg-1 expression as a distinguishing marker of HCCs from nonHCCs .The aim of this study is to find out the expression of Arginase-1 in hepatic malignancies and to determine its diagnostic utility in differentiating hepatocellular carcinomas (HCC) from non-hepatocellular carcinomas (non-HCC).*
Materials and Methods*: This was a descriptive study and included a total of 31 cases of hepatic malignancies diagnosed on biopsies and FNA samples in the department of Pathology, Government Medical College,Kannur from January 2020 to January 2021. Of these 14 cases were HCCs and 17non HCCs (2 Cholangiocarcinomas & 16 Metastatic malignancies). The cytological and histopathological slides were reviewed and the tumours were classified as hepatocellular and non hepatocellular carcinomas.The representative blocks of biopsies & smears of cytology slides were subjected to immunohistochemical (IHC) study with Arg-1 antibody. The results were subsequently analysed.*
Results*: Arg-1 expression was found to be positive in 13 out of 14 cases (92.8%) and all belonged to the category of well / moderately differentiated HCCs. 87.5% of the well differentiated and 60% of moderately differentiated HCCs showed strong Arg-1 positivity (2+) . Arg-1 expression was negative in one case of poorly differentiated HCC. 76.4% of nonHCCs (13/17) were Arg-1 negative and 23.5% (4 cases) showed 1+ positivity of which 2 were cholangiocarcinomas, 2 were metastatic adenocarcinomas. Sensitivity of Arg-1 in differentiating HCCs from non HCCs was 92.85% and the specificity was 76.47% .The positive predictive value was 76.47% & Negative predictive value was 92.8%.The association between Arg-1 expression and HCC was found to be statistically significant on chi square analysis (p<0.001).*
Conclusion: *Our results indicate that Arg-1 is a sensitive marker of hepatic differentiation and hence can be utilized as a diagnostic marker in differentiating HCCs from nonHCCs.*
Key Word*: Arginase1,Hepatocellular carcinoma,immunohistochemistry*

Date of Submission: 12-03-2022 Date of Acceptance: 27-03-2022

I. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver accounting for 70%-85% of total liver cancers[1]. It is the sixth most common cancer worldwide and the fourth most common cause of cancer death. The disease occurs more frequently in underdeveloped countries of Asia, Africa and China.[2]The morphologic features of hepatocellular carcinoma (HCC) often overlap with those of metastatic tumors and intrahepatic cholangiocarcinoma on one hand, and with those of benign or borderline entities like focal nodular hyperplasia (FNH), hepatocellular adenoma (HCA), and high-grade dysplastic nodule (HGDN) on the other.[3]The distinction of HCC from cholangiocarcinoma and other types of adenocarcinoma metastatic to the liver is a relatively frequent, often challenging dilemma for surgical pathologists and very crucial, as the treatment goal for these tumors are different. Several treatment modalities, including surgical resection, radiofrequency ablation, and transarterial chemoembolization/radioembolization, are available for hepatocellular carcinoma[4] .Due to its markedly aggressive features and poor survival outcome, HCC remains an essential public health problem all over the world. Immunohistochemistry plays a critical role in the differential diagnosis of hepatic malignancies.[5]

II. Material And Methods

This descriptive study was done in Department of Pathology,Government medical college, Kannur from January 2020 to January 2021. A total 31 cases of hepatic malignancies were included in this study.

Study Design: Descriptive study.

Study Location: This study was done in Department of Pathology,Government medical college, Kannur, Kerala,

Study Duration: January 2020 to January 2021.

Sample size: 31.

Sample size calculation: Sample size calculated was 50. Records showed that there was an average of 50 liver samples (including FNA samples) at our centre each year. All cases of Hepatocellular carcinomas, Cholangiocarcinomas and Metastatic carcinomas to liver received in the Department of Pathology, during the above-mentioned period were included. Using the convenient sampling method, 31 consecutive samples were included in this study

Subjects & selection method: All patients with Hepatocellular carcinomas, Cholangiocarcinomas and Metastatic carcinomas of liver diagnosed in the Department of Pathology, GMC Kannur during the period from January 2020- January 2021.

Inclusion criteria:

All diagnosed cases of hepatic malignancies which includes

1.Resection specimens

2. Small biopsies

3.FNACs

Exclusion criteria:

Poorly processed samples are not included in the study.

Procedure methodology

Informed consent in both mother tongue and English was taken from the patients before performing the study. The relevant clinical data, results of laboratory investigations already done, operative findings etc were recorded from the request form for histopathological and cytological examination. FNA samples are collected in minimum two slides. One is air-dried followed by staining with a Romanowsky-type stain (MGG, Giemsa), other alcohol-fixed followed by Papanicolaou (Pap) or hematoxylin and eosin (H&E) staining , Biopsy specimens are received in 10% neutral buffered formalin. Representative sections are taken, labelled, processed and stained with H&E stain. The slides are examined under the microscope and the representative areas are marked and subjected to IHC staining with Arginase 1 mouse monoclonal antibody. Arg-1 staining pattern and intensity were noted. Only cytoplasmic or cytoplasmic and nuclear reactivity was considered as positive staining for Arg-1.The staining intensity of each IHC reaction were scored semiquantitatively :(as per reference number 11)

- 0 (no staining) -- absent
- 1+ (weak staining) – granular cytoplasmic staining
- 2+ (strong staining) – diffuse cytoplasmic and nuclear staining.

The pattern of staining was recorded as focal or diffuse.

- Focal staining -reactivity in < 10% of tumor or lesional cells.
- Diffuse staining- reactivity in >10% of tumor or lesional cells.

Statistical analysis

Statistical analysis was carried out using SPSS version 20 (SPSS Inc., Chicago, IL). Qualitative variables are expressed as frequencies and percentage. Validity of Arginase-1in distinguishing HCC from non-hepatocellular malignancies (CC and MC) was calculated using diagnostic performance depending on sample 2×2 contingency tables. The sensitivities, specificities, positive predictive values (PPV), negative predictive values (NPV), and accuracies with their respective 95% confidence intervals were calculated. The histological diagnosis designated as the gold standard .Chi square test was used to examine the relationship between categorical variables. The level $P < 0.05$ was considered as the cutoff value or significance.

III. Result

Table no 1 shows histological distribution of nonHCCs. Out of the 17 cases, cholangiocarcinoma-2 ,intraductal papillary neoplasm of bile duct-1,other metastatic carcinomas-14 (which included Gastric adenocarcinoma-3,pancreatic adenocarcinoma-2,mucinous colonic adenocarcinoma-1,neuroendocrine carcinoma-2,GIST-1,metastasis from unknown primary-5).

Table no 1: Histological distribution of non HCCs**.**

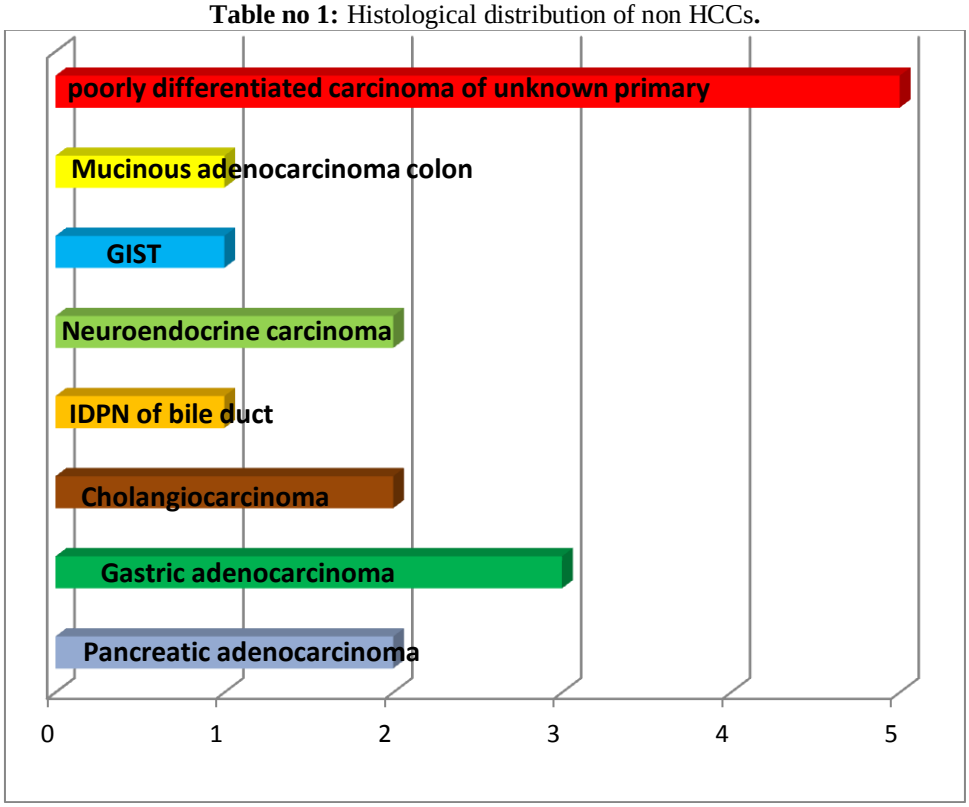

Table no 2: Arg -1 expression in HCCs and non HCCs

ARG- 1 expression	HCC	Non HCC
POSITIVE	13	4
NEGATIVE	1	13

Table no3 shows majority of the well differentiated and moderately differentiated HCCs showed strong Arg-1 immunoreactivity (2+).

Table no3: Histological grading of HCCs & Arg-1 expression

HCC GRADE	GRADING OF ARG-1 EXPRESSION			TOTAL
	O (absent)	1+ (weak)	2+ (strong)	
Well differentiated	0	1	7	8
Moderately differentiated	0	2	3	5
Poorly differentiated	1	0	0	1

Table no4 Shows negative Arg-1 expression in 13 cases of non HCCs and 1+ positivity in 2 cases of cholangiocarcinoma, 2 metastatic adenocarcinoma.

Table no4 : Arg-1 expression in non HCCs

NON HCC	ARG-1 EXPRESSION			TOTAL
	0 (Absent)	1+ (weak)	2+ (strong)	
HEPATIC MALIGNANCIES				
Cholangiocarcinoma	0	2	0	2
Intraductal papillary neolplasm of bile duct	1	0	0	1
METASTASIS				

Gastric adenocarcinoma	2	1	0	3
Pancreatic adenocarcinoma	2	0	0	2
Mucinous adenocarcinoma- colon	1	0	0	1
Neuroendocrine carcinoma	2	0	0	2
Gastrointestinal stromal tumor	1	0	0	1
Adenocarcinoma-unknown primary	4	1	0	5

Table no 5 : Shows the association between Arg-1 expression and HCC done using chi square test and was found to be statistically significant(p value < 0.001).

Characteristics		Group				P value
		HCC		NON HCC		
	Grade	Count	Percentage	Count	Percentage	
Arginase-1 expression	0	1	7.3%	13	76.5%	<0.001
	1+	3	21.3%	4	23.5%	
	2+	10	71.4%	0	0.0%	

Table no6: Validity of Arg-1 expression

Statistics	Value	95% CI
Sensitivity	92.85%	66.132% to 99.819%
Specificity	76.47%	50.101% to 93.189%
Positive Likelihood Ratio	3.946	1.655 to 9.412
Negative Likelihood Ratio	0.093	0.014 to 0.629
Prevalence	45.16%	27.32% to 63.97%
Positive Predictive Value	76.47%	57.675% to 88.573%
Negative Predictive Value	92.85%	65.879% to 98.870%
Accuracy	83.87%	66.3% to 96.55%

IV. Discussion

In this study Arg-1 expression was analysed in 31 cases of hepatic malignancies of which 14 were HCCs and 17 non HCCs (2 cholangiocarcinomas and 15 metastatic malignancies) . The pattern and intensity of Arg-1 expression was evaluated in all.

The mean age of presentation of HCCs in this study was 64 $\pm$ 11.7 and the median age was 65.5years. Among nonHCCs the mean age was 63 $\pm$ 11.5 and the median was 65years, which was similar to the study of Obiorah E et al [2] . Shams U M et al[4] found that 53.6 and 56.9 years were the mean ages of the study population of HCCs and non HCCs respectively.

In our study 71.4% of the HCCs occurred in males and 28.6% in females ,which was similar to the studies of Bita Moudi et al[6] , Hegazy et al[7] and Labib et al[8].

Majority of the HCCs were solitary lesions (71.4%) .HCCs which presented as solitary lesions (75%) predominated in the study of Obiorah E et al [2] also.

Arginase-1 immunoreactivity was detected in 13 of 14 cases (92.8%) of HCCs in our study which was similar to the study of Mcknight et al[1] where it was detected in 37 of 44 cases (84.1%) . In the metaanalysis conducted by Nelson G. Ordo´n˜ez[9] 91% cases showed Arg-1 immuoreactivity in HCCs and Yan et al[5] found the reactivity in 95.9% cases.

Table no 7: Previous Studies of Arginase-1 expression in HCCs in comparison with the Current Study

Study	Year	No: HCC cases	Arg1 positive cases	Type of specimen
Yan et al	2010	151	145(96%)	TMA
McKnight et al	2011	44	37(84%)	FNA
Radwan andAhmed	2012	50	42 (84%)	Whole section
Timek et al	2012	29	23(79%)	FNA
Fujiwara et al	2012	37	30(81%)	FNA
Geramizadehet al	2015	43	43(100%)	Whole section
Nguyen et al	2015	79	78(98.7%)	FNA & whole section

Labib et al	2019	30	27(90%)	Whole section
Current study		14	13(92.8%)	FNA& biopsies.

In our study , the sensitivity of arginase-1 in distinguishing HCCs from non-HCCs was 92.8% and the specificity was 76.47% with a PPV of 76% and NPV of 92.8%. This was similar to the results obtained by Mcknight et al[1] (sensitivity 84.1% and specificity 72.9%). Our findings were also similar to the results of Nguyen et al[10], Yan et al[5] and Radwan & Ahmed[11] who evaluated a larger study population .Nguyen et al[10] also found that Arg-1 was the most sensitive marker in all grades of HCCs.

The 95% confidence interval for sensitivity was in the range of 57 -98 in our study and was 50 -93 for specificity. Fujiwara et al[12] were also of similar opinion.

We found Arg-1 expression to be positive in all well and moderately differentiated HCCs (13/13 cases) which was similar to study of Nguyen et al [10](54/54cases) who inferred that Arg1 is an excellent marker for hepatic differentiation . Geramizadeh et al[13] also obtained 100% positivity and found that the staining of Arg1 was strong in majority of cases, inferring its high sensitivity.

In the study of Timek et al[14] on FNA samples ,they failed to demonstrate a better sensitivity of Arg-1 for higher–grade HCCs, and explained that it may be because of smaller sample size in the category of moderate to poorly differentiated HCCs & limited number of cell clusters on cytology slides .We had a single case of poorly differentiated HCC which showed Arg-1 negativity. On the contrary, the studies of Radwan et al[11] and Yan et al[5] showed that arginase-1 had better sensitivity in identifying higher grade HCC .

Majority of metastatic Adenocarcinomas in the present study were Arg-1 negative (76.47 %) and our results were comparable to that of Geramizadeh et al[13] , who demonstrated Arg-1 negativity in 77.8% of metastatic Adenocarcinomas .

Table no 8: Previous Studies of Arginase-1 expression in Non HCCs in comparison with the Current Study

Study	Year	No: Non HCCcases	Arg-1 negative cases	Type of specimen
Yan et al	2010	99	99 (100%)	TMA
McKnight et al	2011	35	35 (100%)	FNA
Radwan & Ahmed	2012	38	37 (97.6%)	Whole section
Timek et al	2012	28	28 (100%)	FNA
Fujiwara et al	2012	61	55 (90%)	FNA
Geramizadeh et al	2015	27	21(77.8%)	Whole section
Labib et al	2019	30	28(93.3%)	Whole section
Current study		17	13(76.4%)	FNA & biopsies

Both cases of cholangiocarcinomas in our study showed weak Arg-1 immunoreactivity (1+) Shiran et al[15] claimed that the occasional positivity in CC was because of the common progenitor cell of HCC and CC. In addition, Lida et al[16] found that Arg-1 was expressed at a higher rate in CCs -39.28% (11 of 28 cases) and highlighted the importance of being cautious while using Arg-1 as a hepatocyte marker for distinguishing poorly differentiated hepatocellular carcinoma and intrahepatic CC. On the contrary, Fujiwara et al[12] reported negative immunoreactivity of Arg-1 in all their cases of CCs.

Fujiwara et al[12] as well as Radwan and Ahmed[11] found strong Arg-1 reactivity in 20% & 2.6% cases of pancreatic adenocarcinomas respectively. In our study both the pancreatic adenocarcinomas were negative for Arg-1, which is similar to studies of Fathima et al[17] & Timek et al[14].The Arg1 expression in metastatic adenocarcinomas from pancreas is thought to be due to the persistence of common hepatopancreatic stem cells in the adult liver and pancreas[17].

We observed diffuse and strong immunostaining for Arg-1 in the non neoplastic liver tissues adjacent to HCCs and metastatic ACs. This supports the study of Fujiwara et al[12] and Timek et al[14] who reported that Arg-1 has no role in distinguishing well-differentiated hepatocellular carcinoma from benign hepatic lesions.

V. Conclusion

In conclusion the present study demonstrates that Arginase 1 has high sensitivity and specificity in diagnosing hepatocellular carcinoma and can be utilized as a diagnostic marker in differentiating HCCs from nonHCCs.

References

[1]. McKnight R, Nassar A, Cohen C, Siddiqui MT. Arginase-1: a novel immunohistochemical marker of hepatocellular differentiation in fine needle aspiration cytology. Cancer Cytopathol. 2012 Aug 25;120(4):223-9. doi: 10.1002/cncy.21184. Epub 2012 Feb 1. PMID: 22298472.

[2]. Obiorah,.Chahine,J.,KO, K.,Park B.,Deguzman,J,Kallakury,Prognostic Implications of Arginase and Cytokeratin 19 Expression in Hepatocellular Carcinoma After Curative Hepatectomy: Correlation With Recurrence-Free Survival. Gastroenterol Res. 2019;12(2):78-87.
[3]. Quaglia, A.Hepatocellular carcinoma: a review of diagnostic challenges for the pathologist. Journal of Hepatocellular Carcinoma, Volume 5, 99–108. doi:10.2147/jhc.s159808.
[4]. Shams U M,Loya A,Waqas O,Rias S. Arginase-1: A Useful Immunohistochemical Marker in Differentiating Hepatocellular Carcinomas from Non-Hepatocellular Carcinomas of Liver S.Z.P.G.M.I. Vol: 31(1): pp. 41-46, 2017.
[5]. Yan BC Gong C, Song J, et al. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol 2010;34:1147–54.
[6]. Moudi, B., Mahmoudzadeh-Sagheb, H. & Heidari, Z. Hepatocyte paraffin 1 and arginase-1 are effective panel of markers in HBV-related HCC diagnosis in fine-needle aspiration specimens. BMC Res Notes 13, 388 (2020).
[7]. Abdelrahman, Aziza & Abdel-Aziz, Hisham & Hegazy, Abdelmonem. (2016). Immunohistochemical Distinction of Hepatocellular Carcinoma Using Arginase-1, Hepatocyte Paraffin Antigen -1 and Glypican-3. Journal of Tumor. 10.17554/j.issn.1819-6187.2016.04.75.
[8]. Labib, O.H., Harb, O.A., Khalil, O.H. et al. The Diagnostic Value of Arginase-1, FTCD, and MOC-31 Expression in Early Detection of Hepatocellular Carcinoma (HCC) and in Differentiation Between HCC and Metastatic Adenocarcinoma to the Liver. J Gastrointest Canc 51, 88–101 (2020).
[9]. Ordóñez NG. Arginase-1 is a novel immunohistochemical marker of hepatocellular differentiation. Adv Anat Pathol. 2014 Jul;21(4):285-90.
[10]. Nguyen T, Phillips D, Jain D, et al. Comparison of 5 immunohistochemical markers of hepatocellular differentiation for the diagnosis of hepatocellular carcinoma. Arch Pathol Lab Med 2015;139:1028–34.
[11]. Radwan NA, Ahmed NS. The diagnostic value of arginase-1 immunostaining in differentiating hepatocellular carcinoma from metastatic carcinoma and cholangiocarcinoma as compared to HepPar-1. Diagn Pathol 2012;7:149.
[12]. Fujiwara M, Kwok S, Yano H, Pai RK. Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 and glypican-3 in fine-needle aspiration biopsies. Cancer Cytopathol. 2012;120(4):230–7.
[13]. Geramizadeh B, Seirfar N. Diagnostic Value of Arginase-1 and Glypican-3 in Differential Diagnosis of Hepatocellular Carcinoma,Cholangiocarcinoma and Metastatic Carcinoma of Liver. Hepat Mon. 2015 Jul 23;15(7):e30336.
[14]. Dana T. Timek, MD, Jianhui Shi, MD, Haiyan Liu, MD, Fan Lin, MD, Arginase-1, HepPar-1, and Glypican-3 Are the Most Effective Panel of Markers in Distinguishing Hepatocellular Carcinoma From Metastatic Tumor on Fine-Needle Aspiration Specimens, American Journal of Clinical Pathology, Volume 138, Issue 2, August 2012, Pages 203–210.
[15]. Shiran MS, Isa MR, Sherina MS, Rampal L, Hairuszah I, Sabariah AR: The utility of hepatocyte paraffin 1 antibody in the immunohistological distinction of hepatocellular carcinoma from cholangiocarcinoma and metastatic carcinoma. Malays J Pathol 2006, 28(2):87–92.
[16]. Lida H, Hata M, Kakuno A, Hirano H, Yamanegi K, Yamada N, Ohyama H, Terada N, Yasui C, Yamanaka N, Nakasho K: Expression of hepatocyte markers in mass-forming peripheral and periductal-infiltrating hilar intrahepatic cholangiocarcinomas. Oncol Lett 2011, 2(6):1041–1046.
[17]. Fatima N, Cohen C, Siddiqui MT. Arginase-1: a highly specific marker separating pancreatic adenocarcinoma from hepatocellular carcinoma. Acta Cytol. 2014;58(1):83-8. doi: 10.1159/000355629. Epub 2013 Nov 20. PMID: 24281232.

Saidha Thajudeen, et. al. "Diagnostic utility of Arginase-1 in differentiating hepatocellular from nonhepatocellular carcinoma." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(03), 2022, pp. 11-16.