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Value of core needle biopsy in histopathological and immunohistochemical assessment of liver tumors

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***Abstract*---**Introduction: Liver is the most commonly involved organ by metastatic disease. Secondary tumors are more frequent lesion forming more than 75%, while primary cancers of liver formed less than 25%. Imaging according to LI-RAD can give a clue for the nature of the primary lesion of live particularly hepatocellular carcinoma. Liver biopsy is still the golden standard surveillance for histopathological diagnosis of liver tumor; however, it is a traumatic procedure, but its value in reaching the definite diagnosis is superior to imaging technique. Tru cut biopsy is less traumatic than open biopsy and considered nowadays as the preferable technical way in sampling liver tissue that can be applied for both histopathology and immunohistochemistry. Secondary liver lesions were encountered to be originated from lung, breast, Pancreatobiliary tract and GIT, female genital tract, prostate and others. Objectives of the Study: To assess the efficacy of Tru cut biopsy for both histopathological examination and immunohistochemical study identification the type and origin of liver tumors. Methodology: This is a cross sectional study conducted in the Al-Sadr Teaching Hospital in Al-Najaf City, Iraq from January 2019 till December 2021. A total of 79 cases of liver tumors were submitted for liver biopsy, most of them (76 cases) were underwent US guided Tru cut biopsies by G19 width core needles, while 3 cases were done by open biopsy. All clinical data as sex, age, radiological findings were collected. Histopathological and immunohistochemical study were proceeded for all cases. Results were analyzed and correlated with clinical presentation, age and gender using X2 and exact Fischer tests with P value of less than 0.05. Results: Secondary tumors were encountered more frequently than primary tumors, particularly in females. Known origins of these tumor were encountered in 58.23%,

while unknown origins of liver malignant lesions were encountered in 41.77%. Immunohistochemical study revealed that all known origin of secondaries were confirmed as it, while the origin of 91% of cases of unknown primary were identified according to their original site as breast, lung, pancreas and others.Conclusions: Core needle biopsy is an easy and rapid applicable tool for both diagnosis and identification the origin of liver tumors, and considered as sufficient technique for both histological and immunohistochemical study.

***Keywords*---**liver tumors, core needle biopsy, hepatocellular carcinoma.

Introduction

Liver is one of the organs that are commonly involved in metastatic disease. This high incidence of involvement can be attributed to several factors related to the various hypotheses of the spread of metastatic disease [1]. For instance, in the "mechanical/hemodynamic hypothesis" of metastatic spread, the dual blood supply of the liver by the hepatic artery and portal vein provides entrapment mechanism for the cancerous cells in the circulation [2, 3, 4, 5]. On the other hand, primary liver cancer accounts for a significant proportion of liver cancers. Hepatocellular carcinoma (HCC) is the most common form of primary cancer of the liver. Several risk factors are associated with hepatocellular carcinoma, including chronic viral infections -namely, hepatitis B virus (HBV) and hepatitis C virus (HCV)-, alcoholic liver disease, as well as non-alcoholic fatty liver disease. Males are at higher risk compared to females, and the incidence increases with age [6,7,8].

Liver biopsy is an essential procedure for the diagnosis and management of liver malignancies. One of the most reliable procedures is the core-biopsy using Tru-cut needle, it is superior to open biopsy as it is rapid easy and less traumatic [9,10,11,12]. However, in diagnosis of liver malignancies, hematoxylin and eosin-stained tissue section alone does not provide sufficient information for reaching the definitive diagnosis and identification of the origin of tumor. Therefore, application of immunohistochemistry as supporting tool can be substantially effective in achieving this target. [13,14]. There was a lot of histological overlapping among different types of secondary tumors particularly adenocarcinomas, so it is not easy to differentiate according to their primary site of origin. For confirmation of hepatocellular carcinoma, hepatocyte paraffin 1 (HepPar1) is the commonly used immunohistochemical marker. In spite of its high sensitivity (> 95%), it is less sensitive in poorly differentiated tumors [15]. Other markers used for primary hepatocellular carcinoma include alpha-fetoprotein (AFP), glypican-3, polyclonal carcinoembryonic antigen (p-CEA), and CD10 [16].

Application of immunohistochemistry is more essential and valuable identification of origin of secondary liver tumors[17]. Metastatic carcinoma of liver were reported in many malignancies as lung, breast, thyroid, pancreas, GIT, and female genital tract[18]. Application of immunohistochemical markers is usually

used in determination of the origin these secondaries. Cytokeratin (CK) markers are commonly used in the diagnosis of adenocarcinoma, particularly CK7 and CK20. Other markers as TTF1 and Napsin A were used for identification for lung origin, ER, PR and HER 2/neu were used for identification of breast origin. CA125, CDX2, neuroendocrine markers and others are the further step station in differentiation of ovarian ,GIT and other sites [19]. The treatment of both primary and secondary tumors depends on definite diagnosis of its histological type and origin. Upon this point, it is essential to do biopsy and immunohistochemical study for more differentiation and specification of the histopathological type of tumors, as most of recent modulated treatments (immunotherapy) depend on the specific histological type [20].

Material and method

This study is a cross sectional type conducted at Department of Pathology in Al-Sadr Medical City/ Al-Najaf, Iraq, during January 2019 to December 2021. This study was approved by the scientific committee of Histopathology – Arab Board of Health Specializations. All obtained data were handled with privacy and confidentiality. Samples of set of patients with their available clinical data were collected at Department of Pathology in Najaf center. A total of 79 cases with liver tumors were included in this study (76 were Tru cut and 3 were open biopsies), while samples with inadequate clinical information were excluded from this study. Data details were concentrated on the type of biopsy, sex, age, US findings, CT findings and history of any previous disease of operation.

All samples underwent histopathological tissue processing and section of paraffin blocks. H&E and immunohistochemical staining were performed. Histopathological examination of both H&E and immunohistochemical slides were done, for diagnosis and assessment of positivity of the tumor markers21,22. Classification of primary and secondary tumors according to final diagnosis was tabulated accordingly. Statistical analysis was done using SPSS® software (version 23.0 For Linux® operating system) to assess the continuous variables as means ± SD and frequencies and percentages for categorical variables. Student's t-test was used to compare means between two groups, while chi-square test was used to assess the relationship between categorical variables. P-value of ≤ 0.05 was considered statistically significant .

Results and Discussion

This study included 79 tissue samples of patients with liver tumors, 47 females and 32 males, aging from 17 to 90 years (mean 59.92 ± 15.21, median of 62.50). Most of them (>80%) were presented above age of 50 years (table.1). This result is not different from other documented findings worldwide [1,23], and also supported locally by Altaee (2011), conducted in Baghdad / Iraq, in which the mean age of patients was (57.1) years [24]. Most studies explained that the biological evolution and aggressiveness of malignancies were well correlated to age and related to accumulative mutation effect of related genes that be more prominent after age of 50 years [25].

In this study, female patients were found to be involved by liver tumors specifically secondary tumors more than males without significant difference between both (P<0.05) (Table.1). This finding is in controversy to many other studies [26,2], and can be explained by high frequency of breast and female genital tract cancers in female presented with liver secondaries [28]. while both male and female were affected insignificantly different by lung and colonic tumor with liver metastasis[1,29].

According to the available clinical data and radiological pattern of liver lesions, multiple liver lesions formed 58.23% of cases, most of them (95.83%) were of known primary origin other than liver, while solitary liver lesion formed 41.77% of the presented cases, all of them (100%) were of unknown primary origin. There was a significant difference (< P.0.05) between these two groups of multiple liver lesions or solitary lesions, as detailed in Table.2. So we can say that most of presented multiple lesions were of known origin while most of solitary lesions were of unknown origin with strong negative correlation between these two patterns (R>0.3) (table.2). This is an important and vital point regarding the management of liver cancers, that any suspicious lesion of liver, whether multiple or solitary should be taken in consideration and treated accordingly.

Regarding the origin of tumors, known suspected secondary tumors have formed 58.23% of total cases (Table.3), this percentage of secondaries get marked increase after histopathological and immunohistochemical examination (92.7%), while only 5 (16.12%) out 31 cases of solitary lesion were confirmed as primary hepatic carcinoma (HCC) by histopathology and immunohistochemistry (table.3). There was a significant difference (<P.0.05) between these two groups of primary and secondary tumors that were not defined clearly before, as detailed in Table (3-2).

In contrast, it has been found that there was no significant difference between conventional histopathology and IHC technique from statistical point of view, but actually 4 cases out 9 have been missed diagnosed as primary tumors (HCC) and really were secondary tumors after application of IHC, particularly in some multiple lesions (table.3). Upon this findings, we can say that most multiple and solitary lesions were secondary in origin, while all primary hepatic tumors were solitary lesion. This result makes the application of IHC tool necessary for proper histological diagnosis. This result is also an important for dealing with the origin of tumors, particularly in solitary lesions, that should undergo through investigation to rule out any other primary tumors than the liver as breast, lung, ovary or others.

The vast majority of cases (96.20%) were diagnosed using Tru-cut biopsy (76 cases), while the remaining three cases were diagnosed with open liver mass excision, without any significant difference in efficacy of diagnosis between these types of biopsy (P>0.05) (Table1). This evidence can support that Tru cut biopsy is an effective and sufficient technical procedure for rapid sampling the liver tissue with reduced risk of major surgery[30] .

Examination by histopathology and immunohistochemistry has confirmed diagnosis of these tumors, and defined the origin of both solitary and multiple

liver lesions. Hepatocellular carcinoma has been diagnosed in 6.3% of cases, confirmed by immunohistochemical study of HepPar1 and Alfa fetoprotein, all of them were forming radiological presentation of solitary lesions. Secondary carcinoma has been diagnosed in all (100%) multiple liver lesions and in 92.7% of solitary lesions, aided by the application immunohistochemical study of tumor markers according to known or suspected primary lesion other than liver (table.4). So submission patients for liver biopsy and histopathology aided by immunohistochemistry is an important and essential step in management of liver tumors for proper histopathological diagnosis and identification of the tumor origin. Histopathology revealed that most (>90 %) of the clinically presented cases with either known or unknown origin, multiple or solitary lesions of liver were secondary carcinoma, and IHC study was concordant with the origin of these tumors in 100% , while only 5 (55.5%) out 9 cases with histological diagnosis of HCC were confirmed immunohistochemically in solitary unknown lesions (Table 2).

Histopathological examination revealed that adenocarcinoma was the commonest histopathological type of secondary tumor, forming (59.49%) of samples, most of them were moderately differentiated adenocarcinoma. Small Cell Carcinoma has formed 6.33%, Neuroendocrine Tumors 10.13%. The Primary origin of these secondary tumors were confirmed to be distributed as follow; breast formed 31.64%, lung24.05 %, pancreatobiliary 17.21%, GIT 3.79%, female genital tract 2.17% and undetermined 3.79% (Table 4).

All these secondaries (92.3%) have been confirmed histologically and immunohistochemically, of them, the known primary tumors (58.%) were confirmed and corresponding to their primary origin, while 83.8% of those with unknown primary origin were confirmed to have unsuspected origin of tumor. This reflects that the unknown liver lesions formed a considerable proportion of cases, and needs to be thoroughly clarified for proper diagnosis. There is an essential need for application of IHC study as a histopathological panel for proper diagnosis of majority of liver lesions.

Conclusion

From the above results we can conclude that Tru cut biopsy in liver lesions was adequate facility and sufficient for both histopathological and immunohistochemical study . in addition to its role in identifying the site of primary origin . secondary carcinoma of liver was more frequent than primary and many cases of unknown multiple and solitary lesions were really secondary in origin , while primary hepatocellular carcinoma were presented as solitary liver lesion in this study.

Table (1): Distribution frequency of liver biopsies according to the available clinical data (N=79)

Clinical and radiological presentation	Frequency (N)	Percentage (%)
Gender N=79		

Male Female	32 47	40.51% 59.49%
Age N=79 <50 >50	 14 65	 17.72% 82.28%
Multiple lesions* N=48 - Known origin - Unknown origin	 46 2	 95.83% 4.17%
Solitary lesion*N=31 - Known origin - Unknown origin	 0 31	 0.0% 100%
Type of biopsy N=79 - Tru cut - Open	 76 3	 96.3% 3.7%

*P value < 0.0001

Table.2. The relationship between the pattern of liver lesions and the primary origin of tumors

Pattern of lesion	Known origin (secondary)	Unknown origin	Total	P value
Multiple	46 (95.8%)	2 (4.2%)	48	<0.05
Solitary	0 (0.0%	31 (100%)	31	<0.05
Total	46	33	79	

*P value < 0.00001 , R = > 3

Table.3. Histological and immunohistochemical classification of liver tumors in relation to clinical findings

Histological technique (N=79)	Pattern of liver lesions Multiple solitary 48 31	Suspected origin Known unknown 46 33	P value
Histopathology* -Primary N= 9 (11.4%) -Secondary N=70 (89.6%)	 3 6 45 25	 0 9 46 22	 P < 0.05
IHC * -Primary N= 5 (6.3%) -Secondary N=74 (92.7%)	 0 5 48 26	 0 5 46 28	 P < 0.05

Table (4): Distribution frequency of known and unknown cases according to their confirmed sites by IHC study.

Site of origin	IHC confirmed known cases	IHC confirmed unknown cases	Total	Percentage (%)
Breast	14	11	25	31.64%
Lung	15	4	19	24.05%
Pancreatobiliary	12	2	14	17.21%
NET	0	6	6	7.60%
FGT	3	3	6	2.17%
Liver	0	5	5	6.33%
GIT	2	1	3	3.79%
Undetermined	0	3	3	3.79%
Total	46	33	79	100%

References

1. Ridder J, Wilt J, Simmer F, Overbeek L, Lemmens V, Nagtegaal I. Incidence and origin of histologically confirmed liver metastases: an explorative case-study of 23,154 patients. *Oncotarget*. 2016;7(34):55368-55376.
2. Hugen N, Velde C, Wilt J, Nagtegaal I. Metastatic pattern in colorectal cancer is strongly influenced by histological subtype. *Annals of Oncology*. 2014;25(3):651-657.
3. Liu Q, Zhang H, Jiang X, Qian C, Liu Z, Lou D. Factors involved in cancer metastasis: a better understanding to “seed and soil” hypothesis. *Molecular Cancer*. 2017;16:176.
4. Costa-Silva B, Aiello N, Ocean A et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nature Cell Biology*. 2015;17(6):816-826.
5. Eveno C, Hainaud P, Rampanou A et al. Proof of prometastatic niche induction by hepatic stellate cells. *Journal of Surgical Research*. 2015;194(2):496-504.
6. Herbst D. Reddy K. Risk factors for hepatocellular carcinoma. *Clinical Liver Disease*. 2012;1(6):180-182.
7. Yang J, Hainaut P, Gores G, Amadou A, Plymoth A, Roberts L. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nature Reviews, Gastroenterology & Hepatology*. 2019;16(10):589-604.
8. Janevska D, Chaloska-Ivanova V, Janevski V. Hepatocellular Carcinoma: Risk Factors, Diagnosis and Treatment. *Open Access Macedonian Journal Of Medical Sciences*. 2015;3(4):732-736.
9. Cevik F, Aykin N, Naz H. Complications and efficiency of liver biopsies using the Tru-Cut biopsy Gun. *Journal of Infection in Developing Countries*. 2010;4(2):91-95.
10. Adali Y, Eroglu H, Makav M, Karayol S, Guvendi G, Gok M. Comparison of tru-cut biopsy and fine-needle aspiration cytology in an experimental alcoholic liver disease model. *Revista da Associacao Medica Brasileira*. 2020;66(8):1030-1035.

11. Stift J, Semmler G, Woran K et al. Comparison of the diagnostic quality of aspiration and core-biopsy needles for transjugular liver biopsy. *Liver, Pancreas, and Biliary Tract*. 2020;52(12):1473-1479.
12. Sherman K, Goodman Z, Sullivan S et al. Liver biopsy in cirrhotic patients. *American Journal of Gastroenterology*. 2007;102(4):789-793.
13. Lo R, Ng I. Hepatocellular Tumors: Immunohistochemical Analyses for Classification and Prognostication. *Chinese Journal of Cancer Research*. 2011;23(4):245-253.
14. Lopez G, Boggio F, Ferrero S, Fusco N, Gobbo A. Molecular and Immunohistochemical Markers with Prognostic and Predictive Significance in Liver Metastases from Colorectal Carcinoma. *International Journal of Molecular Sciences*. 2018;19(10):3014.
15. Takahashi Y, Dungubat E, Kusano H et al. Application of Immunohistochemistry in the Pathological Diagnosis of Liver Tumors. *International Journal of Molecular Sciences*. 2021;22(11):5780.
16. Gonzalez A, Salomao M, Lagana S. Current concepts in the immunohistochemical evaluation of liver tumors. *World Journal of Hepatology*. 2015;7(10):1403-1411.
17. Fanni D, Gerosa C, Faa G. The Histomorphological and Immunohistochemical Diagnosis of Hepatocellular Carcinoma. In: Wan-Yee Lau J, ed. Hepatocellular Carcinoma - Clinical Research. *London: IntechOpen*; 2012;4:65-88.
18. Golubnitschaja O, Sridhar K. Liver metastatic disease: new concepts and biomarker panelsto improve individual outcomes. *Clinical & Experimental Metastasis*. 2016;33:743-755.
19. Selves J, Long-Mira E, Mathieu M, Rochaix P, Lile M. Immunohistochemistry for Diagnosis of Metastatic Carcinomas of Unknown Primary Site. *Cancers*. 2018;10(4):108.
20. National Institute for Health and Care Excellence. Chapter 2: Diagnosis, in Diagnosis and management of metastatic malignant disease of unknown primary origin. Cardiff (UK): National Collaborating Centre for Cancer (UK). 2010;2:21-32.
21. The manual of surgical pathologists. Third edition ,Chapter 19:gastrointestinal specimen including hepatobiliary and pancreatic specimen
22. ancroft's theory and practice of histopathological techniques. Eighth edition ,appendix III Applications of immunohistochemistry .
23. Wang ZG, He ZY, Chen YY, Gao H, Du XL. Incidence and survival outcomes of secondary liver cancer: a Surveillance Epidemiology and End Results database analysis. Transl Cancer Res 2021;10(3):1273-1283. doi: 10.21037/tcr-20-3319
24. Altaee W. Hepatocellular Carcinoma Presentation & Management, A Prospective Study in the Medical City Baghdad - Iraq. The Iraqi Postgraduate Medical Journal. 2011;10(2):253-259
25. Zhang W, Sun B. Impact of age on the survival of patients with liver cancer: an analysis of 27,255 patients in the SEER database. Oncotarget. 2015;6(2):633-641
26. Hefaiedh R, Ennaifer R, Romdhane H et al. Gender difference in patients with hepatocellular carcinoma. La Tunisie Medicale. 2013;91(8-9):505-508

27. riscom JT, Wolf PS. Liver Metastasis. [Updated 2021 Apr 13]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553118/
28. Horn SR, Stoltzfus KC, Lehrer EJ, Dawson LA, Tchelebi L, Gusani NJ, Sharma NK, Chen H, Trifiletti DM, Zaorsky NG. Epidemiology of liver metastases. Cancer Epidemiol. 2020 Aug;67:101760. doi: 10.1016/j.canep.2020.101760. Epub 2020 Jun 17. PMID: 32562887.
29. Altajer, A., Efendi, S., Jabbar, A., Oudah Mezan, S., Thangavelu, L., M. Kadhim, M., F. Alkai, A. (2021). Novel Carbon Quantum Dots: Green and Facile Synthesis, Characterization and its Application in On-off-on Fluorescent Probes for Ascorbic Acid. Journal of Nanostructures, 11(2), 236-242. doi: 10.22052/JNS.2021.02.004
30. Neuberger J, Patel J, Caldwell H, et al. Guidelines on the use of liver biopsy in clinical practice from the British Society of Gastroenterology, the Royal College of Radiologists and the Royal College of Pathology. Gut. 2020;69(8):1382-1403.