القيمة التشخيصية لأضداد واسم الخلية الكبدية (HeP1) ولتظاهرات الواسم ألفا فيتو بروتين (αFP) لتفريق سرطان الخلية الكبدية البدئية من سرطان الأقنية الصفراوية باستعمال خزعات الرشافة بالإبرة الدقيقة(FNAB) وخزعات الكبد المفتوحة الجراحية

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الملخص

الهدف من هذه الدراسة هو تقييم الميزات النسجية والمناعية النسجية لسرطان الخلية الكبدية البدئي (HCC) وكذلك تصنيفها وتفريقها عن السرطانات غير الكبدية والنقائل إلى الكبد باستعمال خزعات الرشافة بالإبرة الدقيقة (FNAB) والخزعات الجراحية الكبدية عن طريق تعابير الواسمات HeP1 و αFP وبعض الواسمات الأخرى مثل CEA و EMA والسايتو كيراتين.

إن تشخيص سرطان الخلية الكبدية البدئية (HCC) على الأغلب صعب لأسباب متعددة، أهمها، أولا: أن الكبد يعدُّ أحد ثلاثة أماكن لانتقال السرطانات، ولأن سرطانات الخلية الكبدية قليلة، لذلك فإن معظم أورام الكبد نقائل ورمية/ ثانياً: سرطانات الخلية الكبدية البدئية وسرطانات الأقنية الصفراوية (Ch C) تبدي مظاهر متشابهة، وثالثاً: لأن سرطان الخلية الكبدية (HCC) تبدي تظاهرات متغايرة يمكن أن تتشابه مع سرطانات متعددة، مما يزيد في التباس التشخيص خاصة إذا كان الورم البدئي غير معروف، لذا فإن تشخيص سرطان الخلية الكبدية (HCC) يمكن أن يتم عن طريق خزعات الرشافة بالإبرة الدقيقة (FNAB) أو خزع الإبرة المفتوحة الجراحية او الطريقتين معاً، علماً أن الإبرة الدقيقة (FNAB) جيدة، لكنها يمكن أن تؤدي إلى إشكالات مثل زوال البنية النسيجية أو نقص النسيج المطلوب لإجراء دراسات أخرى، حيث تبدي الخلايا المأخوذة بالإبرة الدقيقة (FNAB) تركيباً مشابهاً للخلايا الكبدية الطبيعية، لذلك يمكن تطبيق التلوينات المناعية على الإبرة الدقيقة (FNAB) كما طُبقَتْ على المقاطع النسجية الكبدية.

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– أبدى الواسم αFP إيجابيةٍ على خلايا الـ HCC دون أن يبدي إيجابية لخلايا الكبد الطبيعية، وكذلك النقائل نادراً ما تبدي إيجابية له، ولكن لسوء الحظ فإن الواسم αFP يبدي إيجابية قليلة نسبيا (25–40%) لا يمكن الاعتماد عليها وكذلك أبدى الواسم (CEA) إيجابية بنسبة (50–70%) للـ (HCC) وهناك واسمات أخرى مثل EMA والسايتو كيراتين (كوكتيل) يمكن تطبيقها خاصة في التشخيص التفريقي لـ (HCC) عن سرطان الأقنية الصفراوية (ChC) والنقائل.

– في السنوات الأخيرة أنتج العالم واينبرغ وزملاؤه واسماً وحيد النسيلة للخلية الكبدية سمي (Hep- parl) يطبق على المقاطع المدمجة بالبرافين حيث يَظهَرُ تحبب واضح في هيولى الخلية الكبدية السرطانية (شكل 1 و2) وكذلك الطبيعية، بعد ذلك دراسات عديدة أثبتت أن (Hep1) واسم نوعي لسرطان الخلية الكبدية مع حساسية عالية (جدول IV).

دُرسَت 68 حالة سرطان خلية بدئية (HCC) (30 منها حصل عليها بواسطة الـ FNAB) و10 حالات لسرطان الأقنية الصفراوية (Ch. C)(7 منها بَواسطة الـ FNAB (جدول I) جميع هذه الحالات دُرسَت ولونت مناعيا بالواسمات HeP1، αFP، PCEA، EMA، والسايتو كيراتين (كوكتيل) وذلك على مقاطع برافين محضرة للتلوين المناعي.

68 من 68 حالة لـ HCC أظهرت إيجابية للواسم HeP1 بنسبة (100%) وذلك بإظهار أكثر من 5% خلايا إيجابية من الخلايا الورمية وهذه تتضمن 30 حالة HCC خزِعَت بالـ FNAB (جدولII).

ظهرت إيجابية الواسم αFP في 68/23 حالة HCC (بنسبة 34%). 10 حالات من ChC (7 منها بواسطة الـ FNAB) لم تبد أي إيجابية للواسمات HeP1 و αFP لكن لوحظت إيجابية عالية للـ CEA (60%) و الـ EMA (90%) والـ CK (90%) في حالات سرطان الأقنية الصفراوية (جدول III).

واستنتاجا لما ذكر يمكن اعتبار تعابير الـ HeP1 و الـ αFP بأنها نوعية لسرطان الخلية الكبدية مع حساسية عالية للـ (HeP1) مما يجعلها مفيدة في تشخيص سرطان الكبد البدئي وتفريقه عن السرطانات غير المميزة وذلك عن طريق استعمال الـ FNAB وخزعات الكبد المفتوحة الجراحية.

يعدُ الـ HeP1 واسماً نوعيا للخلايا الكبدية وخاصة سرطانات الكبد جيدة التمايز ومتوسطة التمايز. إن حساسية HeP1 على السرطانات الكبدية قليلة التمايز نسبيا قليلة ولكن لوحظ أن تعبير الـ αFP على السرطانات قليلة التمايز الكبدية HCC أنها أكثر إيجابية.

Diagnostic value of hepatocyte Paraffin 1 antibody (hep1) reaction and alfa feta protein (αFP) expression to differentiate primary hepatic cell carcinoma from cholangiocarcinoma in FNAB and opened liver biopsies.

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Abstract

Background: The aim of this study is to evaluate the histological, and immunohistochemical characterization of primary hepatic carcinoma (HCC), classification and differentiation from non hepatic and metastatic carcinoma, in FNAB and open liver biopsies, by Hep1, and αFP expression.

Material and methods: 68 cases of HCC(30 of them were FNAB)and 10 cases of non hepatic cholangiocarcinoma (7 of them were FNAB),all were stained with Hep1, αFP,PCEA,EMA and cytokeratin.

Results: Sixty eight(/68) cases of HCC were Hep1 positive(100%)with positive cells >5%, this including 30 cases of FNAB. αFP was positive in 23/68,(34%) of HCC cases. 10 cases of cholangiocarcinoma(7 of them FNAB) exhibited no reactivity for Hep1 nor αFP ,but reactive for CEA(60%),EMA(90%),CK(90%).

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القيمة التشخيصية لأضداد واسم الخلية الكبدية (HeP1) ولتظاهرات الواسم ألفا فيتـــو بـــروتين (αFP) لتفريق سرطان الخلية الكبدية البدئية من سرطان الأقنية الصفراوية باستعمال خزعات الرشافة بـــالإبرة الدقيقة(FNAB) وخزعات الكبد المفتوحة الجراحية

Conclussion: Expression of HeP1 antibody and αFP were found to be specific immunostains that may be useful in the diagnosis of all HCCs and most undifferentiated liver tumours biopsied by(FNAB) and opened liver biopsies. HeP1 is a specific marker for hepatocellular tissue, including well and moderately differentiated HCC,its sensitivity in poorly diffrentiated HCC is relatively low. αFP expression was more frequent in poorly differentiated HCC.

Key words:
Hepatocellular carcinoma, cholangiocarcinoma, hepatocyte paraffin 1, alfa feto protien, fine needle aspiration biopsy.

Abreviations:
Hepatocellular carcinoma(HCC),cholangiocarcinoma(Ch C),hepatocyte paraffin 1 (HeP1),alfa feto protien(αFP),fine needle aspiration biopsy(FNAB), cytokeratin (CK), well differentiated (WD), moderate differentiated (MD) , poorly differentiated (PD) .

Introduction:

The pathologic diagnosis of hepatic cell carcinoma(HCC)is usually difficult for many reasons:

First : the liver represents one of three most common sites of metastasis, while the incidence of HCC is relatively low. Indeed,most of the liver tumours are metastasis.

Second: hepatic cholangiocarcinoma and HCC frequently share overlapping morphologic appearances ,which may cause diagnostic problem(20).

Third: there is a variety of histological patterns of HCC mimiking a wide variety of malignant tumours and complicating the diagnostic processes. Therefore,liver masses frequently cause a diagnostic problem,particularly when no primary tumour is known.In addition HCC may present as a metastasis of unknown origin.The differential diagnosis can be made by fine needle aspiration biopsy (FNAB)(3,17),core and open liver biopsy(5,14),or a combination of both(33) .Even though FNAB of the liver is generally accurate(2),it suffers from the same problems as FNAB in any other body locations,namely, loss of tissue architecture and lack of material ,to perform additional studies . Poorly differentiated liver tumour present a particular challenge because they may represent either HCC,CH.C or metastasis .

Therefor distinguishing among them is important.

In FNAB material ,the most useful diagnostic criteria is similarity of tumour cells to liver cells(5,16,33).

Slide diagnosis can be aided by performing immunohistochemical stains on FNAB material similarly as on paraffin embeded tissue.

Different immunohistochemical studies have been applied in an attempt to differentiate HCC from hepatic cholangiocarcinoma of liver metastasis(9,11,23,25). αFP immunostaining is expressed in neoplastic hepatic tissue(6,12,15,21),but not in normal hepatic tissue.

Other tumours rarely express αFP(9). Unfortunately only about 25- 40% of cases of HCC are positive for αFP(6,19,20).

Intercellular canalicular expression found in neoplastic hepatic tissue and have been reported to be positive for PCEA(9,18,21,23)and can help distinguish HCC from metastatic adenocarcinoma, aproximately(50-

70%)of cases of HCC . This feature may not be always present on FNAB material.

Poorly differentiated HCC has been reported to have the lowest expression on canalicular PCEA(32).

Another immunostains like EMA(13),and cytokeratin (13),have been used to discriminate (HCC)from other non hepatic cholangiocarcinoma and metastasis.

Recently Winnerberg et al(31),produced a monoclonal antibody named Hepatocyte Paraffin 1 antibody (Hep Par 1)which reacts with paraffin embedded normal and neoplastic liver tissue, exhibiting an intracytoplasmic granular pattern .A few subsequent studies confirmed that the Hep1 was a relatively specific marker for HCC with a high sensitivity(6,31,34) .

It is not clear,however,how Hep1 expression correlate to HCC differentiation because approximately (10-20%)of HCC cases are respectively negative for Hep1(10,26).

In the current study we examined Hep1 expression in 68 cases of HCC (including 30 cases of FNAB) and 10 cases of non hepatic cholangiocarcinoma(including 7 cases of FNAB). In additon, αFP, EMA, PCEA,and cytokeratin expression were expressed using paraffin sections immuno-hestochemistry.The study demonstrates that Hep1 is a specific marker for HCC, with high sensitivity.

Material and methods:

Cases: sixty eight cases of HCC and ten cases of non hepatic cholangiocarcinoma, were obtained from surgical pathology department files at AL-ASSAD teaching hospital – Damascus – Syria and files of surgical pathology dept of hospital – clinico San Carlos – Madrid-Spain from(1998–2005) distributed as fellow:37 cases of FNAB(30 of HCC and 7 cholangio-carcinoma)were selected from Al-Assad.T.H and 41 cases of open surgical biopsies (38 cases of HCC ,3 cases of cholongiocarcinoma)were selected from the hospital – clinico San Carlos (table I).

Table I:

Diagnosis		total No.of cases	No.of positive cases	%
FNAB	HCC	30	30	100
	Ch.C	7	0	0
Wedge biopsies	HCC	38	38	100
	Ch.C	3	0	0

Distribution and staining patterns of cases studied with the HeP1 antibody.

The tissue of open surgical biopsies had been routinly fixed in (10%)neutral formaline and embedded in paraffin,one block from the open surgical biopsies was selected from each case.The diagnosis of HCC was confirmed by two senior pathologists in all FNAB and surgical biopsies.

The cases distribution is summrised in (Table I),the original cytological diagnosis was rended on the bases of morphology clinical history,immuno histochemical with HeP1,αFP,CEA,EMA and cytokeratin markers. Cytological nuclear changes,tumour differentiation and grading,growth patterns were assessed according to the criteria proposed by(chu pg et al)(10,18,19,34).

Immunohistochemical stains:

The antibodies and dilutions we used were:

1. Monoclonal antibodies to Hep1(1/100)(Dako corporation).
2. αFP (1/100,Dako).
3. Cytokeratin (AE1-AE3-cocktail-Dako)(1/100).
4. PCEA-Dako(1/1000).
5. EMA(1/800).

In the current study sections were cut on to positively charged slides,and immunohistochemistry was performed using the avidin-biotin complex method on an optimax plus automated immunostainer performed by Zimmerman et al(34).

Slides were graded in a blind fashion regarding the percentage of tumour cells that exhibited strong expression of Hep1. Strong expression was

defined as unequivocally positive coarsely granular cytoplasmic staining that could not be confused with back ground staining or with endogenous peroxidase staining.

Results:

From 78 cases included in the current study (table II)shows the result of our study for the 68 cases of HCC. (25 cases were well and moderately differentiated HCC obtained by FNAB including one case of hepatoblastoma that react positively with Hep1 and vimentine,and 5 cases were poorly differentiated HCC obtained by FNAB), 38 cases of HCC obtained with wedge surgical resection (28 cases were well and moderately differentiated HCC,and 10 cases were poorly differentiated HCC).

Table II:

Diagnosis		No.	No.of positive cases			
			>10%cells+		5-10%cells+	
			No.	%	No.	%
FNAB	HCC(W.D+MD)	25	25	100	0	0
	HCC(PD)	5	3	60	2	40
Histological-biopsey	HCC(W.D+MD)	28	28	100	0	0
	HCC(PD)	10	8	80	2	20
Total		68	64	93	4	7

HeP1 antibody staining pattern of hepatocellular carcinoma(HCC) by tumour grade.
(WD:well differentiated/MD:moderately differentiated/PD:poorly differentiated).

The study revealed that 68 out of 68 cases(100%)exhibited immunoreactivity for Hep1in at least some malignant cells,which demonstrate diffuse cytoplasmic granular positivity(Fig 1). Sixty four out of 68 cases(93%) exhibited Hep1 in more than (10%) of tumour cells these cases were mainly well and moderately differentiated HCC(Fig 2).

Poorly differentiated HCC stained less frequently with Hep1(4cases of PD HCC showed a less than 10%expression with an averege of 5% of malignant cells immunoreactive)(table II). However ,these 4 cases of PD HCC would have been considered as positive according to Chu et al(10).

Figure 1:

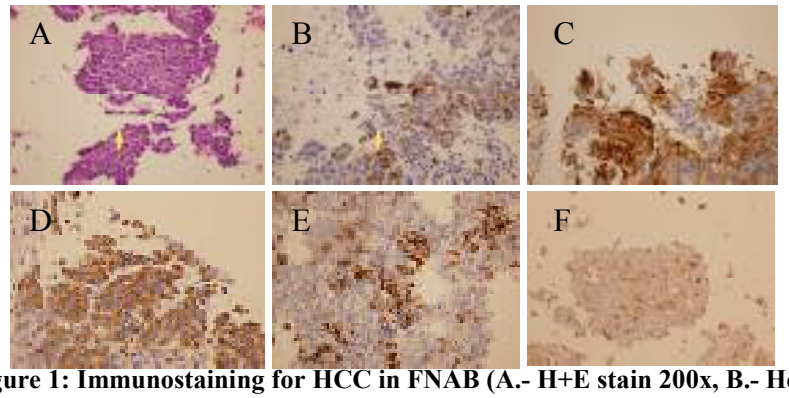

Figure 1: Immunostaining for HCC in FNAB (A.- H+E stain 200x, B.- HeP1 positive 200x, C.- Alpha feto protein 200x, D.- Cytokeratins positive, 200x, E.- EMA positive, 200x; F.- CEA positive, 200x).

Figure 2:

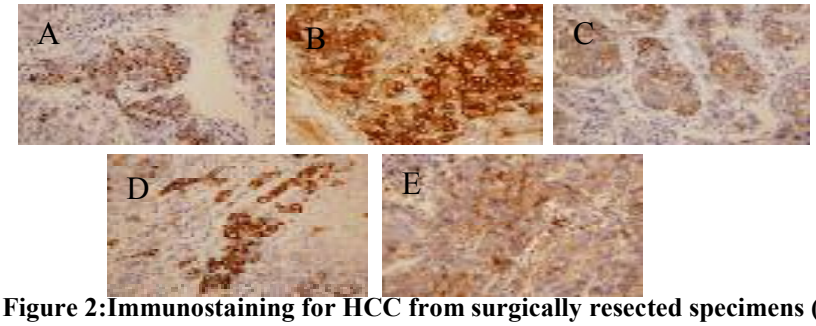

Figure 2:Immunostaining for HCC from surgically resected specimens (A.- HeP1 positive 200x, B.- Alpha feto protein 200x, C.- Cytokeratins positive, 200x, D.- EMA positive, 200x; E.- CEA positive intracanalicular, 200x;).

Twenty three/68 cases of HCC showed cytoplasmic positivity for αFP (34%)(3,7), most of them focal,αFP was more frequent in poorly

differenti-ated HCC and less in well and moderately differentiated cases. CEA (polyclonal) was positive in 34/68 cases of HCC(50%)with intercellular,canalicular pattern(14,27). EMA was expressed in 24/68 cases of HCC (45.8.13)(37%),while cytokeratin(AE1,AE3)reacted positively in 19/68 cases (28%).This finding is in keeping with the proposal that HCC and cholangiocarcinoma arise from a common pluripotent stem cells(20).

In comparison 10 cases of non-hepatic cholangiocarcinoma exhibited no immunoreactivity for Hep1,and αFP,while cytokeratin (19,24),and EMA(4.5.8.13), were strongly positive 9/10 cases (90%), PCEA shows immunoreactivity in 6/10 cases (60%)of cholangiocarcinoma(14,27).

Overall sensetivity of Hep1 for HCC was (93-100%) and specificity was relatively very high (about 100%).(Table III).

Table III:

Immunostains	HCC(68)		CH.C(10)	
	NO	%	NO	%
FP	23	34	0	0
HP1	64-68	93-100	0	0
CEA	34	50	6	60
EMA	24	37	9	90
CK(cocktail)	19	28	9	90

Immunostains expression in(HCC)and(Ch.C).

Discussion:

FNAB is an accepted and practical procedure for the diagnosis of liver tumours,in addition to core and wedge surgical biopsies. However distingushing HCCs from cholagiocarcinoma or metastases may create a challenge,specially if the malignancy is a poorly differentiated type .

The use of αFP and other markers such as PCEA,CK,EMA may help to resolve these difficult cases, although the canalicular staining pattern by PCEA(32), may not always be apparent. From current study and other studies,αFP is not a highly sensitive immunostains(34% of cases)(6,26). EMA and various combination of keratin (and other markers) have been tried, but have not gained acceptance.

HeP1 is a monoclonal immunostains exhibiting a coarsely granular cytoplasmic staining pattern.HeP1 has proved useful in diagnosis of HCC in various surgical pathology settings(10).The use of HeP1 in cytology has been widely explored particularly on FNAB(3,5,14,16,17,18,31,33,34).
This study demonstrate that HeP1 is a specific immunostains of benign and malignant hepatocyte with high sensitivity(93-100%)(10.22.26.31.34),in cell block material.Thus HeP1 exhibit greater sensitivity than αFP (34%) and PCEA (50%) in staining HCC.
In their original reports,Zimmerman et al(34),found that the sensitivity of HeP1 was(79%)and its specificity was(96%). Chu et al(10),indicating sensitivity of HeP1 on HCC (92%), Loeng et al(22), (95%),Wennerberg et al(31),reported (93%),Minervini et al(26),also found HeP1 (81%)while Marakata et al(25a),reported of HeP1(90%)sensitivity(Table IV).

Table IV:

Refrences	HeP1 expression in HCC	
	No.	%
Zimmerman et al(34)	33/40	79
Chu PG et al(10)	88/96	92
Leoing t al (22)	35/37	95
Wennerberg et al(31)	41/43	93
Minervini et al(26)	17/21	81
Marakuta et al(25a)	9/10	90
Currunt study	64-68/68	93-100

Comparison of hepatocyte sensitivity and specificity for HCC.

This study also suggest that Hep1 deos not immunoreact with cholangiocarcinoma and most metastatic carcinoma from other site(34), cholangiocarcinoma cases exhibit no reactivity to HeP1 and αFP,but shows strong reactivity to cytokeretin(90%),EMA (90%) with less sensitivity to PCEA (60%).
As conclussion Expression of HeP1 antibody and αFP were found to be specific immunostains that may be useful in the diagnosis of all HCCs and most undifferentiated liver tumours biopsied by(FNAB) and opened liver biopsies. HeP1 is a specific marker for hepatocellular tissue,including well and moderately differentiated HCC,its sensitivity in poorly diffrentiated HCC is relatively low. αFP expression was more frequent in poorly differentiated HCC.

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