

Impact of CA19-9 in Diagnosis of Combined Hepatocellular Carcinoma and Cholangiocarcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide. Intrahepatic cholangiocarcinoma (ICC) accounts for about 10-15% of all primary hepatic malignancy. Combined hepatocellular carcinoma and cholangiocarcinomas (cHCC-CCs) are rare malignancies representing 1.0-4.7% of all primary liver cancers. ICC and cHCC-CC preoperative diagnosis is desirable because frequency of lymph node metastasis. Also they have been considered a contraindication for liver transplantation (LT). The study aimed to assess the importance of CA19-9 in diagnosis of cHCC-CC "non-invasively" in patients with typical radiological features of HCC which may alter the interventional procedure. This observational cross-sectional study was conducted on 62 patients with typical radiological criteria of HCC and elevated CA19-9 level, who attended to National Hepatology and Tropical Medicine Research Institute (NHTMRI); Cairo, Benha university hospitals within the period between October 2017 and April 2019. All studied subjects underwent history taking, clinical examination, biochemical workup, tumor markers, abdominal ultrasound, and dynamic study (CT/MRI). Target liver biopsy was done for histopathology and immuno-histochemistry. The studied patients were classified into three groups based on pathological diagnosis. 20 patients were diagnosed as HCC, 40 patients were diagnosed as ICC, and 2 patients were diagnosed as cHCC-CC with statistically significant difference between HCC and ICC as regard CA19-9 and alpha-fetoprotein (AFP). CA19-9 and AFP cut offs were > 64.8 U/mL and < 23.6 ng/mL, respectively favoring the diagnosis of ICC. CA19-9 level was 176.3 and 156.7 U/mL while AFP level was 460 and 170 ng/mL in cHCC-CC cases, respectively. The use of CA19-9 can help in diagnosis of ICC and suspicion of cHCC-CC in cases of typical radiological criteria of HCC.

Keywords Hepatocellular carcinoma, Intrahepatic cholangiocarcinoma, Combined hepatocellular carcinoma and cholangiocarcinoma, Alpha-fetoprotein, Carbohydrate Antigen 19-9.

1. Introduction

HCC represents the sixth most common cancer worldwide.¹ In Egypt; it represents the fourth common cancer.²

ICC is one of the subtypes of cholangiocarcinoma, which accounts for about 10–15% of all primary hepatic malignancies.³

Misdiagnosis of ICC as HCC may result in selection of a treatment option (such as TACE or RFA) that is effective for HCC but not as effective for CCA.⁴

ICCs are associated with poor outcomes. ICCs considered a contraindication for LT.⁵

cHCC-CC is a rare primary liver cancer containing elements of both HCC and ICC and accounting for 1.0–4.7% of all primary liver cancers.⁶

The presence of imaging features of both HCC and CCA in the same tumor may alert the radiologist to the possibility of cHCC-CC though.⁷ But the definite diagnosis of cHCC-CC can be made by histopathological examination only with use of Immuno-histochemistry (IHC).⁸

Correct preoperative diagnosis is desirable because the high frequency of lymph node metastasis, making its dissection a necessity for curative resection.⁹

LT has no role and is not a good management option for cHCC-CC.¹⁰ Outcomes are worse when compared with HCC due to associated higher recurrence rates after transplant.¹¹

So, this study was conducted aiming to assess the significance of elevated CA19-9 in suspecting diagnosis of cHCC-CC "non-invasively" in patients with typical radiological features of HCC which may alter the interventional procedure.

2. Patients and methods

This observational study was conducted on 62 patients with radiological criteria of HCC and elevated CA19-9 level, who attended to National Hepatology and Tropical Medicine Research Institute (NHTMRI); Cairo, Benha university hospitals within the period between October 2017 and April 2019.

They were divided into three groups: Group (1) included 20 patients diagnosed as HCC, Group (2) included 40 patients diagnosed as ICC and Group (3) included two patients diagnosed as cHCC-CC.

All patients were underwent history taking, clinical examination, detailed biochemical investigations (complete blood count, bilirubin level (T,D), serum albumin, INR and renal functions), viral markers, tumor markers (AFP and CA19-9), abdominal ultrasound, and dynamic study.

Liver biopsy was done using 18-gauge core biopsy needle and the collected liver tissue was put into formalin immediately and the blocks were prepared from the collected tissue specimen and the sections were stained with Hematoxylin and Eosin (H&E).

Sections were treated by using specific monoclonal antibodies against GPC3, Hep-par1, CK7

and CK19. Chromogen and hematoxylin was applied as counter-stain for IHC staining.

2.1 Ethical approved

This work was approved by the scientific committee of Benha University Hospitals. All included patients were informed about the procedure of the study and gave a written informed consent to participate in the study.

2.2 Statistical analysis

Patients' data were analyzed using Statistical Program for Social Science (SPSS) version 15.0 for windows. Quantitative data were expressed as mean \pm standard deviation (SD) and median. Qualitative data were expressed as frequency (No.) and percentage (%). Chi-square test (X²) was used when comparing between non-parametric data. P-value was set at ≤ 0.05 for significant results and > 0.05 for insignificant results. ROC curve was used to determine cut off value and the following statistics can be defined: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

3. Results

The 62 patients were chosen from 622 patients with typical radiological criteria of HCC, for them CA19-9 was done and who showed elevated level was included in this study.

The studied patients were classified into three groups based on pathological diagnosis. 20 patients were diagnosed as HCC, 40 patients were diagnosed as ICC, and 2 patients were diagnosed as cHCC-CC.

Two patients were diagnosed as cHCC-CC with an incidence 0.32% from all primary liver tumors patients visited the NHTMRI during the study period.

They were 58 and 45 years old males; none of them showed signs of liver cell failure during clinical examination and laboratory assessment. Both cases serology was positive for HCV and negative for HBV.

AFP level was 460 and 170 ng/mL, and CA19-9 level was 176.3 and 156.7 U/mL for both cases, respectively.

For both cases liver biopsy was done and histopathological result was HCC grade II with mucin production was noted. On IHC, malignant cells were positive for GPC3, Hep-par1, CK7 and CK19; these results were consistent with cHCC-CC.

As regard the other groups; the mean age was 63.27 ± 8.76 years in HCC and 59.60 ± 9.79 years in ICC patients, and 63.6% of HCC and 73.3% of ICC patients were males, with no statistically significant difference (*P*-value was 0.16 and 0.45, respectively).

All patients of both groups complained of significant weight loss, none of HCC and 26.75% of ICC patients had jaundice, 63.6% of HCC and only 6.7% of ICC patients had esophageal varices, with

statistically significant difference (*P*-value was 0.008 and 0.001, respectively).

As regard laboratory findings; the mean Hb level was 11.5 ± 1.87 gm/dL in HCC and 11.61 ± 1.53 gm/dL in ICC, the mean WBCs count was 7.23 ± 4.12 thousands/Cmm in HCC and 7.78 ± 4.18 thousands/Cmm in ICC, the median ALT was 45 U/L in HCC and 47.5 U/L in ICC, the mean serum albumin was 3.61 ± 0.44 gm/dL in HCC and 3.43 ± 0.57 gm/dL in ICC, the mean total bilirubin was 1.56 ± 0.37 mg/dL in HCC and 1.35 ± 0.71 mg/dL in ICC, the mean INR was 1.2 ± 0.17 in HCC and 1.18 ± 0.23 in ICC, the mean serum creatinine was 0.86 ± 0.31 mg/dL in HCC and 1.22 ± 0.31 mg/dL in ICC, with no statistically significant difference (*P*-value was 0.8, 0.89, 0.92, 0.12, 0.24, 0.37 and 0.6, respectively).

As regard the mean platelets count was 165.55 ± 78.09 thousands/Cmm in HCC and 280.87 ± 105.57 thousands/Cmm in ICC patients, with statistically significant difference (*P*-value was < 0.001).

As regard tumor markers; the median AFP was 46.3 ng/mL in HCC and 8.7 ng/mL in ICC patients, while the median CA19-9 was 67.4 U/mL in HCC and 155.7 U/mL in ICC patients, with statistically significant difference (*P*-value was 0.002 and 0.003, respectively).

As regard histopathological results of HCC patients; 63.6% were HCC grade I, 27.3% were HCC grade II and 9.1% were HCC grade III. And as regard ICC patients; 6.7% were moderately differentiated adenocarcinoma, 60% were poorly differentiated adenocarcinoma and 33.3% were undifferentiated malignant tumor.

As regard IHC; all cases of HCC showed positive staining for GPC3 and Hep-par1 and negative staining for CK7 and CK19, while all cases of ICC showed positive staining for CK7 and CK19 and negative staining for GPC3, and Hep-par1, there was statistically significant difference (*P*-value was < 0.001).

Using ROC curve, it was shown that CA19-9 can be used in discrimination between HCC and ICC patients at a cut off level of > 64.8 U/mL, with 95.2% sensitivity, 57.4% specificity, 71.1% PPV and 87.06% NPV (*P*-value was 0.002).

Using ROC curve, it was shown that AFP can be used in discrimination between HCC and ICC patients at a cut off level of < 23.6 ng/mL, with 88.1% sensitivity, 61.8% specificity, 71.11% PPV and 79.6% NPV (*P*-value was 0.002).

4. Discussion

This study was conducted on 62 patients with typical radiological criteria of HCC and elevated CA19-9 aiming to evaluate its significance in suspecting cHCC-CC.

Two cases were diagnosed as cHCC-CC with an incidence 0.32% from all primary liver tumors, and this was in agreement with Wang et al.¹² who

reported combined HCC-CC incidence accounts for 0.4 - 14.2% of primary liver cancer cases.

AFP level was 460 and 170 ng/mL, and CA19-9 level was 176.3 and 156.7 U/mL for both cases, and this was in agreement with Sciarra et al.¹³ who reported the combination of elevated CA19-9 and AFP should alert investigators for the possibility of cHCC-CC.

For both cases a target liver biopsy was done and histopathological result was HCC grade II with mucin production was noted, and this was in agreement with IARC WHO 4th edition.¹⁴ who reported differentiated hepatocellular and biliary components in the tumor is required for histopathological diagnosis of cHCC-CC.

On IHC, malignant cells were positive for GPC3, Hep-par1, CK7 and CK19; these results were consistent with cHCC-CC, and this was in agreement with Tickoo et al.¹⁵ who reported the same IHC for both malignant components in cases of cHCC-CC.

As regard the other groups the mean age was 63.27±8.76 years in HCC and 59.60±9.79 years in ICC patients, with no statistically significant difference between the two groups, and this was in agreement with Jung et al.¹⁶ who reported that mean age were no different between two groups.

In the current study 63.6% of HCC and 73.3% of ICC patients were males, with no statistically significant difference between the two groups, and this was in agreement with Wakizaka et al.¹⁷ who reported the majority of HCC and ICC cases occur in men.

Esophageal varices were more common in HCC than ICC patients (63.6% and only 6.7%, respectively), with statistically significant difference between the two groups, and this was in agreement with Wakizaka et al.¹⁷ and Lee et al.¹⁸ who reported that esophageal varices and liver cirrhosis were predominant in HCC patients.

Jaundice was predominant in ICC (26.75%) than HCC patients, with statistically significant difference between the two groups, and this was in agreement with Li et al.¹⁹ who reported predominance of jaundice in ICC patients.

PLT count was lower in HCC than ICC patients (mean was 165.55±78.09 and 280.87±105.57 thousands/Cmm, respectively), there was statistically significant difference between the two groups, and this was in agreement with Aljumah et al.²⁰ who reported mild thrombocytopenia in HCC patients, and Gigante et al.²¹ who reported normal PLT count to mild thrombocytosis in ICC patients.

In this study AFP was higher in HCC than ICC patients (median was 46.3 and 8.7 ng/mL, respectively), there was statistically significant difference between the two groups, and this was in agreement with Hsieh et al.²² who reported the median AFP was 59.6 ng/mL in HCC patients, and Gigante et al.²¹ who reported the median AFP was 9.1 ng/mL in ICC patients.

But CA19-9 was higher in ICC than HCC patients (median was 155.7 and 67.4 U/mL, respectively), there was statistically significant difference between the two groups, and this was in agreement with Lee et al.²³ who reported the median CA19-9 was 56.1 U/mL in HCC patients, and Lumachi et al.²⁴ who reported the median CA19-9 was 170.5 U/mL in ICC patients.

Histopathological results in HCC patients; 63.6% were HCC grade I, 27.3% were HCC grade II and 9.1% were HCC grade III, and this was in agreement with Zhang et al.²⁵ who reported the same pathological grading in HCC cases.

But histopathological results of ICC patients; 6.7% were moderately differentiated adenocarcinoma, 60% were poorly differentiated adenocarcinoma and 33.3% were undifferentiated malignant tumor, and this was in agreement with Sapisochin et al.²⁶ who reported the same pathological differentiation in ICC patients.

As regard IHC; all HCC cases showed positive staining for GPC3 and Hep-par1 and negative staining for CK7 and CK19, while ICC cases showed positive staining for CK7 and CK19 and negative staining for GPC3, and Hep-par1, with statistically significant difference between the two groups, and this was in agreement with Ryu et al.²⁷ comparative study who reported the same results and differences between HCC and ICC cases.

Using ROC curve analysis revealed that CA19-9 can be used in discrimination between HCC and ICC patients at a cut off level of > 64.8 U/mL, with 95.2% sensitivity, 57.4% specificity, 71.1% PPV and 87.06% NPV; area under the curve was 0.77 and P-value was 0.002 denoting good predicative value of CA19-9 in prediction of ICC, and this was in agreement with Li et al.²⁸ who reported a CA19-9 cut off level of > 125.07 U/mL, with 76.67% sensitivity and 80% specificity for diagnosis of CCA.

Using ROC curve analysis revealed that AFP can be used in discrimination between HCC and ICC patients at a cut off level of < 23.6 ng/mL, with 88.1% sensitivity, 61.8% specificity, 71.11% PPV and 79.6% NPV; area under the curve was 0.76 and P-value was 0.002 denoting good predicative value of AFP in prediction of ICC, and this was in agreement with Li et al.²⁸ who reported a AFP cut off level of < 15.4 ng/mL, with high sensitivity and specificity for diagnosis of CCA when combined to CA19-9.

5. Conclusion

CA19-9 is important in the diagnosis of the cHCC-CC in patients with typical radiological criteria of HCC.

CA19-9 and AFP are significant in diagnosis of pure cases of ICC in patients with typical radiological criteria of HCC.

Esophageal varices, jaundice, platelet count, and immuno-histochemistry are significant to differentiate between HCC and ICC patients.

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