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# Influence of Cytokeratin-19 Level on Pattern of Hepatocellular Carcinoma in Patients with Liver Cirrhosis

**Ahmed Adel Saad[*1], Salah El Din Abdel hakim El Gamal[1], Shimaa R. Hendawy[2], Ahmed Saleh[1]**
Departments of [1]Internal Medicine, Hepatology and Gastroenterology and
[2]Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt
***Corresponding author:** Ahmed Adel Saad, **Mobile:** (+20) 01111884841, **E-Mail:** dr.a_adelsaad@yahoo.com

## ABSTRACT

**Background:** In hepatocellular carcinoma (HCC), CK19 is a marker of hepatic progenitor cells and acts as a key player in tumor invasion, indicating poor prognosis. Early diagnosis of HCC heavily affects the clinical outcome of patients. The widely accepted serological marker for HCC diagnosis is alpha-fetoprotein (AFP). However, its diagnostic accuracy is controversial and unsatisfactory because of its low sensitivity. The objective of the current study is to evaluate the influence of CK19 level on Pattern of HCC in patients with liver cirrhosis.

**Patients and methods:** This was a prospective case -control study that was conducted on patients attending at early detection of HCC Outpatient Clinic or admitted to Hepatology and Gastroenterology Unit, Specialized Medical Hospital Mansoura University, over past year. The current study included 75 participants divided into 3 groups: *Group 1* (HCC), *Group 2* (cirrhosis only) and *Group 3* (healthy people without any medical disease).

**Results:** There was statistically significant increase as regard median CK19 level, between degrees of aggressiveness index (A, B and C) ($P < 0.05$). Regarding the validity of CK19 in differentiating the studied groups, there was no statistically significant difference as regard median CK19 level in cirrhosis and control groups with Sensitivity 56% and Specificity 40%. There was no statistically significant difference as regard median CK19 level in HCC and control groups with Sensitivity 64% and Specificity 40%. There was no statistically significant difference as regard median CK19 level in HCC and cirrhosis groups with Sensitivity 64% and Specificity 44%. There is weak significant relationship between the levels of CK19 and AFP in HCC cases (P-value 0.07).

**Conclusion:** CK19 associated carry a poor prognosis as it associated with more aggressive pattern of HCC. CK19 is good negative marker of early HCC, So CK19 negative HCC patients has no priority for treatment.

**Keywords:** Hepatocellular carcinoma, liver cirrhosis, alpha-fetoprotein, CK19.

## INTRODUCTION

HCC is a serious public health issue and the fourth leading cause of cancer mortality worldwide [(1)]. HCC accounts for about 80% of the primary liver cancer while the other types include cholangiocarcinoma (10–20%) and angiosarcoma (1%) [(2)].

There are a wide variety of tumor markers for HCC. Studies are ongoing regarding the roles of tumor markers in screening, diagnosis, treatment, and prognostic prediction of HCC. Serum AFP can be very useful for HCC surveillance, prognostic prediction, and treatment response evaluation in high-risk patients with HCC. Other tumor markers such as PIVKA-II and AFP-L3% have also been proven to be effective in HCC diagnosis, follow-up, and prognostic prediction; these markers are increasingly used with serum AFP in clinical trials. However, the roles of tumor markers in surveillance are poorly understood and still controversial, requiring further research. HCC surveillance in at-risk population is a critical issue in management of HCC. Further data regarding the predictive value and cost-effectiveness of tumor markers could facilitate their uses in HCC surveillance [(3)]**.** Particularly novel biomarkers, such as microRNAs, over the last two decades are of profound importance. Both traditional tumor markers including AFP, glypican-3 and transforming growth factor (TGF)-β and novel biomarkers including microRNAs provide useful clinical data, not only on prognosis, but also on pathogenesis and treatment efficacy. Furthermore, specific biomarkers may be potential therapeutic targets [(4)]**.**

AFP level usually starts to increase approximately 6 months before diagnosis of HCC. Adopting a lower cut-off value of AFP level at 6 Ug/L .Elevated AFP to >20 Ug/L has a very high specificity for HCC [(5)]**.**

HCC characteristics are generally considered in assessing an individual patient's tumor-related management and prognosis. They are: maximum tumor diameter (MTD), number of tumor nodules, portal vein invasion (PVI) and blood AFP levels (as well as presence or absence of metastasis, as with most solid tumor types). 'HCC Aggressiveness' scoring system was described, which incorporated all 4 of these parameters and related them to survival [(6)]**.**

Cytokeratins have been extensively used as serum tumour markers for monitoring of disease progression in cancer patients. The source of cytokeratins in the circulation as well as the mechanisms of release from cells has long been unclear. Recent evidence suggests that cytokeratins present in the circulation of cancer patients are released from apoptotic or necrotic tumour cells [(7)]**.**

CK19 is suggested to be an epithelial stem cell marker as it correlates with differentiation potential. Its level is highest in epithelial stem cells decreasing during differentiation and becomes absent in specialized cells. A reverse process is observed during carcinogenesis where there is an increase in CK19 levels as the



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dedifferentiation progress with poorly differentiated cancers showing highest CK19 expression [8].

Several other studies also confirmed a correlation between increased CK19 expression and a lower survival rate and/or a shorter remission period in HCC patients. It is worth noting that CK19 expression was found to coincide with an increase in tumorigenic potential in preneoplastic hepatocytes, which could offer a mechanistic rationale for its use as a potential HCC biomarker [9].

The objective of the current study was to evaluate the influence of CK19 level on Pattern of HCC in patients with liver cirrhosis.

## PATIENTS AND METHODS

This was a prospective case -control study that was conducted on patients attending at early detection of HCC Outpatient Clinic or admitted to Hepatology and Gastroenterology Unit, Specialized Medical Hospital Mansoura University, over past year.

**Inclusion criteria:** The study was conducted on patients with age> 18 y both gender with evidence of liver cirrhosis, the diagnosis of cirrhosis was settled either with compensated or decompensated cirrhosis.
**Exclusion criteria**: Age <18, Patient with previous interventional managements of HCC, Focal lesion not a primary tumor (metastatic tumor in liver), and Patients with any tumor other than HCC.

**Sample size:** Sample size calculation was based on 5 year-overall survival of HCC cases with cytokine 19 positive and negative cases derived from previous research (80.4% and 28.9%, respectively) carried out by **Lee *et al.* [10].** Using G*power version 3.0.10 using z test to detect difference between 2 proportions, 2-tailed, with α error =0.05 and power = 90.0%, the calculated sample size will be 22 in each arm and by adding 5% to compensate drop out then sample size will be approximately 25 in each group at least.

**The included participants were divided into 3 main groups:** ***Group 1*** patients with HCC proved with radiological signs of HCC by Tri phasic CT abdomen, ***Group 2*** patients with cirrhosis only, and ***Group 3*** healthy group between age >18y and <70y, both gender without any medical disease.

**Diagnosis of liver cirrhosis among studied patients was based on:**

- Physical examination suggestive cirrhosis:
  - Temporal MS wasting.
  - Palmar erythema, Spider nevi…..etc.
  - Splenomegaly.
  - Asterixis.
  - Small liver span.
  - Fetor hepaticus.
  - Caput medusa.
  - Ascites.
- Laboratory finding suggestive of cirrhosis:
  - Plt <140.000.
  - Albumin <3.5mg/dl.
  - INR> 1.2.
  - Bilirubin >1.5mg/dl.
- Imaging findings suggestive of cirrhosis by US:
  - Nodular surface of liver.
  - Widening of fissure.
  - Irregularity of the borders.
  - Splenomegaly.
  - Ascites.
  - Collaterals.

**Diagnosis of HCC among studied patients was based on:**

- Clinical examination suggestive of HCC:
  - Abdominal mass.
  - Hepatomegaly with hard irregular borders.
  - Vascular bruit.
  - Paraneoplastic manifestations:
    - Hypocalcemia.
    - Hypoglycemia.
    - Watery diarrhea.
- AFP Level.
- Radiological modalities suggestive of HCC:
  - US and Color Doppler:
    - Hypoechoic focal lesion.
    - Increased vascularity with greater flow velocity and for evaluation of portal vein.
  - CT scan:
    - Non-peripheral washout appearance – Hypoenhancement when compared to liver in portal venous or delayed phase of contrast administration.
    - Enhancing capsule appearance – Smooth border around most or all of the lesion that enhances visibly in the portal venous or delayed phase of contrast administration.

**All patients were subjected to:**

- **Full history taking**: including age, sex, special habits, drug abuse and routine Clinical evaluation specially history of hepatitis, Hepatic encephalopathy, Ascites, Hematemesis & Melena and Upper GIT endoscopy.
- **Radiological investigations**: abdominal U/S with special comment on liver and Tri-phasic CT scan of abdomen.
- **Laboratory investigations**: Basic investigations including; complete blood count, liver function tests (Albumin, SGOT, SGPT, Bilirubin, INR) and AFP level.

**Patients' evaluation:** Patients either with liver cirrhosis or HCC were evaluated using the Child-Pugh score.

HCC group were evaluated for the pattern of HCC using the AgI.

**CK19:** Strip plate ELISA (enzyme-linked immunosorbent assay) Kit for analyzing the presence of the CK19 ELISA Kit target analytes in biological samples.

**Ethical consent:**

**An approval of the study was obtained from Mansoura University Academic and Ethical Committee (IRB code number (MS.20.03.1090). Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.**

***Statistical analysis***

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) and inter quartile range for non-parametric data, and mean and standard deviation (SD) for parametric data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at 0.05 level.

Chi-Square test for comparison of 2 or more groups. Monte Carlo test and Fischer exact test were used as correction for Chi-Square test when more than 25% of cells have count less than 5. Student's t-test was used to compare 2 independent groups. One Way ANOVA test was used to compare more than 2 independent groups with Post Hoc Tukey test to detect pair-wise comparison. Mann-Whitney U test was used to compare 2 independent groups. Kruskal Wallis test was used to compare more than 2 independent groups with Mann Whitney U test to detect pair-wise comparison. Spearman's correlation: The Spearman's rank-order correlation is used to determine the strength and direction of a linear relationship between two non-normally distributed continuous variables and / or ordinal variables. Receiver Operating Characteristic (ROC) curve analysis: The diagnostic performance of a test, or the accuracy of a test to discriminate diseased cases from non-diseased cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity and Specificity were detected from the curve and PPV, NPV and accuracy were calculated through cross tabulation. P value $\leq$0.05 was considered significant.

## RESULTS

**Table 1** shows that there was no statistically significant difference as regard age and sex between the three studied groups (P >0.05).

**Table (1): Socio-demographic characteristics of the 3 studied groups:**

| Variable | Cirrhosis | HCC | Control | Test of significance |
|---|---|---|---|---|
| Number of Cases | n=25 | n=25 | n=25 | |
| CK-19 | 0.925 ± 0.98 | 0.795 ± 0.43 | 0.796 ± 0.62 | |
| Age/years Mean ± SD | 62 ± 5 | 61.60 ± 6 | 60.32 ± 5.99 | F=0.641 p=0.530 |
| Sex n (%) Male | 17 (64.0) | 18 (72) | 17 (68) | F=0.58 p=0.56 |
| Female | 8 (36) | 7 (28) | 8 (32) | F=2.76 p=0.086 |

F: One Way ANOVA test, $\chi^2$ : Chi-Square test.

**Table 2** shows that there was no statistically significant difference, between cirrhosis and HCC groups, as regard grades of ascites, hepatic encephalopathy and Child Pugh classification (P >0.05).

**Table (2): Comparison of ascites, encephalopathy and child -Pugh classification between studied cases:**

| Variable | Cirrhosis | HCC | test of |
|---|---|---|---|
| | n=25 (%) | n=25(%) | significance |
| Ascites | | | |
| No | 8 (32) | 4 (16) | MC |
| Mild | 2 (8) | 4 (16) | P=0.273 |
| Moderate | 8 (32) | 8 (32) | |
| Marked | 5 (20) | 9 (36) | |
| Tense | 2 (8) | 0 | |
| Encephalopathy | | | |
| No | 16 (64) | 15 (60) | MC |
| Grade 1 | 3 (12) | 4 (16) | P=0.950 |
| Grade 2 | 2 (8) | 3 (12) | |
| Grade 3 | 2 (8) | 1 (4) | |
| Grade 4 | 2 (8) | 2 (8) | |
| Child Pugh | | | |
| A | 5 (20) | 0 | |
| B | 8 (32) | 8 (32) | $\chi^2$=5.86 |
| C | 12 (48) | 17 (68) | P=0.053 |

$\chi^2$: Chi-Square test , Monte Carlo test

**Table 3** shows illustrates the aggressiveness index among HCC cases (n=25) showing that 2 cases have A score, 6 cases have B score and 17 cases have C score (8%, 24% and 68% respectively).

**Table (3): Aggressiveness index among hepatocellular carcinoma cases:**

| Variable | n=25 | % |
|---|---|---|
| Aggressiveness index | | |
| A | 2 | 8.0 |
| B | 6 | 24.0 |
| C | 17 | 68.0 |

**Table** (4) shows that there is no statistically significant difference, between cirrhosis, HCC and control groups, as regard median Cytokeratin-19 level (0.6, 0.7 and 0.6 respectively) (P >0.05).

**Table (4): Comparison of CK19 among studied groups:**

| Variable | Cirrhosis n=25 | HCC n=25 | Control n=25 | Test of significance |
|---|---|---|---|---|
| CK 19 median (range) (IQR) | 0.6 (0.2-14.5) (0.35-1.35) | 0.70 (0.10-1.9) (0.45-1.1) | 0.60 (0.2-2.9) (0.4-0.9) | KW=0.197 P=0.906 |

KW: Kruskal Wallis test, IQR: Interquartile range

**Table 5** shows that there was very weak correlation between CK19 with (PLT, Hb, WBC, Bilirubin and AFP), weak correlation between CK19 with (Age, INR, Albumin, Ascites, Encephalopathy, Child-pugh, AST and ALT).

**Table (5): Correlation between CK19 and demographic & laboratory findings among cirrhosis group:**

| **Variable** | | |
|---|---|---|
| | | **CK 19** |
| age/years | r | 0.272 |
| | P | 0.189 |
| Platelets (x10^9) | r | -0.030 |
| | P | 0.887 |
| Hemoglobin(gm/dL) | r | -0.032 |
| | P | 0.879 |
| WBCS(x10^9) | r | 0.103 |
| | P | 0.623 |
| INR | r | 0.256 |
| | P | 0.227 |
| Albumin(gm/dL) | r | 0.269 |
| | P | 0.193 |
| Bilirubin(mg/dL) | r | -0.114 |
| | P | 0.588 |
| Ascites | r | 0.201 |
| | P | 0.336 |
| Encephalopathy | r | 0.339 |
| | P | 0.097 |
| Child-pugh | r | 0.252 |
| | P | 0.224 |
| AFP(ng/mL) | R | -0.060 |
| | P | 0.775 |
| AST(U/L) | R | 0.245 |
| | P | 0.239 |
| ALT(U/L) | R | 0.300 |
| | P | 0.146 |

r: Spearman correlation co-efficient.

**Table 6** shows that there was very weak correlation between CK19 with (Age, PLT, WBCS, INR, Albumin, Ascites, Encephalopathy, Child-Pugh and AFP), weak correlation between Ck19 with (HB, bilirubin, ALT, AST and Aggressive index score)

**Table (6): Correlation between CK19 and demographic & laboratory findings among HCC group:**

| **Variable** | | **CK-19** |
|---|---|---|
| Age/years | R | 0.120 |
| | P | 0.569 |
| Platelets (x10^9) | R | 0.139 |
| | P | 0.507 |
| Hemoglobin(gm/dL) | R | 0.306 |
| | P | 0.137 |
| WBCS(x10^9) | R | -0.093 |
| | P | 0.659 |
| INR | R | 0.110 |
| | P | 0.599 |
| Albumin(gm/dL) | R | 0.037 |
| | P | 0.860 |
| Bilirubin(mg/dL) | R | 0.251 |
| | P | 0.227 |
| Ascites | R | 0.124 |
| | P | 0.554 |
| Encephalopathy | R | 0.166 |
| | P | 0.428 |
| Child-Pugh | R | 0.084 |
| | P | 0.691 |
| AFP(ng/mL) | R | 0.154 |
| | P | 0.463 |
| AST(U/L) | R | 0.281 |
| | P | 0.174 |
| ALT(U/L) | R | 0.293 |
| | P | 0.155 |
| Aggressive index score | R | 0.391 |
| | P | 0.053 |

r: Spearman correlation co-efficient

**Table 7** there was statistically significant difference as regard median CK-19 level, between degrees of aggressiveness index (A, B and C) (P <0.05) and there is relation found between CK19 and aggressiveness index where the mean value of the aggressive index increases with increasing of CK-19.

**Table (7): Relation between CK19 & aggressiveness index:**

| Variable | Aggressive index group | | | Test of significance | Within group significance |
|---|---|---|---|---|---|
| | A | B | C | | |
| CK -19 | 0.35 (0.1-0.6)<br>(0.10-0.60) | 0.550 (0.3-1.1)<br>0.375-0.800 | 0.9 (0.20-1.9)<br>0.5-1.15 | t=16.84<br>p=4.24e-15 | P1=0.402<br>P2=0.125<br>P3=0.159 |

t-test, P1: difference between A &B, P2: difference between A &C, P3: difference between B &C.

**Table 8** show that there was no statistically significant difference as regard median CK19 level between cirrhosis and control groups with Sensitivity 56% and Specificity 40%. There is no statistically significant difference as regard median CK19 level between HCC and control groups with Sensitivity 64% and Specificity 40%. There was no statistically significant difference as regard median CK19 level between HCC and cirrhosis groups with Sensitivity 64% and Specificity 44%.

**Table (8): Validity of CK-19 in differentiating studied groups:**

| Variable | AUC<br>(95% CI) | P value | Cut off points | Sensitivity% | Specificity% |
|---|---|---|---|---|---|
| Between cirrhosis & control | | | | | |
| CK 19 | 0.506 (0.343-0.670) | 0.938 | 0.55 | 56.0 | 40.0 |
| Between HCC & control | | | | | |
| CK 19 | 0.546 (0.383-0.708) | 0.580 | 0.55 | 64.0 | 40.0 |
| Between HCC& cirrhosis | | | | | |
| CK 19 | 0.511 (0.346-0.676) | 0.892 | 0.55 | 64.0 | 44.0 |

AUC: Area under Curve.

**Table 9** shows that the p-value is >0.05, so that there was no significant relation between CK19 level, aggressiveness degree, and AFP of HCC cases.

**Table (9): Relation between CK-19, Aggressive index and Alpha fetoporotien**

| Variable | CK-19 | AI | AFP | test of significant |
|---|---|---|---|---|
| Mean | 0.776 | 8.32 | 2318.52 | F=2.15<br>p=0.12 |

F: One way Anova

**Table 10** reveals that the p-value was 0.07 indicating that there is weak significant relationship between the levels of CK19 and AFP in HCC cases.

**Table (10): Relation between CK-19 and Alpha fetoporotien**

| Variable | CK-19 | AFP | test of significant |
|---|---|---|---|
| Mean | 0.776 | 2318.52 | t=1.71<br>p=0.07 |

t: t-test

## DISCUSSION

The mean age of groups; HCC group 61.60, cirrhotic groups 62.04 and control group 60.32 (P-value 0.53) and according the gender HCC group; female 7 (28%), male 18 (72%), cirrhotic group; female 9 (36%), male 16 (64%) and control group; female 8 (32%), male 17 (68%) [P-value 0.83].

The current study evaluated the socio-demographic characteristics of the studied groups showing that there was no statistically significant difference as regard age and sex between the three studied groups (P >0.05).This is extremely important as the studied groups should be age and sex matched, so any difference in the level of our marker is related to pattern of HCC.

In comparison to **Holah *et al.*** [11] study that included 92 HCC patients who had undergone surgical intervention. Results revealed that 51.1% of the studied HCC patients were at least 58 years old, 81.5% male and 18.5% female, 51.2% of the patients had an AFP level of at least 200 ng/ml and 95.7% were positive for hepatitis viral infection.

We stress that cirrhotic patients and HCC patients are cross matched, as regard age and sex, to abort any effect of these factors on the pattern of HCC.

In contrast to these results, **Attallah *et al.*** [12] performed a study to investigate whether serum cytokeratin-1 (CK1) could complement AFP to improve the diagnosis of (HCC). 150 consecutive HCC patients and 100 LC patients and 50 healthy individuals were enrolled in the study. There was a significant difference between the age of patients with HCC and LC; patients with HCC were associated with older age. There was a male predominance in cirrhotic and HCC patients.

The current study illustrated that there was no statistically significant difference, between cirrhosis and HCC groups, as regards grades of ascites, hepatic encephalopathy and Child Pugh classification (P >0.05).

**El Raziky *et al.*** [13] performed abdominal ultrasonography showing that ascites, was present in 50%, of the advanced HCC group but not present in other groups (cirrhotic patients with early HCC or cirrhotic with no evidence of HCC)

Interestingly, the current study evaluated the aggressiveness index among HCC cases (n=25) showing that 2 cases had A score, 6 cases had B score and 17 cases had C score (8%, 24% and 68%, respectively).

Liver biochemical parameters and the liver index correlated with HCC Aggressiveness and may be directly involved in the biology (growth and invasiveness) of HCC cells. However, this index will need to be externally validated [14].

The liver index correlated significantly with HCC aggressiveness and may thus be involved in human HCC biology and is likely another example of microenvironment influencing HCC behavior [15].

The current study evaluated the CK19 level among the studied groups showing that there was no statistically significant difference, between cirrhosis, HCC and control groups, as regard median CK19 level (0.6, 0.7 and 0.6, respectively) (P >0.05).

Similarly **El Raziky *et al.*** [13], found that there was no significant difference between all groups before any intervention regarding the CK-19 levels.

Study correlation performed showed that there is very weak correlation between CK-19 with PLT, Hb, WBC, bilirubin and AFP, weak correlation between CK19 with Age, INR, Albumin, ascites, encephalopathy, Child-pugh, AST and ALT among cirrhotic group; while there is very weak correlation between CK19 with Age, PLT, WBCS, INR, albumin, ascites, encephalopathy, Child-Pugh and AFP, weak correlation between Ck19 with HB, bilirubin, ALT, AST and AgI score among HCC group.

CK-19 is an HCC-cancer stem cell marker that plays an integral role in carcinogenesis, metastases, and recurrence [16]. In **El Raziky *et al.*** [13] study although there was no significant difference in baseline serum CK-19 levels among the three groups, its combination with AFP improved their sensitivity to 93.9% that came in harmony with our results.

The current study found that there was a statistically increase as regard median CK19 level, between degrees of aggressiveness index (A, B and C) (P <0.05).

In contrast to our results, it was found that CK19 is a marker for bile duct cells, hepatic progenitor cells (HPCs), and early hepatoblasts. Additionally, its expression is linked to the poor prognosis of patients diagnosed with HCC [17].

CK-19 is well correlated with tumor aggressiveness and is an important marker of proliferative subtypes, suggesting a poor prognosis in patients with HCC. Many studies have shown that the overall survival (OS) rate in CK-19 negative is greater than CK19 positive patients with HCC [18].

Importantly, the current study evaluated the validity of CK-19 in differentiating the studied groups. There was no statistically significant difference as regard median CK-19 level between cirrhosis and control groups with sensitivity 56% and specificity 40%. There was no statistically significant difference as regard median CK-19 level between HCC and control groups with sensitivity 64% and specificity 40%. There was no statistically significant difference as regard median CK-19 level between HCC and cirrhosis groups with Sensitivity 64% and Specificity 44%.

**El Raziky *et al.*** [13] showed that CK19 level was of sensitivity 63.4% and specificity 55% in studied patients (early HCC, advanced HCC or cirrhosis).

Conversely, **Zhuo *et al.*** [19] demonstrated that patients with CK-19 positive HCC have poorer prognosis compared with CK-19 negative ones.

A substantial number of studies demonstrated that CK-19 was associated with early tumor recurrence and

worse overall survival after surgical resection or liver transplantation. **Govaere *et al.* [20] and Kim *et al.* [21]**. A large meta-analysis was done by **Sun *et al.* [22]** to investigate the association between CK-19 expression in tissue and the prognosis of HCC patients. It included 17 studies with a total of 2943 patients. The results showed that tissue CK-19 overexpression was significantly associated with poor overall survival rate and early tumor recurrence rate in HCC patients.

Overexpression of CK-19 in HCC cells is related to metastatic behavior. Serum CK-19 level might reflect the pathological progression in some HCC and may be a useful marker for predicting tumor metastasis and a therapeutic target for the treatment of HCC patients with metastases [23].

Our study indicating that there is weak significant relationship between the levels of CK-19 and AFP in HCC cases p-value is 0.07.

Moreover, **El Raziky *et al.* [13]** performed a study to verify the effect of combination of both AFP and CK-19 levels on increase the diagnostic accuracy of suspected HCCs, it show that CK-19 levels are good predictors of ablation/recurrence in patients who underwent interventional procedures minimizing the need for follow-up imaging modalities.

These differences in results may be attributed to many factors: one of them is the number of candidates that was smaller in our study, and another factor is the method of detection of CK-19 which was done by measuring its serum level by ELISA in our study but in other studies was done by immune-histochemistry of surgically excised tumors or using microarrays. Another factor is a longer period of follow-up in other studies, and this may reveal cases of recurrence or new lesions on long follow-up period and also different underlying cause of Chronic liver disease.

In conclusion, this study added an evidence for the role of CK-19 level in diagnosing the pattern of HCC in patients with liver cirrhosis. Significant increase was found between the median CK-19 level and degrees of aggressiveness index (A, B and C) (P >0.05). CK-19 level has Sensitivity 64% and Specificity 40%to differentiate between HCC and healthy people and Sensitivity 64% and Specificity 44% to differentiate between HCC and cirrhosis. Finally, CK-19 is not a good marker for diagnosis of HCC, but a good prognostic marker of HCC.

**According to the findings of the present study, the following recommendations are suggested:**

1. This work represents a small sized sample in our center. So, additional studies with larger number of patients would be useful to confirm the influence of CK-19 level on Pattern of HCC in Egyptian patients with liver cirrhosis.
2. Research on role of CK-19 level on pattern of HCC in patients with liver cirrhosis is still in early stage, and it needs to be clarified with differentiation between detection of serum CK-19 levels by simple ELISA technique or by the reported tissue expression method.
3. We suggest CK-19 is good marker for differentiation between early and advanced stage of HCC, although this does not reach to statically significance.
4. CK-19 associated carry a poor prognosis as it associated with more aggressive pattern of HCC.
5. CK-19 is good negative marker of early HCC, So CK19 negative HCC patients has no priority for treatment.

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