The possible role of Cytochrome c and programmed cell death protein 4 (PDCD4) on pathogenesis of hepatocellular carcinoma

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Abstract Background: Hepatocellular carcinoma (HCC) is regarded as one of the most common malignancies and among the leading causes of cancer death in the whole world. Apoptosis is a fundamental process controlling cell death and plays a critical role in normal development of multicellular organisms. When abnormalities occur in apoptosis, a variety of diseases are caused, including cancer. The aim of the current study was to determine the serum expression of Cytochrome c and PDCD4 among patients with hepatocellular carcinoma and chronic hepatitis.

Patients and methods: A total of 40 serum and tissue samples (17 samples from chronic hepatitis and 23 samples from HCC patients) were collected. Apoptotic markers in serum were carried out using the quantitative sandwich enzyme immunoassay technique.

Results: We found that serum levels of PCDC4 and Cytochrome c were increased in patients with HCC when compared to chronic hepatitis patients. They were also increased in patients with chronic hepatitis when compared to controls (p < 0.05, significant). Analyzing the impact of HCC characters on serum values of PDCD4 and Cytochrome c revealed that the mean values of both PDCD4 and Cytochrome c are significantly higher in cases with single lesion of HCC (p < 0.05, significant). Right lobe location of HCC lesions has the highest mean values of PDCD4 (p < 0.05, significant). As regards grade of differentiation, grade II has higher mean values of Cytochrome c (p < 0.05, significant).

Conclusion: Serum levels of Cytochrome c and PDCD4 are increased in patients with cirrhosis and hepatocellular carcinoma and could be used as diagnostic aid for HCC.

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1. Introduction

Apoptosis is a fundamental process controlling cell death and plays a critical role in normal development of multicellular organisms. When abnormalities occur in apoptosis, a variety of diseases are caused, including cancer [1].

Hepatocellular carcinoma is a slow progressing disease. During the initiation phase of this disease the balance between apoptosis and cell proliferation of hepatic cells is disrupted and favors proliferation, whereas hepatocytes undergo high levels of hepatocytic cell death. In response to this injury, innate immune cells migrate to the site of damage and release a plethora of proinflammatory cytokines and free radicals generating an inflammatory microenvironment, which promotes cancer progression. After chronic exposure to rounds of liver injury and inflammation hepatocytes develop mechanisms to evade apoptotic death; this results in the accumulation of damaged hepatocytes that eventually become HCC. These mechanisms include the persistent down regulation of proapoptotic molecules and upregulation of anti-apoptotic proteins [2].

The mitochondria turned out to participate in the central control or executioner phase of the cell death cascade. Cytochrome c was identified as a component required for the crucial steps in apoptosis, caspase-3 activation and DNA fragmentation. Cytochrome c was shown to redistribute from mitochondria to cytosol during apoptosis in intact cells [3,4].

Cytochrome c has a well-defined role in triggering apoptosis and as a marker of apoptosis [5], though it was recently shown that Cytochrome c exists in a complex in serum with leucine-rich alpha-2-glycoprotein-1 [6].

Programmed cell death protein 4 (PDCD4) is a protein that in humans is encoded by the PDCD4 gene. This gene encodes a protein localized to the nucleus in proliferating cells. Expression of this gene is modulated by cytokines in natural killer and T cells. The gene product is thought to play a role in apoptosis but the specific role has not yet been determined [7]. The aim of the current study was to determine the serum expression of Cytochrome c and PDCD4 among patients with hepatocellular carcinoma and chronic hepatitis.

2. Patients and methods

Between June 2011 and June 2013, a total of 60 serum samples (25 samples from controls, 17 samples from hepatitis and 23 samples from HCC patients) were collected from patients who underwent liver resection or donor liver transplantation (LDLT) at the First Affiliated El Sahel Teaching Hospital and Dr. Refaat Kamel Hospital (Cairo, Egypt). Serum samples were also collected from 25 healthy volunteers who served as the control group. All of the HCC patients were diagnosed by liver biopsy or by the findings of at least two radiological tests of HCC, including abdominal ultrasound, magnetic resonance imaging (MRI), hepatic angiography and contrast-enhanced dynamic computed tomography or by increased AFP (AFP > 200 µg/mL). Patients with secondary or recurrent tumors, a history of other malignant tumors or being included in other studies were excluded from this study. For the 17 chronic hepatitis cases, the diagnosis was based on the serum tests. Serum hepatitis B surface antigens (HBsAg) and anti-HCV antibody were assayed by micro particle enzyme immunoassay using commercial kits to determine hepatitis B or hepatitis C infection. A total of 25 cancer-free controls were attached at the physical examination center. Controls that had clinical liver diseases were excluded. After signing an informed consent, all subjects were asked to fill a questionnaire to investigate the demographic characteristics, disease history, and the history of cancer and alcohol or tobacco use. The tumor characteristics including tumor differentiation, tumor size, metastasis, Child-Pugh class, were collected from medical records.

2.1. Histopathological examination

Tumor staging was performed according to the American Joint Committee on Cancer and International Union against Cancer (AJCC/UICC) staging system (6th edition, 2002). Histological tumor grading was performed according to the Edmondson-Steiner classification: grade 1–2 (well differentiated), grade 3 (moderately differentiated), and grade 4 (poorly differentiated) [8].

2.2. Detection of Human Programmed cell death protein-4 (PDCD-4) and Human Cytochrome-c in blood samples (serum)

The procedure for each parameter is as follows:

Apoptotic markers in serum were carried out using the quantitative sandwich enzyme immunoassay technique. The kits (Glory Science Co., Ltd, USA) used double – antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Programmed cell death protein 4 (PDCD4) and Human Cytochrome c in blood samples (serum).

- PDCD4 was added to monoclonal antibody Enzyme well which was pre-coated with Human Programmed cell death protein monoclonal antibody, incubation; or Cytochrome c was added to monoclonal antibody Enzyme well which is pre-coated with Human Cytochrome c monoclonal antibody, then
- Add PDCD4 or Cytochrome c antibodies labeled with biotin, and combine them with Streptavidin-HRP to form immune complex; then carry out incubation and wash again to remove the uncombined enzyme. Then Chromogen solution was added A, B, the color of the liquid changes into the blue, and the effect of acid, the color finally becomes yellow. The chroma of color and the concentrations of the human substance programmed cell death 4 (PDCD4) or Cytochrome c of sample were positively correlated.
- We calculate the O.D. value of all the wells with the standard and the wells with samples, make the standard curve diagram with concentration of the standard from the low to the high and the left to the right as abscissa \(X\) and O.D. values of the wells at 450 nm as ordinate \(Y\) axis.
- Then we found out the corresponding concentration range of each sample on this standard curve diagram according to their O.D. values.

2.3. Statistical analysis

Data were expressed as mean ± SD unless otherwise indicated. Categorical data were described as frequency of the subjects with a specific characteristic. Chi-square test or Fisher’s exact test was used for comparing categorical data and Student’s
-test, Mann–Whitney–U test, one-way ANOVA or Kruskal–Wallis test, when appropriate, was used for comparing continuous variables. Two-tailed \( p \)-values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

### Table 1  Patient characteristics.

| Variables            | HCC group               | Chronic hepatitis group |
|----------------------|-------------------------|-------------------------|
|                      | mean ± SD               | N = 23                  | N = 17                  |
| Age in years         | 54.27 ± 9.176           | 45.23 ± 9.35768         |
| Sex                  |                         |                         |
| Male No (%)          | 22 (95.7)               | 15(88.2)                |
| Female No (%)        | 1 (4.3)                 | 2 (11.8)                |
| PH OF anti-Sch. ttt   |                         |                         |
| Negative No (%)      | 9 (39.1)                | 9 (52.9)                |
| Positive No (%)      | 14 (60.9)               | 8 (47.1)                |
| AST IU/L mean ± SD   | 104.44 ± 82.93          | 54.20 ± 21.28           |
| ALT IU/L mean ± SD   | 63.10 ± 62.39           | 64.60 ± 30.27           |
| Albumin gm/dl mean ± SD | 2.2 ± 2.4    | 2.1 ± 2.3               |
| Total bilirubin mg/dl mean ± SD | 2.1 ± 1.3 | 1.5 ± 1.8               |
| AFP ng/ml mean ± SD  | 537.88 ± 1848.63        | 15.33 ± 22.59           |
| CA19-9 IU/ml mean ± SD | 28.76 ± 20.03           | 53.56 ± 33.77           |
| CEA IU/ml mean ± SD  | 2.01 ± 1.28697          | 5.71 ± 6.15             |
| CHOL mg/dl mean ± SD | 108.00 ± 44.69          | 121.66 ± 33.29          |
| TRG mg/dl mean ± SD  | 91.20 ± 44.69           | 74.00 ± 13.11           |
| HDL mg/dl mean ± SD  | 41.00 ± 18.29           | 28.00 ± 15.11           |
| LDL mg/dl mean ± SD  | 42.30 ± 18.46           | 114.00 ± 16.35          |
| Cause of chronic liver disease |          |                         |
| HCV No (%)           | 20 (86.9)               | 10 (58.8)               |
| HBV No (%)           | 4 (13.1)                | 5 (29.4)                |
| PBC No (%)           | 1 (5.9)                 |                         |
| PSC No (%)           | 1 (5.9)                 |                         |
| HCC lesions          |                         |                         |
| Number               |                         |                         |
| 1 No (%)             | 11 (47.8)               |                         |
| Multiple No (%)      | 12 (42.2)               |                         |
| Site                 |                         |                         |
| Rt. lobe No (%)      | 10 (43.5)               |                         |
| Lt. lobe No (%)      | 1 (4.3)                 |                         |
| Both No (%)          | 12 (42.2)               |                         |
| Size cm              | 3.65 ± 1.75             |                         |
| Grade                |                         |                         |
| I No (%)             | 4 (17.4)                |                         |
| II No (%)            | 19 (82.6)               |                         |
| Type                 |                         |                         |
| Trabecular No (%)    | 9 (39.1)                |                         |
| Mixed No (%)         | 14 (40.9)               |                         |
| Liver background     |                         |                         |
| 1-Mixed cirrhosis with mild activity No (%) | 2 (8.7) | 5 (29.4) |
| 2-Mixed cirrhosis with moderate activity No (%) | 21 (91.3) | 12 (70.6) |

PH OF anti-Sch. ttt: past history of antischistosomal treatment.
AST: aspartate transaminase.
ALT: alanine transaminase.
AFP: \( \alpha \)-fetoprotein.
CHOL: cholesterol.
TRG: triglycerides.
HDL: high density lipoprotein.
LDL: low density lipoprotein.

### 3. Results

Two groups of patients were enrolled in the current study; HCC group consists of 23 patients, 22 males and one female with mean age of 54.27 ± 9.17 years. Chronic hepatitis group


consists of 17 patients, 15 males and 2 females with mean age of 45.23 ± 9.35 years. 25 subjects, 23 males and two females with mean age of 43.6 ± 6.8 years were enrolled as a control group. Frequency of past history of Schistosomiasis was 60.9% among HCC group while it was 47.1% among patients of chronic hepatitis group. Distribution of causes of chronic liver diseases among the studied groups of patients revealed that the most common cause was HCV infection especially among the HCC group followed by HBV infection. One case of primary biliary cirrhosis (PBC) and another case of primary sclerosing cholangitis (PSC) were found among the chronic hepatitis group as shown in Table 1. There was no significant difference between two studied groups of patients as regards liver function tests. As a traditional marker for HCC, AFP was significantly increased among the HCC group of patients. Although mean values of CA19-9 and CEA were increased among patients with chronic hepatitis but this difference did not reach significant level. Macroscopic examination of hepatocellular malignant lesions revealed that distribution of these lesions was more prevalent in both right and left lobes, then the right lobe and was least found in the left lobe with mean size of 3.65 ± 1.75 cm. The frequency of single lesion was more than that of multiple lesions. Microscopic examination of liver tissues of the HCC group of patients revealed that grade 2 of differentiation and mixed type was more prevalent than grade 1 and trabecular type. The frequency of mixed cirrhosis with chronic hepatitis was more among the two studied groups of patients than mixed cirrhosis with mild activity as shown in Table 1. We found that serum levels of PCDC4 and Cytochrome c were increased in patients with HCC when compared to chronic hepatitis group as shown in Table 1. There was no significant effect of HCC characters on serum values of PDCD4 and Cytochrome c as shown in table 4. As regards grade of differentiation, grade II has higher mean values of Cytochrome c (p < 0.05, significant). Otherwise, there was no significant correlation of serum Cytochrome c; PDCD-4 to other studied variables as shown in Table 3. Analyzing the impact of HCC characters on serum values of PDCD4 and Cytochrome c proved that the mean values of both PDCD4 and Cytochrome c were significantly higher in cases with single lesion of HCC (p < 0.05, significant). The right lobe location of HCC lesions has the highest mean values of PDCD4 (p < 0.05, significant). As regards grade of differentiation, grade II has higher mean values of Cytochrome c (p < 0.05, significant). Otherwise, there was no significant effect of HCC characters on serum values of PDCD4 and Cytochrome c as shown in table 4. As regards PDCD4, cut off value of 7.650 could be a diagnostic marker for HCC with 91.3% sensitivity; 70.6% Specificity and Area under the curve of 0.867. Meanwhile, Cytochrome c cut off value of 9.660 could be a diagnostic marker for HCC with 60.9% sensitivity, 88.2% specificity and area under the curve of 0.792. It was noticed that specificity of both apoptotic markers were higher than that of α-fetoprotein as shown in Table 5 and Figs 1 and 2.

4. Discussion

It was found that serum levels of PCDC4 and Cytochrome c were increased in patients with HCC when compared to chronic hepatitis patients. They were also increased in patients with chronic hepatitis when compared to controls. During carcinogenesis, cell death rates are changed according to disease stage and individual conditions. Although proliferation of malignant cells often already starts to increase years before the manifestation of cancer disease, effective immune-

### Table 2 Serum levels of Cytochrome c and PDCD-4 in the different studied groups with its statistical significance.

| Groups of patients | Cytochrome c ng/ml | PDCD-4 ng/ml |
|--------------------|--------------------|--------------|
| Controls (25) A    |                    |              |
| Range              | 2.8–5.6            | 2.4–7.2      |
| Mean ± SD          | 4.09 ± 0.92        | 4.37 ± 1.13  |
| HCC (23) B         |                    |              |
| Range              | 6.087–21.1         | 6.473–23.76  |
| Mean ± SD          | 11.94 ± 4.69       | 12.92 ± 5.69 |
| P value            |                    |              |
| A:B                | <0.05              | <0.05        |
| B:C                | <0.05              | <0.05        |
| Liver cirrhosis (17) C |        |              |
| Range              | 4.5–10.5           | 3.9–107      |
| Mean ± SD          | 7.34 ± 1.89        | 6.77 ± 1.98  |
| P value            |                    |              |
| A:C                | <0.05              | <0.05        |

AST: aspartate transaminase.
ALT: alanine transaminase.
GGT: gamma glutamyl transferase.
CHOL: cholesterol.
TRG: triglycerides.
HDL: high density lipoprotein.
LDL: low density lipoprotein.
CEA: carcinoembryonic antigen.
PC %: prothrombin concentration.

| Variables | PDCD-4 | Cytochrome c |
|-----------|--------|--------------|
|           | r      | p            | r    | p |
| Age       | .455** | .004         | .273 | .093 |
| Tumor size| .359   | .12          | 0    | 1   |
| α-Fetoprotein | .405  | .029         | .068 | .727 |
| ALT       | -.472  | .076         | -.667 | .007 |
| AST       | .104   | .725         | .11  | .708 |
| GGT       | .679   | .094         | .071 | .879 |
| PC %      | .173   | .521         | .202 | .453 |
| Bilirubin | .541   | .086         | .717 | .013 |
| CA19-9    | -.099  | .748         | -.253 | .404 |
| CEA       | -.629  | .038         | -.405 | .216 |
| CHOL      | -.262  | .531         | -.563 | .146 |
| TRG       | .476   | .233         | .216 | .608 |
| HDL       | -.400  | .505         | -.100 | .873 |
| LDL       | -.300  | .624         | -.300 | .624 |

**p is highly significant.
*p is significant.

Table 3 Correlation between Cytochrome c; PDCD-4 and the studied variables.
surveillance leads to the elimination of transformed cells by a simultaneously elevated cell death rate. To break this counterbalance, tumor cells look to evade these controlling mechanisms by lowering their susceptibility to apoptosis as well as by manipulating the immune response e.g. by reducing specific surface ligands and subsequently by directly attacking immune cells [9] In addition, genetic or epigenetic alterations lead to impairment of cell death and acceleration

### Table 4 Impact of HCC characters on serum values of PDCD4 and Cytochrome c.

| HCC characters | PDCD4 Mean ± SD | P value | Cytochrome c Mean ± SD | P value |
|----------------|-----------------|---------|------------------------|---------|
| Number         |                 |         |                        |         |
| Single         | 16.44 ± 5.47    | 0.0001* | 13.59 ± 4.94           | 0.002*  |
| Multiple       | 8.28 ± 1.21     |         | 9.58 ± 3.63            |         |
| Site           |                 |         |                        |         |
| Rt.lobe        | 16.06 ± 5.99    | 0.005*  | 13.24 ± 5.24           | 0.313   |
| Lt.lobe        | 11.20           |         | 11.57                  |         |
| Both           | 8.28 ± 1.21     |         | 10.33 ± 3.99           |         |
| Grade          |                 |         |                        |         |
| I              | 9.37 ± 1.23     | 0.568   | 7.00 ± 1.30            | .040*   |
| II             | 12.59 ± 5.78    |         | 12.25 ± 4.64           |         |
| Type           |                 |         |                        |         |
| Trabecular     | 15.11 ± 6.23    | 0.101   | 15.00 ± 4.70           | 0.022*  |
| Mixed          | 10.70 ± 4.67    |         | 9.94 ± 3.69            |         |

Rt.lobe: right lobe.
Lt.lobe: left lobe.
* p value is significant.

### Table 5 The sensitivity/specificity of α-fetoprotein; PDCD4 and Cytochrome c as diagnostic markers for HCC.

| Parameter       | Area under the curve | Cutoff value | Sensitivity (%) | Specificity (%) | OR   | PPV (%) | NPV (%) |
|-----------------|----------------------|--------------|-----------------|-----------------|------|---------|---------|
| α-Fetoprotein   | 0.832                | 8.050        | 100.0           | 69.2            | 0.200| 80.0    | 100.0   |
| PDCD4           | 0.867                | 7.650        | 91.3            | 70.6            | 25.200| 80.8    | 85.7    |
| Cytochrome c    | 0.792                | 9.660        | 60.9            | 88.2            | 11.667| 87.5    | 62.5    |

![Figure 1](ROC_curve_PDCD4.png)  ![Figure 2](ROC_curve_CYTC.png)
of proliferation mechanisms in cancer cells. Resulting tumor growth and invasion of local structures are then further supported by new formation of blood vessels which provide the required additional energy supply [10]. At stage of apparently invasive and metastasizing cancer, the rate of cell death may remain low if expression levels of regulatory genes for resistance to apoptosis such as Bcl-2 are increased. Alternatively, cell death rates may increase again as a result of the accumulating presence of dysfunctional cells in hyperproliferating tissues as well as by effective counterattack of tumor cells to immune cells [11]. We suggest that the rate of hepatocyte death increases during liver carcinogenesis and this may explain high serum levels of PDCD4 and Cytochrome c among patients with chronic hepatitis and HCC. All HCC cases in the current study have liver cirrhosis as a background. This background with continuous progression of inflammation may have an impact on increased serum values of apoptotic markers among cirrhotic and malignant patients.

Cancer can be viewed as the result of a succession of genetic changes during which a normal cell is transformed into a malignant one while evasion of cell death is one of the essential changes in a cell that cause this malignant transformation. As early as the 1970’s, Kerr and his colleagues had linked apoptosis to the elimination of potentially malignant cells, hyperplasia and tumor progression [12]. Hence, reduced apoptosis or its resistance plays a vital role in carcinogenesis. There are many ways a malignant cell can acquire reduction in apoptosis or apoptosis resistance. Generally, the mechanisms by which evasion of apoptosis occurs can be broadly divided into: (1) disrupted balance of pro-apoptotic and anti-apoptotic proteins, (2) reduced caspase function and (3) impaired death receptor signaling [13].

Ben-Ari and his colleagues concluded that soluble Cytochrome c levels are increased in different types of liver disease. Soluble Cytochrome c is probably derived from the liver and secreted into the bile. Levels correlate with the apoptotic index and are affected by antiviral treatment. Soluble Cytochrome c may serve as a serum marker of apoptosis [14]. Interestingly, elevated Cytochrome c levels were observed in sera from patients with hematologic malignancies [15].

The results showed that the serum levels of Cytochrome c were highly increased in HCC cancer patients while they were increased in chronic hepatitis. An early event in apoptosis induced by death receptor-independent stimuli is the translocation of Cytochrome c into the cytosol. Some investigators have shown that Cytochrome-c is not only released from the mitochondria upon apoptosis induction, but furthermore it leaves the cell and can even be detected in the serum of cancer patients upon chemotherapy Cytochrome-c is released from apoptotic but not from necrotic cells [15,16].

These results were in accordance with Osaka et al. (2009) who reported that serum Cytochrome c level was significantly higher in patients with malignant tumors than patients with benign tumors (20.6 vs. 15.5 ng/mL; \( P = 0.017 \), Mann–Whitney U test). No difference in the levels among subtypes of cancer was found, indicating that the change in serum Cytochrome c levels reflect cancer individually and not specific subtypes of cancer [17].

Cytochrome-c certainly covers all malignancies since no cancer devoid of mitochondria exists. Its broad spectrum is achieved for the price of the selectivity. The increase in serum Cytochrome c indicates, with a good degree of precision, the increase in apoptosis in vivo. Some of the serum Cytochrome c may also be released from healthy tissue because current chemotherapy is also considerably toxic to some types of normal cells; serum Cytochrome-c is a sensitive apoptotic indicator in vivo. Furthermore, high-serum Cytochrome-c appears to be a negative prognostic marker during cancer therapy, probably being indicative of high tumor mass [18,19].

As regards the results of PDCD-4 several studies have been conducted to investigate the role of PDCD4 during tumorgenesis. Over-expression of PDCD4 resulted in suppressed carcinoid cell proliferation through repressing the transcription of the mitosis-promoting factor cyclin-dependent kinase (CDK) 1/cdc2 via up regulation of p21Waf1/Cip1. The role of PDCD4 in cell apoptosis has also been investigated in different studies. PDCD4 was suggested to be a pro-apoptotic molecule involved in transforming growth factor beta-1 (TGF beta-1) induced apoptosis in hepato-cellular carcinoma (HCC). Diminished PDCD4 expression deregulated the normal DNA-damage response, thus preventing DNA-damaged cells from undergoing apoptosis [20].

As increased cell death (PDCD-4) may be an attempt to limit the expansion of the tumor cell population, several studies have linked the rate of apoptosis with the proliferative rate of adenomas and carcinomas, yielding conflicting results. PDCD-4 is up-regulated on induction of apoptosis and down-regulated in certain aggressive tumours including lung, breast, colon, brain, and prostate cancers [21].
Pdc4 suppresses tumorigenesis and tumor progression and invasion and inhibits cap-dependent but not internal ribosomal entry site-dependent global and specific mRNA translation by binding eukaryotic initiation factor 4A and mRNA molecules selectively; the expression of Pdcd4 is lost or suppressed in some tumors, but induced or stimulated in the others [22]. Yet, little is known concerning the expression and function of Pdcd4 protein during apoptosis and its downstream targets as well as upstream regulators.

The studied apoptotic markers in the current research could be used as diagnostic markers for HCC especially PDCD4. Their specificity as diagnostic marker for HCC was higher than that of \( \alpha \)-fetoprotein. PDCD4 has high sensitivity for HCC diagnosis.

The abundance of the literature suggests that defects along apoptotic pathways play a crucial role in carcinogenesis and that many new treatment strategies targeting apoptosis are feasible and may be used in the treatment of various types of cancer. Some of these discoveries are preclinical while others have already entered clinical trials. Many of these new agents or treatment strategies have also been incorporated into combination therapy involving conventional anticancer drugs in several clinical trials, which may help enhance currently available treatment modalities. However, some puzzling and troubling questions such as whether these treatment strategies induce resistance in tumors and whether they will cause normal cells to die in massive numbers still remain unanswered [23].

5. Conclusion

Serum levels of Cytochrome c and PDCD4 are increased in patients with cirrhosis and hepatocellular carcinoma. Their levels were increased in single lesions of HCC. Their specificity as diagnostic marker for HCC was higher than that of \( \alpha \)-fetoprotein, so they could be used as diagnostic aid for HCC.
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