Original article

Estimation of serum Alpha feto-protein (AFP), interlukin-6 and Des-γ-carboxyprothrombin (DCP) in case of hepatocellular carcinoma

Haque SS[1], Kumari R[2], Muzaffar A[3], Kumar U[4], Sharan A[5], Kumari B[6]

Abstract:

Background: Hepatocellular carcinoma (HCC) is one the most common primary malignancy of the liver and represents the third leading cause of cancer-related deaths worldwide. Incidence rates are highest in East Asia and Sub-Saharan Africa. A number of evidence suggests a possible role of interleukin-6 (IL-6), α-Fetoprotein (AFP) and Des-γ-carboxyprothrombin (DCP) in the pathogenesis of hepatocellular carcinoma (HCC). The high DCP may be related to increase tumour behaviour, such as the presence of vascular invasion and intrahepatic metastasis of HCC cells. ***Patients and methods***: We studied IL-6, AFP and DCP in patients with HCC or in healthy controls. AFP was measured by chemiluminescent immunoassay; Serum IL-6 and DCP were measured by enzyme linked immunosorbent assay in 30 patients with primary hepatocellular carcinoma and 30 normal subjects. ***Results***: IL-6, AFP and DCP were found high in the serum of patients initially diagnosed with HCC (18±9.8), (315.99±594.62) and (26.15±5.01) respectively compared with healthy subjects (4.29±2.10), (3.13±1.27) and (4.25±1.22). A significant positive correlation was found between mean levels of IL- 6 & AFP in HCC ($P < 0.05$), Combination of IL-6, AFP and DCP improved the sensitivity in diagnosing HCC or predicting future HCC development. ***Conclusions***: IL-6, DCP along with AFP could be considered a promising tumor marker for HCC. DCP is a well recognized tumor marker for the screening and diagnosis of HCC. In particular, the diagnostic value of the test is significantly increased when combined with AFP.

Keywords: AFP; DCP; IL-6; HCC

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Introduction:

Hepatocellular carcinoma (HCC) most common liver cancers reported world wide. Males are more prone to HCC [1]. There are another risk factor such as chronic liver inflammation due to hepatitis B virus (HBV) and hepatitis C virus (HCV) infection [2, 3]. There are no satisfactory screening procedures for early detection for HCC is available, serum alpha fetoprotein (AFP) and ultrasound scan is commonly recommended[4]. AFP is a major serum glycoprotein comprised of 591 amino and 4% carbohydrate residues, encoded by a gene on chromosome 4q11-q13 with a half-life of 5-7 days, which is synthesized by fetal

1. Dr. Syed Shahzadul Haque, Biochemist, Dept of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India
2. Dr. Rekha Kumari, Asst prof , Dept of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India
3. Dr. Ali Muzaffar, Senior Resident Dept of Pathology, Indira Gandhi Institute of Medical Sciences, Patna-14, India
4. Dr. Uday Kumar, Prof & Head, Dept of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India
5. Dr. Anand Sharan, Prof, Dept of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India
6. Dr. Bandana Kumari, Senior Resident, Dept of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India

Corresponds to: Dr. Syed Shahzadul Haque,Biochemist, Dept of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India. **E-mail:** sshaq2002@yahoo.co.in

liver cells, by yolk sac cells, and in trace amounts by the fetal gastrointestinal tract [5, 6]. AFP can be produced under many circumstances, including other liver diseases [7], and is not present in all those with HCC. Therefore, the use of AFP as a primary screen for HCC has been questioned[8] and more sensitive serum biomarkers for HCC are desired. Recently, afetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) have been widely used for HCC diagnosis and follow-up surveillance as tumor serologic markers in most of the Asian countries.

Des-γ-carboxyprothrombin (DCP), also known as PIVKA-II (protein induced by vitamin K absence or antagonist), is an inactive prothrombin, lacking carboxylation of the 10 glutamic acid residues in the N-terminus, which is the result of an acquired posttranslational defect of the prothrombin precursor in HCC cell lines. DCP is unable to bind calcium ion that is essential for its conformational transition and functional activity. DCP was discovered in serum of patients during their anticoagulant therapy with a vitamin K antagonist. It has been proved that significant concentrations of serum DCP are present in 50%-60% of all HCC patients, but in only 15%-30% of early HCC case[9].

Interleukin-6 (IL-6) is a multifunctional pleiotropic cytokine largely responsible for the hepatic response to infections or systemic inflammation and that plays an important role in hematopoiesis, as well as in the differentiation and growth of a number of cells of different histologic origin, e.g. endothelial cells, keratinocytes, neuronal cells, osteoclasts, and osteoblasts[10]. Serum IL-6 levels are elevated in patients with chronic liver inflammation including alcoholic hepatitis[11], hepatitis B[12], and HCV infections[13]

New biomarkers for earlier diagnosis of HCC with high sensitivity and identification of high risk groups are required.

Patients and methods:

Patients: The study involved 60 subjects who were divided into two groups. The control group consisted of 30 healthy subjects (21 women and 09 men) with an average age of 55.38 years, who were from 30 to 70 years old; they also did not have family history of HCC and they were not medically treated. Rest 30 subjects were diagnosed with HCC. Detailed clinical history and examination were carried out and recorded in preformed Performa. The study conducted in the Department of Biochemistry in collaboration with the Department of Gastroenterology, during the period from Jan 2010 to March 2012.

Blood samples were collected from eighty patients who were attending to Indira Gandhi Institute of Medical Sciences Patna teaching hospital. Sera were separated and stored at -20 ^{o}C until use.

Methods:

AFP was performed by Chemiluminescent Immunoassay Beckman coulter Inc.IL-6 serum titers were evaluated in the peripheral blood of all the above patients; blood samples were taken from an antecubital vein of the forearm of each study subject, after overnight fasting; serum was centrifuged and then frozen at 4 ^{0}C for subsequent analysis. DCP and Serum IL-6 was DCP was performed using a commercial enzyme-linked immunosorbent assay kit (Human IL-6 Immunoassay, R&D Systems, Minneapolis, MN) following the manufacturer's instructions.

The study was approved by the ethical committee of Indira Gandhi Institute of Medical Sciences.

Statistical Analysis: The data of the study subjected to statistical analysis is expressed as mean ± SD. Statistical comparisons were performed by Student't' test.

Results:

From 30 patients, 21 (70%) of them were women while only 9 (30%) of who were men. The mean age of the patients was 55.38 ± 10.05 (55.26 ± 7.93 for women and 54.85 ± 8.23 for men).

The mean serum AFP level in case HCC was (315.99±594.62) and in control was (3.13±1.27). The normal cut-off value is less than 5.0 ng/ml. It is interesting to note that a large number of patients, both males and females with elevated levels of AFP are basically diagnosed with HCV or HBV infections.

Chronic hepatitis C patients had significantly higher serum IL-6 levels than healthy controls (18±9.8) vs. (4.29±2.10), $p < 0.005$) and the difference was similar in male and female and the value of DCP in case of HCC was (26.15±5.01) compared to control (4.25±1.22)

Table 1:

Groups	AFP	IL-6	DCP
Control (N=30)	3.13±1.27	4.29±2.10	4.25±1.22
HCC (N=30)	315.99±594.62**	18±9.8***	26.15±5.01**

***P<0.005, **p<0.05

Discussion:

Since AFP was discovered in the serum of HCC patients in 1964 [14], it has been regarded as the

most useful serum protein thus far for patients at risk for HCC [15-17]. The first quantitative serum assays for AFP were established by Ruoshlati and Seppala [18]. Up to 11 AFP isoforms exist based on variations in the glycan terminal chain[19, 20] More recently, isoelectric focusing has been investigated, which fractionates AFP into four variant bands, I-IV. AFP bands III and IV can be specific for HCC and help differentiate from AFP of cirrhosis or pregnancy [21]. The sensitivity of AFP is low renders it unsatisfactory for this purpose and compels to search for novel biomarkers for the detection of early HCC [22]. Many studies indicated a big role for IL-6 in the process of liver damage and carcinogenesis [23, 24]. Previous studies have confirmed that serum IL-6 level is increased in patients with established HCC.[25-30] High serum IL-6 may promote the development of HCC in hepatitis B patients [31]. For the progression of liver disease, i.e., from chronic hepatitis to cirrhosis to HCC the DCP level increases. PIVKA-II, an abnormal prothrombin discovered in 1984, has been widely proposed to be a useful HCC biomarker[32]. The DCP level was decreased in HCC patients with low AFP value but significantly increases in patients with high AFP value. It is well documented that AFP estimation remains along with IL-6 a useful test for clinicians, oncologists and physicians involved in the management of patients of HCC.

Conclusion:

In conclusion, high serum IL-6 level predates the development of HCC in chronic hepatitis B patients, and has moderate accuracy in predicting future cancer. DCP shows better prognostic marker in those having high AFP level. This may assist clinicians in selecting high-risk patients for HCC surveillance program. Combining the two markers can provide a new perspective in the diagnosis and prognosis of HCC.

Conflict of interest: None declared.

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