Status of serum alpha feto-protein (AFP) and midkine (MDK) levels in patients with hepatocellular carcinoma

Syed Haque[1*], Md Tanweeruddin[2], Bandana Kumari[1], Amod Kumar[3], Santosh Kumar[4], Amarendra Kumar[5], Avinash Kumar[5], Amitesh Kumar[5], Subhashchandra Jha[6], Ali Muzaffar[3]

1. Department of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14 India
2. Department of Anaesthesiology, ECR, Danapur, India
3. Department of Pathology, Indira Gandhi Institute of Medical Sciences, Patna-14, India
4. Department of Orthopedics, Indira Gandhi Institute of Medical Sciences, Patna-14, India
5. Department of Gastroenterology, Indira Gandhi Institute of Medical Sciences, Patna-14, India
6. Department of Pathology, Govt Medical College, Bettiah, Bihar, India

[*]**Corresponding author:** Tel: +91 9934664715; fax: +91 612 2297225
Address: Department of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India
E-mail: sshaq2002@yahoo.co.in
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Abstract

Introduction: Hepatocellular carcinoma (HCC) is sixth common cancers worldwide and predominant in Asia and Africa. A number of evidence suggests a possible role of midkine (MDK) and α-fetoprotein (AFP) in the pathogenesis of hepatocellular carcinoma (HCC).
Materials and methods: We studied MDK and AFP in patients with HCC or in healthy controls. MDK and AFP was measured by enzyme linked immunosorbent assay and chemiluminescent immunoassay, respectively in 30 patients with primary hepatocellular carcinoma and 30 normal subjects.
Results: MDK and AFP were found in high levels in the serum of patients initially diagnosed with HCC (18 ± 9.8 pg/ml and 492.01 ± 298.89 pg/ml) compared with healthy subjects (4.29 ± 2.10 pg/ml and 3.13 ± 1.27 pg/ml), respectively. A significant positive correlation was found between mean levels of MDK and AFP in HCC ($P < 0.05$). Combination of MDK and AFP improved the sensitivity of HCC diagnosis or predicting future HCC development.
Conclusion: MDK along with AFP could be considered a promising tumor marker for HCC. In particular, the diagnostic value of the test is significantly increased when combined with AFP.

Keywords: AFP, HCC, MDK

Introduction

Hepatocellular carcinoma (HCC) most common primary liver cancers reported worldwide. HCC occurs mainly in males and gender is an important risk factor (1). There are number of other cases 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 glycoprotein molecules comprised of 591 amino acids with a half-life of 5-7 days, which is synthesized by fetal liver cells, by yolk sac cells, and in trace amounts by the fetal gastrointestinal tract (5, 6). Reappearance of AFP in adult serum often signals pathologic conditions, particularly the presence of hepatocellular carcinomas (HCC) and germ cell tumors containing yolk sac cell elements (7, 8). In

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60-70% of patients with HCC high serum AFP has been reported, but there are other causes in which increased levels are seen, such as cirrhosis, lung cancer, biliary cancer, gastric cancer, pancreatic cancer, teratocarcinoma of the testis, spherocytosis and tyrosinemia (9). Although AFP has certain limitation which compels to search for other prognostic marker, so we investigated the clinical value of serum MDK in diagnosing primary hepatocellular carcinoma along with AFP.

Midkine (MDK), also known as neurite growth-promoting factor 2 (NEGF2), is a basic heparin-binding growth factor of 13-kDa protein rich in a basic amino acid and cysteine. In humans, it is encoded by the MDK gene on chromosome 11 (10). In majority of cases MK level in serum samples was that 0.6–0.8 ng/mL, whereas the MK levels in the sera of normal human subjects were low or undetectable (11). In addition, the overexpression level of MK in HCC with intra-hepatic metastasis was significantly higher than that in HCC without intra-hepatic metastasis (12). It is noteworthy that a significant increase in serum MK is associated with HCC patients, including those with normal serum AFP concentrations (13). Furthermore, serum MDK levels are reportedly higher in patients with HCC than in those without (14).

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

Materials and methods

Patients: The study involved 60 subjects who were divided into two groups. The control group consisted of 30 healthy subjects (26 women and 14 men) with an average age of 56.34 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. Blood samples were taken from an antecubital vein of the forearm of each study subject, after overnight fasting. Blood was centrifuged and the separated serum then frozen at -20 ºC for subsequent analysis.

Serum AFP level was performed by chemiluminescent immunoassay method on a Beckman coulter. Serum MDK was titered using a commercial enzyme-linked immunosorbent assay kit (Human MDK Immunoassay, R&D Systems, Minneapolis, MN) following the manufacturer's instructions and the results were expressed as pg/ml.

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

Form 60 patients, 26 (65%) of them were women while only 14 (35%) of who were men. The mean age of the patients was 56.34 ± 11.01 years (56.26 ± 6.62 years for women and 53.56 ± 7.13 years for men).The mean serum AFP level in case HCC was (594.62 ± 315.99 pg/ml) and in control was (3.13 ± 1.27 pg/ml) (Table 1).

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 MDK levels than healthy controls (18 ± 9.8 pg/ml) vs. (2.29 ± 4.10 pg/ml), $p < 0.01$) and the difference was similar in male and female.

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Table 1. Serum levels of α-fetoprotein and MDK in patients under study.

Groups	AFP	MDK
Control (N=30)	2.89±1.01	0.69±.10
HCC (N=30)	298.89±492.01***	2.29±4.10**

***P<0.005, **p<0.01

Discussion

AFP was discovered by Bergstrand and Czar (15) in 1956 using paper for its electrophoretic separation from human fetoprotein in serum, and it was first described by Abelev et al. (16) in 1960. The first quantitative serum assays for AFP were established by Ruoshlati and Seppala (17). Up to 11 AFP isoforms exist based on variations in the glycan terminal chain (18, 19). Taketa et al. found AFP-L3 to be positive in about 35% of patients with HCC smaller than 2 cm, which may be present in serum up to 9 months before detection by imaging techniques (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). Chronic hepatitis or cirrhosis raise AFP in 20% and 50% of patients, respectively, and tend to fluctuate in parallel with underlying inflammatory activity (22). 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 (23).

Midkine (MK), a plasma secreted protein, was initially identified in embryonal carcinoma cells at early stages of retinoic acid-induced differentiation. Multiple studies have reported that MK plays important roles in tumor progression, and is highly expressed in various malignant tumors. Because increased serum MK concentrations also have been reported in patients with various tumors, serum MK may have the potential to become a very useful tumor marker.

It is well documented that AFP estimation remains along with MDK a useful test for clinicians, oncologists and physicians involved in the management of patients of HCC.

Conclusion

In conclusion, high serum MDK level predates the development of HCC in chronic hepatitis B patients, and has moderate accuracy in predicting future cancer. 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.

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