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Clinical Evaluation of Serum Alpha Feto-Protein (AFP) and Interlukin-6 in Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the important cancers worldwide and predominant in Asia and Africa. A number of evidence suggests a possible role of interleukin-6 (IL-6) and α-Fetoprotein (AFP) in the pathogenesis of hepatocellular carcinoma (HCC).
Patients and methods: We studied both IL-6 and AFP in patients with HCC or in healthy controls. Serum IL-6, AFP was measured by enzyme linked immunosorbent assay and Chemiluminescent immunoassay respectively in 30 patients with primary hepatocellular carcinoma and 30 normal subjects
Results:IL-6 and AFP was found in high levels in the serum of patients initially diagnosed with HCC (18±9.8), and (315.99±594.62) respectively compared with healthy subjects (4.29±2.10).and (3.13±1.27).A significant positive correlation was found between mean levels of IL- 6 & AFP in HCC ($P < 0.05$), Combination of IL-6 and AFP improved the sensitivity in diagnosing HCC or predicting future HCC development.
Conclusions: IL-6 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, IL-6.

1. Introduction

Hepatocellular carcinoma (HCC) most common primary liver cancers reported world wide. HCC occurs mainly in males and gender is an important risk factor [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 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 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 investigate the clinical value of serum interleukin-6(IL-6) in diagnosing primary hepatocellular carcinoma along with AFP.

Interleukin-6 (IL-6) is a pleiotropic cytokine 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]. Moreover, IL-6 induces the hepatic acute phase response by modulating the transcription of several liver-specific genes during inflammation [11].IL-6 may also regulate cell growth and act as a paracrine and autocrine growth factor in different malignancies [12]. Furthermore, serum IL-6 levels are reportedly higher in patients with HCC than in those without [13].

In chronic hepatitis, IL-6, produced mainly by activated Kupffer cells, intensifies local inflammatory responses and induces compensatory hepatocyte proliferation, facilitating malignant transformation of hepatocytes [14]. Also, IL-6 induces the hepatic acute phase response by modulating the transcription of several liver specific genes during inflammation [15]. Moreover, Giannitrapani et al indicated that IL-6 could be more sensitive marker in identifying HCCs than AFP [16].

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

1.1. Patients 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 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 sampleswere collected from eighty patients who were attending to Indira Gandhi Institute of Medical Sciences Patna teaching hospital. Sera were separated and stored at -20 ºC until use.

2. 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 24 ^{0}C for subsequent analysis. Serum IL-6 was titered using a commercial enzyme-linked immunosorbent assay kit (Human IL-6 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.

3. 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 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) pg/ ml, $p < 0.005$) and the difference was similar in male and female.

Table-1. Serum Alfa feto-protein and Interlukin level in different groups

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

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

4. Discussion

AFP one of the serum glycoprotein that was discovered by Bergstrand and Czar [17] in 1956 using paper for its electrophoretic separation from human fetoprotein in serum, and it was first described by Abelev, et al. [18] in 1960. The first quantitative serum assays for AFP were established by Ruoslahti and Seppälä [19]. Up to 11 AFP isoforms exist based on variations in the glycan terminal chain [20, 21]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 [22]. 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 [23] Chronic hepatitis or cirrhosis raise AFP in 20% and 50% of patients, respectively, and tend to fluctuate in parallel with underlying inflammatory activity [24]. 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 [25]. Many studies indicated a big role for IL-6 in the process of liver damage and carcinogenesis [26, 27]. Previous studies have confirmed that serum IL-6 level is increased in patients with established HCC.[28-33] However, since IL-6 levels were checked after the diagnosis of HCC, it is impossible to differentiate whether IL-6 production is the response to HCC, or IL-6 contributes to the development of HCC. In fact, IL-6 level decreases after surgical resection of HCC, and high IL-6 at the day of surgery is associated with prolonged hyperbilirubinemia after surgery.[34]

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.

5. 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. 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.

References

[1] S. Caldwell and S. Park, "The epidemiology of hepatocellular cancer: From the perspectives of public health problem to tumor biology," *J Gastroenterol.*, vol. 44, pp. 96-101, 2009.

[2] J. Baig, J. Alam, S. Mahmood, M. Baig, R. Shaheen, I. Sultana, and A. Waheed, "Hepatocellular carcinoma (HCC) and diagnostic significance of A-fetoprotein (AFP) World J Surg Oncol 2013, 11:212

[3] H. Lee, J. Jung, Y. Kang, Y. Kim, H. Moon, K. Park, Y. Lee, S. Kim, S. Seo, S. Lee, S. Kim, B. Lee, and N. Kim, "Clinical significance of transiently elevated serum AFP level in developing hepatocellular carcinoma in HBsAg positive-liver cirrhosis *Korean J Gastroenterol*, vol. 43, pp.252-259. 2004

[4] P. Stefaniuk, J. Cianciara, and A. Wiercinska-Drapalo, "Present and future possibilities for early diagnosis of hepatocellular carcinoma," *World J. Gastroenterol.*, vol. 16, pp. 418–424, 2010.

[5] E. Ruoslahti and M. Seppala, Structural properties of α-fetoprotein from human cord serum: the protein molecule at low pH possesses all the properties of the molten globule .*Adv. Cancer Res.*, vol. 29, pp. 275-346, 1979.

[6] D. Gitlin, A. Perricelli, and G. M. Gitlin, Synthesis of α-Fetoprotein by Liver, Yolk Sac, and Gastrointestinal Tract of the Human Conceptus, *Cancer Res.*, vol. 32, pp. 979-982, 1972.

[7] G. I. Abelev, Diagnostic applications of alpha-fetoprotein; Government regulations prevent full application of a clinically useful test**,** *Cancer Res.* vol. 28, pp. 1344-1350, 1968.

[8] R. J. Kurman, P. T. Scardino, K. R. McIntire, T. A. Waldmann, and N. Javadpour**,** Cellular localization of alpha-fetoprotein and human chorionic gonadotropin in germ cell tumors of the testis using an indirect immunoperoxidase technique. *A new approach to classification utilizing tumor markers, Cancer*, vol. 40, pp. 2136-2151, 1977.

[9] G. Mizejewski, "Levels of alpha-fetoprotein during pregnancy and early infancy in normal and disease states," *Obstet Gynecol Surv.*, vol. 58, pp. 804-26, 2003.

[10] S. Akira, T. Taga, and T. Kishimoto, "Interleukin-6 in biology and medicine," *Adv Immunol.*, vol. 54, pp. 1–78, 1993.

[11] J. Gauldie, C. Richards, and D. Harnish, "Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells," *Proc Natl Acad Sci U S A*, vol. 84, pp. 7251–7255, 1987.

[12] O. Martinez-Maza and J. Berek, "Interleukin 6 and cancer treatment," *In Vivo*, vol. 5, pp. 583–588, 1991.

[13] Y. Oyanagi, T. Takahashi, S. Matsui, S. Takahashi, S. Boku, K. Takahashi, K. Furukawa, F. Arai, and H. Asakura, "Enhanced expression of interleukin-6 in chronic hepatitis C," *Liver*, vol. 19, pp. 464–72, 1999.

[14] W. Naugler and M. Karin, "The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer," *Trends Mol Med.*, vol. 14, pp. 109–19, 2008.

[15] M. Malaguarnera, I. Di Fazio, and A. Laurino, "Role of interleukin 6 in hepatocellular carcinoma," *Bull Cancer*, vol. 83, pp. 379–384, 1996.

[16] L. Giannitrapani, M. Cervello, and M. Soresi, "Circulating IL-6 and sIL-6R in patients with hepatocellular carcinoma," *Ann N Y Acad Sci.*, vol. 963, pp. 46–52, 2002.

[17] C. Bergstrand and B. Czar, "Demonstration of new protein fraction in serum from human fetus," *Scand J Clin Lab Invest.*, vol. 8, p. 174, 1956.

[18] G. Abelev, S. Perova, N. Khramkova, Z. Postnikova, and I. Irlin, "Production of embryonal alpha-globulin by transplantable mouse hepatomas," *Transplantation*, vol. 1, pp. 174-180, 1963.

[19] E. Ruoslahti and M. Seppälä, "Studies of carcino-fetal proteins. Development of a radioimmunoassay for -fetoprotein," *Int J Cancer*, vol. 8, pp. 374-383, 1971.

[20] P. Johnson, T. Poon, N. Hjelm, C. Ho, S. Ho, and C. Welby, "Glycan composition of serum alpha-fetoprotein in patients with hepatocellular carcinoma and non-semi-nomatous germ cell tumour," *Br J Cancer*, vol. 81, pp. 1188–95, 1999.

[21] K. Shimizu, H. Katoh, F. Yamashita, F. Tanaka, M. Tanaka, and K. Tanikawa, "Comparison of carbohydrate structures of serum alpha-fetoprotein by sequential glycosidase digestion and lectin affinity electrophoresis," *Clin Chim Acta*, vol. 254, pp. 23–40, 1996.

[22] L. Burditt, M. Johnson, P. Johnson, and R. Williams, "Detection of hepatocellular carcinoma-specific alpha-fetoprotein by isoelectric focusing," *Cancer*, vol. 74, pp. 25–9, 1994.

[23] P. Johnson, "The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma," *Clin Liver Dis.*, vol. 5, pp. 145–59, 2001.

[24] Z. Sun, A. Klein, S. Radaeva, F. Hong, O. El-Assal, H. Pan, B. Jaruga, S. Batkai, S. Hoshino, Z. Tian, G. Kunos, and A. Diehl, "In vitro interleukin-6 treatment prevents mortality associated with fatty liver transplants in rats," *Gastroenterology*, vol. 125, pp. 202–15, 2003.

[25] H. Ataseven, I. Bahcecioglu, N. KuzuM, M. Yalniz, S. Celebi, A. Erensoy, and B. Ustundag, "The levels of ghrelin, leptin, TNF-alpha, and IL-6 in liver cirrhosis and hepatocellular carcinoma due to HBV and HDV infection," *Mediators Inflamm.*, vol. 2006, p. 78380, 2006.

[26] L. Bai, G. Mao, and C. Cao, "Effects of inflammatory cytokines on the recurrence of liver cancer after an apparently curative operation," *J Dig Dis.*, vol. 8, pp. 154–9, 2007.

[27] L. Giannitrapani, M. Cervello, M. Soresi, M. Notarbartolo, M. La Rosa, L. Virruso, N. D'Alessandro, and G. Montalto, "Circulating IL-6 and sIL-6R in patients with hepatocellular carcinoma," *Ann NY Acad Sci.*, vol. 963, pp. 46–52, 2002.

[28] C. Hsia, T. Huo, S. Chiang, M. Lu, C. Sun, J. Wu, P. Lee, C. Chi, W. Lui, and S. Lee, "Evaluation of interleukin-6, interleukin-10 and human hepatocyte growth factor as tumor markers for hepatocellular carcinoma," *Eur J Surg Oncol.*, vol. 33, pp. 208–12, 2007.

[29] C. Porta, M. De Amici, S. Quaglini, C. Paglino, F. Tagliani, A. Boncimino, R. Moratti, and G. Corazza, "Circulating interleukin-6 as a tumor marker for hepatocellular carcinoma," *Ann Oncol.*, vol. 19, pp. 353–8, 2008.

[30] M. Soresi, L. Giannitrapani, F. D'Antona, A. Florena, E. La Spada, A. Terranova, M. Cervello, N. D'Alessandro, and G. Montalto, "Interleukin-6 and its soluble receptor in patients with liver cirrhosis and hepatocellular carcinoma," *World J Gastroenterol.*, vol. 12, pp. 2563–8, 2006.

[31] H. Song le, V. Binh, D. Duy, J. Kun, T. Bock, P. Kremsner, and A. Luty, "Serum cytokine profiles associated with clinical presentation in vietnamese infected with hepatitis B virus," *J Clin Virol.*, vol. 28, pp. 93–103, 2003.

[32] K. Chijiiwa, S. Saiki, and M. Tanaka, "Serum interleukin-6 and hepatocyte growth factor levels in patients after hepatectomy," *Hepatogastroenterology*, vol. 49, pp. 467–71, 2002.

[33] D. Moran, N. Mayes, L. Koniaris, P. Cahill, and I. McKillop, "Interleukin-6 inhibits cell proliferation in a rat model of hepatocellular carcinoma," *Liver Int.*, vol. 25, pp. 445–57, 2005.

[34] D. Moran, M. Mattocks, P. Cahill, L. Koniaris, and I. McKillop, "Interleukin-6 mediates G(0)/G(1) growth arrest in hepatocellular carcinoma through a STAT 3-dependent pathway," *J Surg Res.*, vol. 147, pp. 23–33, 2008.