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# Evaluation of alpha-fetoprotein in patient with chronic hepatitis C infection by using ELFA technology

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## Abstract

Hepatitis known as inflammation of liver is caused by some known viruses, alcohol, drugs and some other infection. Most common hepatitis infections in humans are Hepatitis A, B and C. Chronic HCV infection may lead to liver cirrhosis and Hepatocellular carcinoma (HCC). Alfa feto protein's concentration in patient's serum is useful utility to diagnose HCC. The current study was conducted at Genome Research Centre Lahore for the measurement of AFP concentration in HCV positive patients of Punjab and Khyber Paktun Khwa (KPK) Province of Pakistan. Total of 172 patients, atleast 60 from each province were screened for Alfa feto protein concentration in their blood by using modern ELFA technology that is most rapid, specific and sensitive method. Results show high percentage rate of HCC in Punjab as compared to KPK Province. Age wise Results analyse that age group >60 years show very high percentage rate of HCC and in male patients s compared to females. It is concluded that due to prolong HCV infection, patients of age group >60 are at high risk of liver cirrhosis and HCC. It is also concluded that the concentration of AFP in patient's blood is useful test for diagnosis of HCC and to monitor the patient's condition.

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## Introduction

Hepatitis is referred to as inflammation of liver that is characterized by the presence of inflammatory cells in the tissues of the organ (Ryder and Beckingham, 2001). Hepatitis may be cause by a group of viruses known as hepatitis viruses (A, B, C, D, E, G) but it may also occur due to alcohol, drugs and other infections(Ahmedin *et al.,* 2004).

Hepatitis C, a member of family Flaviviridae can cause severe infection in Humans, first time identified in 1989 by Choo and his co-workers (Choo *et al.,* 1989). HCV is transmitted through blood or contaminated blood products. Contaminated surgical instruments, sharing of contaminated razor, sexual contact with infected person are also major routes of its transmission (Perrillo, 1990). HBV is transmitted to healthy population through blood or various body fluids i.e. saliva, semen, or vaginal fluid) (Ahmad *et al.,* 2006). Chronic hepatitis B and C infection is major cause of Hepatocellular carcinoma (*HCC) so* when screening for HCC is being done, these factors should be considered. According to guidelines of the American association for the Study of Liver Diseases (AASLD),concentration of alpha-fetoprotein (AFP)>200 ng/mlin serum, acts as marker for diagnosis of Hepatocellular carcinoma (*HCC) (*Beasley *et al.,* 1981; Davila JA, 2004; Sherman M, 2001; Tsukuma *et al*., 1993).

In Humans, Alpha-fetoprotein is encoded by AFP gene that is present on the q arm of chromosome (4). In fatal development stage, AFP is produced by liver and yolk sac. It is also produced in different types of tumours i.e. hepatoblastoma, germ cell tumors of the ovary, nonseminomatous and hepatocellular carcinoma. Different studies from all over the world show that 70% of the patients with high AFP levels in their body represent Hepatocellular carcinoma (HCC). Evaluated AFP concertation was also observed in serum of patients with severe liver disease i.e. viral hepatitis and cirrhosis (Daniele *et al* 2004; Isselbacher and Dienstag, 1998).

Few number of studies based on limited samples were available from Pakistan. So the current study was conducted to evaluate serum Alfa Feto Protein in HCV positive patients from Punjab and Khyber Paktoon Khwa province of Pakistan.

## Materials and methods

*Source of clinical samples*

Blood samples were collected along with specifically designed data sheets from patients admitted/attending various tertiary collection centres/hospitals situated in different areas of KPK and Punjab province to evaluate AFP value in HCV positive patients. All the samples were centrifuged at 4000 rpm and serum was separated. All the research work described below was conducted at Genome Centre for Molecular Based Diagnostics & Research (GCMBDR) Lahore.

*Study design*

Current research study was designed to analyse the frequency of evaluated APF in HCV positive patients.

*Inclusion criteria*

Only those patients were selected in the present study that were found positive for HCV RNA by real time PCR Method.

*Exclusion criteria*

Haemolysed samples and samples low in quantity were excluded from the study; also those samples whose demographics were unknown were excluded from final analysis of data. In addition the samples which were co-infected with HCV and HBV were also excluded from the study.

*Method*

VIDAS BIOMERIEUX (Biomérieux, Inc. Durham, NC 27712 U.S.A) with kits from bioMérieux France is used to detect AFP level in serum samples of the HCV Positive patients. Vidas is a Compact Automated Immunoassay Analyzer that has become a reference in the immunoassay field. The unique design virtually ensures the robustness of the system and prevents cross-contamination in particular with analyses

which require very low limits of detection. VIDAS® assays offer a rapid, automated and reliable solution to help clinicians enhance patient care. The VIDAS system work on ELFA assay principle, combining the ELISA test method with a final blue fluorescent reading. Vidasis more specific and sensitive technology.

*Statistical analysis*

Statistical analysis was performed by using Statistix 9 version software. P-value <0.05 was considered as significant. One way ANOVA test was performed relationship amongst the categorical parameters.

**Results**

Punjab and KPK province of Pakistan were selected in the present research work (Table 1). From each Province equal number of patient i.e. 86 from Punjab and 86 from KPK were observed for the concentration of AFP in their serum. In Punjab province, of the total 68 patients 80.2 %( n=69) were found with normal AFP concentration in their serum, 3.48% (n=3) with moderate high while 16.2 % (n=14) with very high concentration of AFP. In KPK Province, of the total 86 patients, 84.8% were observed with normal, (5.81) with moderate high and 9.30% with very high concentration of AFP levels in their serum samples.

**Table 1.** Frequency of AFP level in HCV positive patients of KPK & Punjab.

| AFP Level | PUNJAB | KPK | P-VALUE |
|---|---|---|---|
| 0.1-10 ng/ml (Normal) | 69 (80.2%) | 73(84.8%) | 0.0000 |
| 11-400 ng/ml (Moderate High) | 3(3.48%) | 5(5.81%) | Significant |
| >400 ng/ml ( Very High) | 14(16.2%) | 8(9.30%) | |
| TOTAL | 86 | 86 | |

For evaluation of AFP level, total 172 patients were analysed (Table 2) in which 77 male and 95 Female patients were included. It was observed that out of the total HCV infected patient with a viral load >100000 IU/ml, 82.5 % (n=142) HCV positive patient show normal AFP level, 4.65 (n=8) % patients show moderate high, and 12.7 % (n=22) patients were found with high concentration of AFP in their serum.

Age wise frequency of AFP were analysed in table 3. It was observed that in age group >60 years, rate of evaluated AFP level was very high as compare to younger due to chronic HCV infection.

**Discussion**

In certain areas of the world Hepatocellular carcinoma is count to be major cause of tumours, and every year 0.25 million new cases are reported globally (Daniele *et al*., 2004). In Asia and Africa regions approximately 20-30 % cases of Hepatocellular carcinoma are reported annually. Prevalence rate and etiological factor of HCC differ in various regions of the world but many studies have investigated that some viruses, certain toxin and alcohol have effect on this malignancy (Isselbacher and Dienstag, 1998; Benvegnu *et al*., 1994).

**Table 2.** Frequency of AFP concentration in both sexes.

| AFP | MALE | FEMALE | n (%) | P Value |
|---|---|---|---|---|
| 0.1-10 ng/ml (Normal) | 60(77.9%) | 82 (86.31%) | 142(82.5) | 0.0589 non-significant |
| 11-400 ng/ml (Moderate High) | 04(5.19%) | 04(4.21%) | 08 (4.65) | |
| >400 ng/ml ( Very High) | 12(15.5%) | 10(10.5%) | 22 (12.7) | |
| TOTAL | 77 | 95 | 172 | |

Now a days it is well documented that measurement of Alfa feto protein in blood samples is beneficial test for oncologists, physicians and clinicians to manage patients of hepatocellular carcinoma, hepatic infection and cancers of pancreas and germ cell (Abelev GI and Lazarevich NL, 1996). Serum Alfa feto protein is also useful for monitoring of treatment response.

Screening of AFP in blood is recommended for monitoring of chemotherapy and surgery in GCT, Hb and HCC. Most common clinical utility of AFP, is its utilisation for surveillance of HCC in patients with chronic viral hepatitis (HCV, HBV) or risk factor of liver cirrhosis (Johnson PJ, 2001; Engelhardt *et al*., 1973).

Several research works from various countries analysed that in HCV and HBV positive patients, chances of HCC are 20 times greater than individuals and concentration of Alfa feto protein in these infected individuals is more than 400-500 ng/ml. From some research studies it was observed that AFP concentration in serum of HBs Ag positive patients is lower than Anti-HCV positive patient and nowadays role of HCV oncogenesis in Hepatocellular carcinoma induction have been recognized (Davila *et al*., 2004; Daniele *et al*., 2004).

**Table 3.** Frequency of AFP concentration in different age groups.

| AFP Level | 0.1-10 ng/ml (Normal) | 11-400 ng/ml (Moderate High) | >400 ng/ml ( Very High) |
|---|---|---|---|
| 10-30 YEARS | 49(34.5%) | 00 | 01(4.54%) |
| 31-60 YEARS | 62(43.66%) | 01(12.5%) | 07 (31.81%) |
| >60 YEARS | 31(21.83%) | 07(87.5%) | 14 (63.63)% |
| TOTAL | 142(82.5%) | 08(2.32%) | 22(12.7%) |
| P-VALUE | 0.0029 (significant) | | |

In the current study we analysed AFP as a tumour marker in HCV positive patients. Patients from Largest provinces of Pakistan and from Khyber Paktoon khwa were selected for screening of AFP. In Punjab, patients with evaluated AFP level were significantly high as compared to Khyber Paktoon Khwa. Some of the patients were found with very high AFP level i.e. >48484.0 ng/ml that represent Hepatocellular carcinoma. In other patients, AFP levels are between 11-400 ng/ml which show initial stages of HCC and AFP testing are very useful to monitoring of the patients.

In a research study conducted at north Indian analysed that 65% of the patients with HCC were found with high AFP Level (Francioni S, Pastore M, 1989). In another study carried out at north India it is observed that 47.4% of HCV positive patient were found with elevated AFP levels(Li P *et al*, 2011). In study from Pakistan baig *et al* shows that 30.8 % of HCV positive patients were found with high AFP level in their blood. These studies shows correlation with our studies.

In Male and female patients, male patients were observed with high AFP levels than female patients. In male patients 15.58% were found with high AFP level, 5.19% with moderate AFP level and 77.9% of the patients had normal AFP level without HCC.

When we analysed female patients it shows that 82.5% of the patients were present in normal AFP level category, 4.21 % in moderate high while only 10.52% patients were in very high AFP category.

Age wise presentation of the patients show that patients of age group >60 years were at very high risk of HCC because of prolong HCV infection.

Most important observation of the current study is that in this study Enzyme linked Fluorescent Assay (ELFA) technology was used that is most rapid, specific and sensitive method than other methods like ELISA and Chemiluminescence immunoassay.

Prospective research studies need to be performed in order to corroborate our findings and to perform a cost-benefit analysis of AFP test. In future it is necessary to perform different studies to evaluate AFP level in HCV positive patients because if we consider that approximately 5% of HCV positive patients develop Liver cirrhosis that may be lead to HCC within a year. So it is very beneficial test to monitor HCV positive patients and to prevent patient's liver from Hepatocellular carcinoma.

## Conclusion

From the current study it is concluded that AFP level in serum is a useful tool for detection of Hepatocellular Carcinoma. In addition to AFP test liver histology should be performed for confirmation of Hepatocellular carcinoma. It is also concluded that HCC is most common in age group >60 years.

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## References

**Abelev GI, Lazarevich NL.** 1996. Alpha-fetoprotein (AFP): solved and unsolved problems. McGill journal of medicine **2,** 127–34.

**Ahmad I, Khan SB, Rahman HU, Khan MH, Anwar S.** 2006. Frequency of Hepatitis B and Hepatitis C among cataract patients. Gomal Journal of Medical Science **4,** 61–64.

**Ahmedin J, Taylor M, Ram CT.** 2004. A New Section in Cancer Offering Timely and Targeted information. Can J Clin. **54,** 23–25.

**Beasley RP, Hwang LY, Lin CC, Chien CS.** 1981. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet **2,** 1129-33.

**Benvegnu L, Fatovich G, Noveta F.** 1994. Co-current hepatitis B and C virus infection and risk of HCC cirrhosis. Cancer **74,** 2442-7.

**Daniele B, Bencivenga A, Megna AS, Tinessa V.** 2004. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. Gastroenterology **127,** 108-12.

**Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB.** 2004. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology **127,** 1372-80.

**Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB.** 2004. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a papulation based study. Gastroenterology **127,** 1372-80.

**Engelhardt NV, Poltoranina VS, Yazova AK.** 1937. Localization of alpha-fetoprotein in transplantable murine teratocarcinomas. International Journal of Cancer **11,** 448–5.

**Isselbacher K, Dienstag J.** 1998. Tumors of the liver and biliary tract: principles of internal medicine. Fauci Braunwald Wilson *et al* (Eds 14th), Mc Graw Hill Companies, Italia, part six 578-80.

**Johnson PJ.** 2001. The role serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clinics in Liver Disease **5(1),** 145–59. 15.

**Choo QL.** 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science **244,** 359–362.

**Perrillo RP.** 1990. Factors influencing response to interferon in chronic hepatitis B: implications for Asian and western populations. Herpetology **12,** 1433–5.

**Ryder S, Beckingham I.** 2001. ABC of diseases of liver, pancreas, and biliary system: Acute hepatitis. British Medical Journal **322,** 151–153.

**Sherman M.** 2001. Alphafetoprotein: an obituary. Journal of Hepatology **34,** 603-5.

**Tsukuma H.** 1993. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N England Journal of Medicine **328,** 1797-801.

**Francioni S, Pastore M.** 1989. Alpha-fetoprotein and acute viral hepatitis type B. The J nuclear medicine and allied sciences **33,** 103-6.

**Li P, Wang SS, Liu H, Li N, McNutt MA, Li G.** Elevated serum alpha fetoprotein levels promote pathological progression of hepatocellular carcinoma. World J Gastroenterol 2011; **17,** 4563-71.

**Baig.** 2009. Hepatocellular carcinoma (HCC) and Diagnostic significance of α -fetoprotein (AFP). J Ayub Med Coll Abbottabad, **21(1),** 72-75.