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EVALUATING THE SERUM MARKERS FOR EARLY DIAGNOSIS OF HCV INDUCED HEPATOCELLULAR CARCINOMA

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ABSTRACT

Hepatocellular carcinoma is a global public health problem which is intensified at developing countries due to lack of early diagnosis. Since Hepatitis C virus related morbidity accounts five million people in Pakistan, rate of morbidity and mortality of HCV related Hepatocellular carcinoma infections is high in Pakistan, and it is attributed to its diagnosis after its development. The public has the lack of awareness about the importance and benefits of liver biopsy testing; therefore, improved, and non-invasive methods of HCC diagnosis are urgently needed. The aim of the present study was to evaluate the importance of serum markers in early diagnosis of HCV induced Hepatocellular carcinoma and to develop a robust set of serological markers for their early detection and diagnosis. A total of 60 HCV positive patients including Chronic Liver disease (n=20), Cirrhosis (n=20), HCC patients (n=20) and 20 healthy volunteers (n=20) were enrolled in this study after an extensive screening process. Blood samples were collected from all the subjects and processed for serum separation followed by immunological, molecular, and biochemical analysis using Enzyme Linked Immunosorbant Assay, Real-Time PCR, complete blood picture, and a range of biochemical tests. Results showed gender wise variability among the subjects from each of the study groups where in the male subjects remained dominant with male and female ratio as 61.75:38.75. The average age of male subjects was observed between 42y, 43y, 49y and 37 years in comparison of female subjects who were 46y, 53y, 50y and 47y in CLD, cirrhosis, Hepatocellular carcinoma, and control groups, respectively. The data has also demonstrated that Hepatocellular carcinoma patients having Alpha Fetoprotein level >1000 showed the increased level of ALP and GGT with decreased HB and HCT. However, the Hepatocellular carcinoma patients having AFP level in the range of 100-500 had total bilirubin level > 3.0, while those having AFP level <100 has all serological markers in normal range. In addition, the patients of HCC showed increased PT with decreased albumin level. The increases level of albumin level (>3-<4.0) was observed from the serum samples of cirrhosis and Chronic liver disease patients. The increase in albumin up to 4.0 accompanied with the increased level of ALP and PT while decreased HB. In conclusion, different biochemical parameters may be used to distinguish the patients having Hepatocellular carcinoma from those suffering from cirrhosis and chronic liver disease induced by HCV infection. The findings of present study may help to enhance the outcome for patients with HCC by enabling the diagnosis to be made at an earlier stage of the disease.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) has been placed as sixth common cancer type and categorized in first three cancer causing disease that is fatal and leads to death in a short time span.

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The estimated mortality rate associated with hepatitis is 0.5 million persons per year (Graham & Swan, 2015; Lozano et al., 2012). The reason of such high mortality and morbidity is that HCV infection does not show prominent signs and symptoms at an early stage (Blackard, Shata, Shire, & Sherman, 2008; Chung, 2005). Chronic hepatitis results in wound healing process that causes fibrosis in liver and

ultimately the development of cirrhosis. The main cause of cirrhosis due to liver fibrosis is hepatitis C virus (HCV), that may develop into hepatocellular carcinoma (HCC), which is called as HCV induced HCC and that occurs with only 7% survival rate. HCV is a main cause of hepatic malignancy that leads to many deaths worldwide. HCV is categorized into six genotypes which help in therapeutically and epidemiological information (Afridi et al., 2009). Reports show that HCV induced non cirrhotic liver may develop into HCC in up to 20% of cases, but the process of hepato-carcinogenesis still remains a question (Alkofer, Lepennec, & Chiche, 2011; Bralet et al., 2000). Prolonged infection with HCV results in CLD, liver cirrhosis, necrosis, that finally terminated in hepatic failure (Yilma, Saxena, & Mehta, 2022).

HCC is reported as the most common type of cancer, worldwide (Looi et al., 2008). HCC has been placed as sixth common cancer type and categorized in first three cancer causing disease that is fatal and leads to death in a short time span (Chidambaranathan-Reghupaty, Fisher, & Sarkar, 2021; Looi et al., 2008). Unhygienic conditions, improper screening of tests, poor knowledge are the factors that aids in the development of HCC at advanced stage that results in poor prognosis of disease and ultimately high mortality. Many studies have shown that in HCV and HBV infection, the presence of aflatoxin in dietary products and alcohol consumption were the main causative factors for liver cancer (Thorgeirsson & Grisham, 2002). In Pakistan, HCV was found as the main factor responsible for the development of liver cancer (Khan et al., 2009), and about 70% patients of HCC were found infected with HCV whereas HBV co infection has been found in 20% of HCC positive cases (Butt et al., 2013). In Pakistan, the prevalence of hepatic inflammation is alarming showing 4.8% positivity for anti HCV antibodies and for 2.5% HBV surface antigen with (Abbas, Jafri, & Hamid, 2010; Bahadar, Khan, Israr, & Ahmad, 2016). The prolonged untreated infection leads to the development of chronic liver disease that gradually progresses to fibrotic stage, cirrhosis, hepatic damage and finally HCC (Hajarizadeh, Grebely, & Dore, 2013).

The most common serum protein in human is albumin with normal range of 3.1 – 6.0 gm/dl of serum. The overall picture of serum proteins can be drawn by the assessment of the total serum protein which should be in range 5.1 – 9.5 (g/dl) in the serum of a healthy person. Regarding assessment of various clinical illnesses, it is observed that the serum proteins electrophoresis plays a role of initial medical diagnosis. On the basis of biochemical properties, the proteins are differentiated by protein electrophoresis. The SDS-PAGE assessment shows that the albumin as well as globulins dominates the serum protein electrophoresis. The globulins make a minimum portion of proteins in serum with normal range of 1.5 – 4.8 gms/dl. The expression of serum proteins remains variable in electrophoresis based on the disease condition as well as physiological disorders of the patient (Ravel, 1995). Albumin and globulins are two known dominated serum proteins manufactured by the liver. Consequently, the accurate running of kidneys as well liver can be indicated by the values of Albumin protein. The greater levels of globulin and/or albumin can be the signs of different body functioning disorders and infections including leukemia, myeloma, hemolytic anemia, hepatitis, tuberculosis, lymphoma and alcoholism and macroglobulinemia while the values less than normal values may be signs of immune diseases, malnutrition and kidney diseases (Ravel, 1995).

The diagnosis of HCV is usually done by the serological and molecular techniques via the detection of the virus antibodies and nucleic acid in the serum of human, respectively. Serological testing is cost effective and is used to detect antibodies made by body against the infection of virus. It is less sensitive and reliable because the titer of antibodies in human serum changes from time to time, therefore, the accuracy of the testing may be challenged (J.-M. Pawlotsky et al., 2000). The serological testing for HCV involves Enzyme linked immunosorbent assay (ELISA) which is an immunological assay. ELISA antibodies from human serum after four to ten weeks of the infection. The limitations with respect to diagnosis of HCV by the immunological assays (e.g ELISA) occur in case of co-infection in which the immune system of the patient is usually suppressed.

Therefore, false negative results may occur (J. M. Pawlotsky, 2002) Therefore, in addition to serological tests, the molecular tests, such as PCR is required as confirmatory test for HCV in human patients including immunosuppressive individuals, co-infected with HIV, and/or alcoholics. qPCR test is a quantitative test and determine the viral load of HCV in human serum (Dienstag, 2002). However, the detection of viral genotypes is done using qualitative PCR. HCV genotypes may be detected by using ELISA technique but the reliability is still a question (Heydtmann, Shields, McCaughan, & Adams, 2001). Due to lack of sensitive and specific diagnostics for the detection of HCC at an early stage, there is growing interest and need to develop novel HCC serum markers with greater sensitivity and specificity. The present study was carried out to investigate HCV induced HCC by molecular, biochemical and immunological methods to make a consensus about the reliability and sensitivity of these diagnostics test for the patients of Sindh, Pakistan and the efforts were made to identify the protein markers that could be used for early detection and diagnosis of HCV induced HCC. Therefore, the aim of present study was to investigate the serum markers for their role in early diagnosis of HCV induced HCC at Hyderabad, Sindh.

2. MATERIALS AND METHODS

Study design, location and population

This was a prospective cross-sectional study carried out from February 2017 to January 2018 at Hyderabad, Sindh Pakistan. The present study included the samples collected across various cities of Sindh. The subjects from different cities across the Sindh, who were attending the Civil Hospital Hyderabad and Asian Institute of Medical Sciences were enrolled. Information regarding age, gender, ethnic group, birthplace, medication, and history of illness were from each participating patient, with their consent.

A total of eighty blood samples belonging to four groups i.e., CLD (*n*=20), Cirrhosis patients (*n*=20), HCC patients (*n*=20) confirmed known cases and healthy volunteers (*n*=20) were enrolled in this study. All blood samples were subjected for serum separation followed by immunological, molecular, and biochemical analysis using ELISA, Real-Time PCR, and range of biochemical tests. Samples were collected in three types of tubes i.e. Gel tube, EDTA and sodium citrate containing tubes. Gel tubes were used for biochemical testing, EDTA containing blood samples were used for HB/HCT whereas the Sodium containing blood samples were used for Prothrombin time. Samples obtained from healthy volunteers were initially tested for anti-HCV antibodies by ELISA method. Samples collected from CLD, Cirrhosis and HCC were subjected to real time PCR analysis for quantitative detection of HCV. Blood samples were used for separation of serum using BD Vacutainer tubes followed by centrifugation. at 4000 rpm for 10 minutes. the separated serum was collected in fresh eppendorff tube. Samples were either used immediately or stored at -20ºC for future use.

Enzyme linked Immunosorbant Assay

Anti-HCV antibodies were detected using 3rd generation ELISA by using Bio Elisa HCV 4.0 kit as per manufacturer's guidelines. Briefly, the micro plates were coated with recombinant antigens representing epitopes of HCV: Core, NS3, NS4 and NS5.The test serum samples obtained from patients were added to the wells of micro plate. After incubation, the micro plate wells were washed twice with buffer and added the secondary antibody i.e. rabbit anti-human IgG conjugated with peroxidase. Following incubation, the micro plate was washed three time to remove any unbound antibodies or non-specifically bound to the plate. The wells were then added with chromogenic enzyme substrate solution for development of a colour. In case of appearance of blue colour, the result was considered positive while colourless reaction indicated the negative result of the tested sample.

Molecular detection of HCV using Real Time PCR

QIAamp Viral RNA Mini Kit (Qiagen) was used as per protocol supplied by manufacturer, for Viral RNA preparation and subsequent detection of HCV from human serum samples. The kit comprises of the selective binding properties of a silica- based membrane with the speed of microspin or vacuum technology and is highly useful for simultaneous processing of multiple samples. The sample was first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions were then adjusted to provide optimum binding of the RNA to the QIAamp membrane, and the sample was loaded onto the QIAamp Mini spin column. The RNA attached to the membrane, and contaminants were washed away in two steps using two different wash buffers. High-quality RNA (free of proteins, nucleases) was then eluted in a RNase-free buffer, which was either used directly or stored.

Investigation of biochemical markers

The blood samples of all four different categories were subjected to various biochemical tests in order to determine the variation in patient's serum profile. All samples were tested for total bilirubin (TBil), Alanine transaminase (ALT), Gama glutamyle transaminase (GGT), Alkaline phosphatise (ALP), Creatinine, HB/HCT, Albumin, Alfa feto protein, prothrombin time (PT) as per the manufacturer's instructions. Clinical chemistry tests were applied by an automatic biochemical analyzer (AU5400, Olympus, Japan). HB/ HCT was detected using Hematology Analyzer (XE-1800, SYSMEX, Japan). PT was measured in automated coagulation instrument (CA-7000, SYSMEX, Japan). AFP was measured by Automated Immunoassay Analyzer (COBAS6000, ROCHE, Switzerland).

3. RESULTS AND DISCUSSION

The present study focused on investigation of serum markers for early detection of HCV induced HCC. Four groups of individuals belonging to CLD, Cirrhosis, HCC patients and healthy individuals' categories were analyzed for variation in serological markers to assist in diagnosis of HCC at early stage of development. Among all the samples collected from different categories of patients, 63.16 % were males and 36.84% females. Comparative analysis of gender-wise study of all the four groups showed dominancy of male participant. The highest ratio of males was seen in CLD group. Average age of CLD, Cirrhotic, HCC patients and healthy individuals was 42, 48, 49 and 41 years respectively, while the male and female ratio was 72:28%, 55:45%, 60:40% and 64:36% respectively. The ratio of non-symptomatic v/s symptomatic 55: 45%, 33:67%, 20:80%. With no symptoms in healthy individuals respectively. The true prevalence data for HCV could not be ascertained because the most studies are

hospital based with small sample size. However, the highest prevalence was recorded in Hyderabad whereas the lowest prevalence was recorded in Mirpur Khas.

Gender wise distribution of all participants of this study

Data analysis showed that 61.25% (n=49) of the subjects were male individuals while 38.75% (n=31) were females individuals. The gender wise variability was observed among the subjects from each of the four groups of the present study. The male subjects remained dominant in CLD group with 16.25%, Cirrhosis group with 13.75%, HCC group with 15 % and control group with 16.25% with average age 42y, 43y, 49y and 37y respectively in comparison of female subjects who were 8.75% in CLD, 11.25% in Cirrhosis group 10% in HCC while 8.75% in control group with average age 46y, 53y, 50y and 47y respectively (Fig. 1).

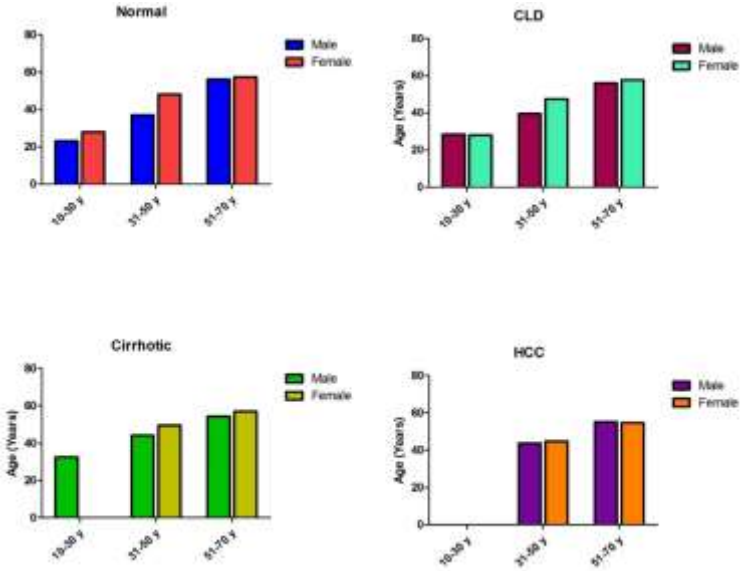

Figure 1. Bar graph showing the gender wise distribution of subjects of all groups of the present study (the mean age of male and female in each group is presented in the graph).

Analysis of the biochemical serological markers

Serum samples from the subjects of the four groups comprising three groups of patients (CLD, Cirrhosis, HCC) and one control group (healthy volunteers) were analyzed for the variations in their serological parameters (Hematological, biochemical, molecular), in order to assist in the diagnosis of HCC at the early stage of the development of deadly disease, HCC. Further analysis showed that in CLD group male and female ratio was 65%:35% while in cirrhosis group, male and female ratio was 55%:45% which different from that of observed from CLD group. Similarly, the analysis of HCC patients showed male and female ratio 60%:40%. The gender wise analysis of control group showed male and female ratio 65%:35%. The comparative analysis of gender wise distribution of all the four groups included in the present study showed dominancy of male participant with the highest ratio seen in CLD group. However, in CLD patients and healthy volunteers the ratio of male was increased up to the age of 50 years as compared to females. All three groups except healthy volunteers (control) shows highest ratio of male at age group more than 30 years as described in next section.

The average HB value obtained from control group was 12.91 ± 1.13 g/dl. However, the subjects from CLD showed 11.96 ± 1.73 g/dl average value of HB which seems lower than the values obtained from the subjects of control group. Similarly, average HB level was much lower in the blood samples of cirrhotic and HCC patients with average values of 10.86 ± 1.44 g/dl and 9.89 ± 2.15 g/dl, respectively (Fig. 2A). The Hematocrit analysis of the four groups, revealed that the average value obtained from normal group was 38.8 ± 4.72 %. However, CLD patients showed 34.88 ±5.86 % which seems lower than the value obtained from normal group of this study. Similarly, Hematocrit level was much lower in cirrhotic and HCC patients with average values of 32.62 ± 5.93 % and 29.13 ± 4.50 %, respectively and it does not fall into the standard normal percentage for this parameter (Fig. 2B). Total bilirubin is a parameter which has got standard range 0.4-0.9 mg/dl. In present study, among the four groups, average value obtained from normal group was 0.6 ± 0.3 mg/dl. However, CLD patients showed 1.40± 0.61 mg/dl which seems higher than the value obtained from normal group of the present study. Cirrhotic group showed 2.36 ± 1.71 mg/dl which was relatively much higher than the values obtained from the CLD group and also does not fall into the standard normal range for this parameter. Similarly, total bilirubin was also higher in HCC group of the present study with average values of 2.81 ± 2.14 mg /dl (Fig. 2C). SGPT is a parameter which has standard value up to 45 U/l. Data showed that the average value obtained from the serum samples taken from the subjects of control (normal) group was 32.15 ± 10.61 U/l whereas 45.85 ± 14.67 U/l from CLD which seems higher than the former. Similarly, cirrhosis patients showed average 52.2 ± 20.43 U/l was observed from cirrhosis group which are also much higher than the values obtained from the normal group and does not fall into the standard normal range for this parameter. In the same manner, SGPT level was much higher in HCC group with average value of 57.25 ± 29.90 U/l (Fig. 2D).

Alkaline phosphatase is a parameter which has standard range 100-290 U/l. In the present study, among the four groups, average value obtained from normal group was 124.20 ± 13.87 U/l. However, CLD patients showed 136.75 ± 26.76 U/l which seems higher than the value obtained from normal group of this study (Fig. 3A). Similarly, GGT level was much higher in cirrhotic and HCC patients with average values of 139.2 ± 31.37 U/l and 148.5 ± 30.44 U/l, respectively and does not fall into the standard normal range

for this parameter (Fig. 3B). GGT is a parameter which has standard value up to 55 U/l. In the present study, among the four groups, average value obtained from normal group was 32.8 ± 9.7 U/l. However, CLD patients showed 41.35 ± 13.9 U/l which seems higher than the value obtained from normal group of this study.

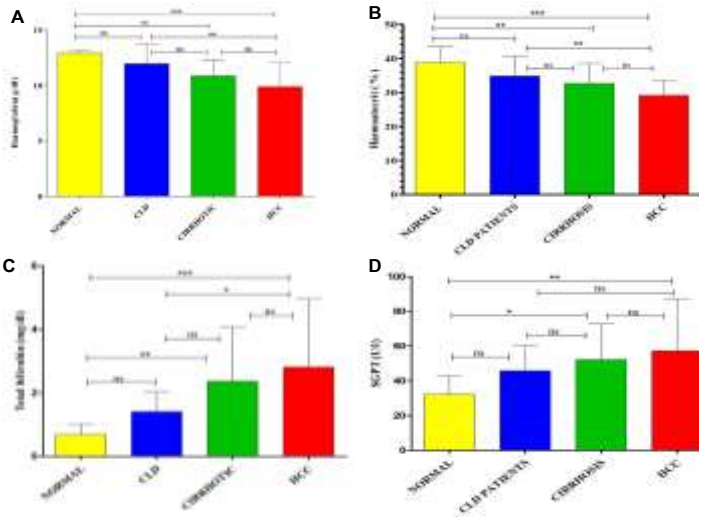

Figure 2. Graph showing the analysis of A) hemoglobin level B) hematocrit level C) serum total bilirubin level D) serum SGPT level in normal, chronic liver disease, cirrhosis, and hepatocellular carcinoma groups. The data is representative of 20 independent individuals of each group (*p< 0.05, ** p <0.01 and *** p< 0.001), ns= non-significant.

Similarly, GGT level was much higher in cirrhotic and HCC patients with average values of 67.35 ± 13.07 U/l and 106.2 ± 11.81 U/l respectively and also does not fall into the standard normal percentage for this parameter (Fig. 3B). Albumin is a parameter which has standard range 3.5-5.5 g/dl. In the present study, among the four groups, average value obtained from normal group was 4.550 ± 0.68 g/dl. However, CLD patients showed 4.52 ± 0.85 g/dl which seems lower than the value obtained from normal group of this study. Similarly, albumin level was much lower in cirrhosis and HCC group that showed average value of 3.63 ± 1.14 g/dl and 3.43 ±1.01 g/dl respectively, which was much lower than the values obtained from the normal group and also does not fall into the standard normal range for this parameter (Fig. 3C). Creatinine is a parameter which has got standard range 0.3-1.0 mg/dl. In present study, the average value obtained from the normal group was 0.63 ± 0.26 mg/dl. However, higher average value of creatinine was observed from CLD, cirrhosis, and HCC groups with 0.87 ± 0.49 mg/dl, 1.31 ± 1.01 mg/dl and 1.62 ± 0.73 mg/dl respectively. All these values do not fall into the standard normal range for this parameter (Fig. 3D).

Prothrombin time is a parameter which has got standard range of 11-13 seconds. In present study, among the four groups, average time obtained from normal group was 12.75 ± 1.51 seconds. However, CLD patients showed 15.5 ± 3.76 seconds which seems higher than the value obtained from normal group of this study. Similarly, Prothrombin time was much higher in cirrhosis and HCC patients with average values of 18.0 ± 6.20 seconds and 20.20 ± 9.63 seconds respectively and also does not fall into the standard normal percentage for this parameter (Fig 4A). Alfa feto protein is a parameter which has got standard value <10ng/ml (Saini, Bhagat, Sharma, Duseja, & Chawla, 2006).

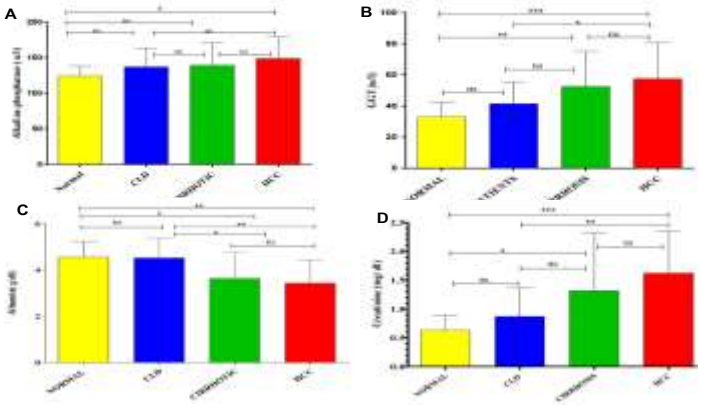

Figure 3. Graph showing the analysis of A) serum alkaline phosphatase level B) serum GGT level C) serum albumin level D) serum Creatinine level in normal, chronic liver disease, cirrhosis and hepatocellular carcinoma groups. The data is representative of 20 independent individuals of each group (*p< 0.05, ** p <0.01 and *** p< 0.001), ns= non-significant.

In present study, average value obtained from normal group was 3.65 ± 1.34ng/ml. However, CLD patients showed 4.70 ± 1.38ng /ml which seems fall in the value obtained from normal group of present study. Conversely, cirrhosis patients showed 308.13± 111.53ng/ml which was much higher than the values obtained from the normal group and does not fall into the standard normal range for this parameter. Similarly, Alfa feto protein level was much higher in HCC patients with average values of 395.51 ± 160ng/ml (Fig 4.14). Furthermore, analysis of serum alfa feto protein level showed statistically significant (P <0.001) in HCC and cirrhotic groups, whereas CLD group was statistically non-significant as compared with healthy group. Among all the groups, except healthy, the highest level of alfa feto protein was observed in HCC group that was statistically highly significant (P <0.001) with that of CLD group of the study. On the contrary, the HCC group showed statistically significant (P <0.05) with Cirrhotic Group The level of alfa feto protein observed in CLD group was statistically highly significant (P <0.001) with cirrhotic group of the present study (Fig 4B). The present study instigates the role of serological marker for diagnosis of HCV induced HCC at earlier stages. Four groups of individuals including patients

of chronic liver disease (CLD, Cirrhosis, hepatocellular carcinoma (HCC) and healthy volunteers (controls) were selected and enrolled in the present study. Except healthy volunteers, all other three groups of individuals were HCV positive. Serum markers were investigated by a wide range of biochemical tests including hemoglobin, hematocrit, Albumin, ALP, PT and AFP.

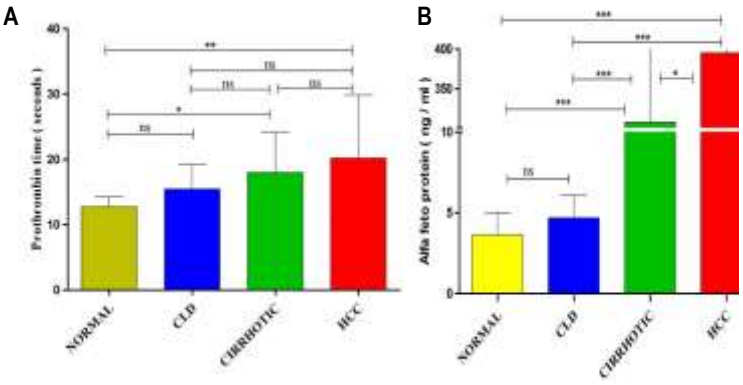

Figure 4. Graph showing the analysis of A) serum Prothrombin level B) Alfa feto proteins in normal, chronic liver disease, Cirrhosis, and hepatocellular carcinoma groups. The data is representative of 20 independent individuals of each group (*p< 0.05, ** p <0.01 and *** p< 0.001), ns= non-significant.

The data demonstrated that in all CLD patients, albumin level was >3.0 and the CLD patients with albumin <4.0 had increased level of ALP than normal mid limit. Increase in ALP was observed with increased PT and decreased HB. In cirrhotic group all patients showed albumin level >2.6. Cirrhotic patients with positive AFP level possessed Total bilirubin level >1.0 and was in decompensated stage. The HB value do not fall into the standard normal range. Among all groups except control (healthy), the highest level of HB was observed. The difference between the HB in CLD and cirrhosis patients was non-significant whereas it was significant between CLD and HCC patients (P <0.01). However, no significant variation in HB was observed between cirrhosis and HCC group of the study.

The analysis of hematocrit showed statistically significant variation in HCC, in cirrhotic group whereas, statistically non-significant in CLD group as compared with the healthy group. Among all groups except healthy, the highest level of hematocrit was observed in CLD group that was statistically non-significant with cirrhotic group and with HCC group. However, no significance in hematocrit level was observed between cirrhotic and HCC group of the study. However, HCC group showed lower mean hematocrit than cirrhotic group of the study. Serum Total bilirubin level analysis revealed that there was marked difference in HCC, (<0.01) in Cirrhotic group in comparison with control group. However, CLD group was statistically non-significant as compared with control group. Among all groups except healthy, the highest level of Total bilirubin was observed in HCC group that was statistically significant (P <0.05) with that of CLD and non-significant with cirrhotic group. Analysis of serum SGPT level showed significant variation in HCC (P<0.01), and in cirrhosis (P <0.05) groups non-significant in CLD group in comparison with control group. However, the average value of the CLD group was higher than the control group of this study. Among all the groups, except control, the highest level of SGPT level was observed. No significant correlation was found between CLD and cirrhosis, between cirrhosis and HCC, between CLD and HCC. However, the mean value was higher in cirrhotic group of the study. Furthermore, analysis of serum ALP level showed that in all the groups, except healthy, no significance was seen among all the three groups of the present study. However, the highest level of ALP was observed in HCC group.

The data analysis of serum GGT level showed that among all groups except healthy, the highest level of GGT was observed in HCC that was statistically significant (P <0.05) with that of CLD and non-significant with cirrhotic group of the study. The level of serum GGT in the CLD group showed non-significant with cirrhotic group of the study however the mean value was higher in cirrhotic group as compared to the CLD group of the study. The serum creatinine level was significantly different in HCC group, (P <0.05) in Cirrhotic as compared with control group. However, CLD group showed non-significant difference as compared with healthy group. Among all groups except healthy, the highest level of creatinine was observed in HCC that was statistically significant (P <0.01) with that of CLD group. However, statistically no significance was observed between CLD Vs cirrhosis and cirrhosis Vs HCC groups of the present study. Furthermore, the analysis of prothrombin time was statistically significant in HCC group (P <0.01), in cirrhotic (P <0.05) group whereas CLD groups was statistically non-significant as compared with healthy group of the present study. Among all the groups, except healthy, no significance was seen among all the three diseased groups of the present study. However, the highest prothrombin time was observed in HCC group of the present study.

4. CONCLUSION

The differentiating characters observed in cirrhotic group with that of CLD was elevation of AFP, Total bilirubin and PT with decrease in albumin and the formation of ascetic fluid in patients with AFP level increased. HB/HCT and SGPT were approximately equal in CLD and cirrhotic patients. Finally, the biochemical data of healthy controls was within normal range as compared with standard range. Overall male ratio was higher as compared to female. Males are more prone to disease due to high risk factors. There is

need to locate the disease at chronic stage so that it cannot progress to the symptomatic stage.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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