Original Article

Hepatitis Infections, Related Hepatic Disorders and Correlated Efficacy of Alpha-feto Protein Estimation

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ABSTRACT

The purpose of the presented study here was to evaluate the prevalence of hepatitis C and B infections in patients diagnosed with or suspected of hepatic carcinoma or any other co-morbid with the objective of correlating diagnostic efficacy of alpha-fetoprotein (AFP). The study covers the period of January 1998 to January 2008. Study requirements such as medical history, reports and related information of patients from both genders (n = 93) with 50 males and 43 females were gathered for analysis and assessment ensuring that diagnostic confirmation of HCC existed with lab test of alpha-feto proteins available. Results shows the distribution analysis of hepatitis infections, the related co-morbid, the high-level AFP results and positive hepatitis profile as; hepatic carcinoma with HCV origin 33.33% (n = 31) and with HBV origin 41.93% (n = 39). For HCC of cirrhotic origin only, 10.75% (n = 10) was noted with no-infection whereas HCC of chronic liver disease [CLD] was around 13.97% (n = 13). As per medical setting (e.g. cirrhosis and chronic liver disease) and hepatic infections (B and C), 50 males patients were segregated as carcinoma patients with HCV and HBV, n = 15 and 24, whereas with cirrhosis and CLD, 6 and 5 respectively. Forty three female patients were also segregated in similar manner, thus resulting in groups of N = 14, 17, 5 and 7, respectively for HCV and HBV related carcinoma and non-malignant co-morbid such as cirrhosis and chronic liver disease. Regarding AFP levels, in males, 10.00% (n = 10) patients have AFP in the highest range of > 300.00 to < 768.00 ng/ml in HCV group; 46.00% (n = 23) in the range of > 150 to < 300 ng/ml in HBV and cirrhosis groups and 8% (n = 4) in the range of >300 to <520 ng/ml in HBV alone group. In females, 20.90% (n = 9) showed AFP ranges between >300 to 612 ng/ml in HCV group; and

18.60% (n = 8) in range of >150 to <300 ng/ml in HBV and CLD groups. Nonetheless the present studies neither irrevocably instate direct correlation between presence of hepatitis infections and formation of HCC nor ascertain that hepatitis infection were the 'only' cause of HCC. However efficacy of AFP has been found to be significant as a diagnostic tool where HCC was related to the hepatitis infections. The analytical data which was assessed after combining all factors is demonstrated here according to medical conditions diagnosed, risk factors obtained and the etiological factors related to the carcinoma and alpha-feto protein concentration characteristics.

Keywords: HCC, AFP, Hepatitis, HBV, HCV, hepatoma, CLD, cirrhosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is known to develop as a result of continual exposure to risk factors such as hepatic C and B viral infections. The commonly stated development mechanism of HCC is the incorporation of viral nuclear material into the genomic components of infected patients, particularly hepatocytes.[1,2,3,4,5,6] Globally, it is now acknowledged that hepatic viral infections, especially B-virus and C-virus are pandemic estimating upto multiple million populous as carriers with 300,000 in USA alone per year. In addition, C-viral infection is a major cause of hepatic ailments across the world.[7,8] Around 150 thousands to 170 thousands new cases of hepatic infection of C-virus origin occurring annually in USA alone and approximately 2.7 million with chronic HCV world-over.[6,9] Present clinical scenario represent that hepatoma, also known as hepatocellular carcinoma is the most widespread and leading hepatic malignancy.[10] It has been reported that the predominance of HCC increased in the USA as well, thus accounts for growing number cancer-related death.[3,4,6,11]

Previous reviews and research relating to hepatitis infections confirms that prevalence of HCC is 3 to 7 cases per 100,000 per year in Americas, several parts of Europe, and Australia.[7,2,10,13,14,15,16] Furthermore, it was suggested that in population adjacent to the Mediterranean, around twenty cases of HCC per 100,000 annually has been detailed. Moreover, one fifty cases per 100,000, the highest as compared to others, were reported from several pacific and African countries.[7,15,17,18] Several African states too show considerable frequency of hepatitis infection and resultant hepatoma.[2,3] Regarding mostly considered basis and source, the large-scale assessment of distribution of HCC has sturdily been linked to the predominance of Hepatitis viral infectivity. As far as clinical setting is concerned, prognosis of subjects with hepatocellular carcinoma is allied with the extent of liver dysfunction, tumor size, and overall functional status.[6,11,19,20,21] In this regard, several significant studies, including three from own group, also have examined serum α-fetoprotein (AFP) as a modality for HCC surveillance as well as a prognostic factor for HCC.[2,10,13,15,20,21] These studies have shown that higher AFP levels at the time of HCC diagnosis are connected to worse fortitude.[22,23,20,24,21,25] As reported earlier,[6] after thorough assessment, evaluation and documented case studies,

AFP has been incorporated into at least three major staging and prognostic scoring systems of HCC: namely the "Italian Program for Liver Cancer" (CLIP), the "Treatment for Hepatocellular Carcinoma Group" (GRETCH), and the Chinese-University-Prognostic-Index (CUPI), as reported earlier.[14,26,27]

It has been comprehensively deliberated that the actual pathogenesis of HCC may vary even between high-incidence populations of HBV/HCV positive cases. Likewise, several established clinical and pathogenic differences exist between HBV and HCV-related HCC.[3,6,25] Keeping in view these noted observations and the fact that AFP is a reasonably priced, widely-existing, and an easily interpretable test, it is imperative that the efficacy of AFP in a hepatitis infected patients needs to be evaluated systematically nonetheless in selected population comprising both gender. Therefore the objective of study presented in this article is to evaluate the occurrence of hepatitis viral infections, namely C-virus and B-virus, in patients diagnosed with or suspected of hepatoma (hepatocellular carcinoma) and correlation of AFP efficacy with its diagnosis and prognosis.

MATERIALS AND METHODS

Study design and patient's selection

The research period was from January 1998 to January 2008. Patients' selections either from wards of OPD-clinics was performed as per description provided earlier,[10] inclusive of all lab results and hepatitis profiling. Protocols of previously described studies by our group[2,10,13] and[6] were used for standardization and comparison. For characterization of patients according to gender and disease/infection state, they were grouped in the age range of greater than 20 yr and less than 70 yr. concise medical details and history of patients with initial diagnosed cases of hepatoma was selected. It was made mandatory to classify the patients into groups such as cirrhosis, hepatitis C or B-infected or in more detailed cases, suspected of hepatitis infections with co-existence of hepatoma.

Final evaluation depicts that around 1429 suspected patients were evaluated and assessed during the course of the study. They were noted to have either hepatoma or continuance of hepatitis viral infections or both. Out of 1429, 142 fall fully into the accessible standards detailed by us. However, during the course of present study 49 patients (29 males 20 females) could not be followed for evaluations or diagnosis as they left the city for their villages or other parts of the country. Therefore, the final total number of patients included in the study was 93 (50 males, 43 females).

Exclusion criteria: Those subjects who were in ages of less than 20 yrs and greater than 70 yr, alcoholics, patients' undergone recent surgeries, bronco-spasmatic (with or with steroid therapies) were designated as ineligible for the study. Those suffering from other malignancy or broad spreading of cancer to other parts of the body were also not registered.

Clinical and Diagnostic data

Brief medical history of all groups was documented, entirely detailing the points of verification of HCC by all or either diagnostic tests' described earlier.[10] Additionally, confirmation of hepatitis infections, B-virus, C-virus profiling, molecular diagnosing, related hematological, biochemical and microbiological tests were also included in data. Treatments information may also be taken were possible for hepatocellular carcinoma and Hepatitis infections. It was gathered that all 142 patients were lab-tested for hepatitis profile. Out of 142, 41 also have confirmation of HBV or HCV through PCR. Related tests also includes urine DR, urobilinogen, blood Cultures etc.

Sample Collection

Collection of blood was done according to prescribed methods in clot activated tubes for AFP testing and hepatitis profiling. Serum was separated and stored at – 20^{o}C until analyzed.

Analysis and Calculation

Alpha-feto protein was analysed on Automated ELISA analyzers (Cobas-Core, Elecsys 1010 and 2010, Roche-Diagnostics) with two -point calibration and controls with definite cut-off values. Standardized AFP values which were greater than 20 ng/ml [range 10 ng/ml] in smokers and greater than 10 ng/ml [range 5 ng/ml] in non-smokers were considered significant. For hepatitis infections, tests were performed on Axsym (Abbott lab, USA) and Vetros using analytical controls. For clarity, assessments and comparison, the data generated and accumulated were depicted as percent-occurrence. Significance level of analytical results was calculated using student *t*-test (SPSS ver 13).

RESULTS

The results are presented in Figures 1-2 and Table 1. Percent distribution precedent of medical setting of patients with high AFP levels and positive Hepatitis profile is as follows; HCC with HCV origin was 33.33% (n = 31), whereas with HBV origin 41.93% (n = 39) (Fig 1). For HCC of cirrhotic origin only, 10.75% (n = 10) was noted with no-infection whereas HCC-Chronic liver disease [CLD] only, 13.97% (n = 13) were found with no-infection. The study comprised of a total of 1429 suspected patients that were evaluated and included in the study where noted to have either, hepatoma and co-morbidity of viral infections of B and C origins. Out of 1429, 142 follow fully into the standards finalized through prescribed methodology. However, during the course of present study 49 patients (29 males 20 females) could not be followed for evaluations or diagnosis as they left the city for their villages or other parts of the country. Therefore, the final total number of patients included in the study was 93 (50 males, 43 females).

Segregation according to medical anomalies, infectivity, cirrhosis and chronic liver disease, the numeral of males, with total of fifty, either with HCC or with C-virus and B-virus, was grouped as 15, 24, and with cirrhosis and liver disease as 6 and 5 respectively. On the other hand, female subjects, totaling forty three, were segregated as 14, 17, 5 and 7, respectively, for C-virus, B-virus, cirrhosis and chronic hepatic anomalies. Combined allocation among male and female category is exhibited as; HCV = 30 % (n = 15) in males and 32.55% (n = 14) in females, HBV = 48.00% (n = 24) and 39.53% (n = 17), Cirrhosis = 12.00% (n = 6) and 11.66% (n = 5) and CLD = 10.00% (n = 5) and 16.27% (n = 7), respectively (Fig 2). Arguably, it was assessed that nevertheless the patients that were diagnosed with hepatocellular carcinoma were in-fact infected with C and B viral agents, however the finding doesn't entail that this co-morbid is lone contributory factor of the incidence of hepatoma. Furthermore, certain technical restraint of research doesn't permit us to ascertain a confirmed affiliation of hepatitis infections and establishment of hepatoma to a definitive extent.

AFP values were found to be elevated in every subject, both male and female, varying from 21.20 to 768.00 ng/ml in males (mean = 281.82 ± 96.11 ng/ml) and 15.00 to 612 ng/ml in females (mean = 201.00 ± 78.10 ng/ml) (Table 1). However, no major disparity was established between the elevated ranges of two categories at $P < 0.05$. In males, around 10.00% (n = 10) have AFP in the highest range of > 300.00 to < 768.00 ng/ml in HCV group; 46.00% (n = 23) in the range of > 150 to < 300 ng/ml in HBV and

cirrhosis groups and 8% (n = 4) in the range of >300 to <520 ng/ml in HBV alone group. In females, 20.90% (n = 9) showed AFP ranges between >300 to 612 ng/ml in HCV group; and 18.60% (n = 8) in range of >150 to <300 ng/ml in HBV and CLD groups. It was observed that there were 41 patients (22 males and 19 females) who underwent rehabilitation for cancer associated medical impediment during last 60 days to one year. Similarly 21 patients (15 and 6, males and females, respectively) went through interferon therapy within 3 months to one year, after diagnosed with hepatitis infection. HCC diagnosis was taken place in these patients in last two to three months of therapy procedures-completion. Moreover, it was analyzed that those hepatoma subjects that were not infected hepatitis viral infections were either suffering from cirrhosis or chronic liver disease of un-known origin. As noted earlier cirrhosis related hepatoma patients were suspected of being malignant due to complications originating from biliary impediment, drug induced, some also with hemochromatosis and cystic fibrosis respectively. Furthermore, subjects indicted in chronic liver disease group were systematically examined for the period of their stay in hospital or OPD visits for any hepatitis infectivity, interrelated illness or associated malignancy, however no substantiation noted. Therefore, it was decided that such patients would be categorized as hepatoma associated chronic liver disease-only with none of the medically important cirrhotic manifestation or contagion. Tiredness, fever, hepatic enlargement, few cases of ascites and pain were the associated signs and symptoms that were seen in all subjects categorized in the present study.

DISCUSSION

The general outcome of present study was the verity that alpha-feto protein analysis not only provided significant support for the substantiation of hepatic malignancy but also in cases of viral hepatitis related hepatoma, the denomination were categorically high, as compared to those with no infection. However, it was argued that alpha feto-protein levels doesn't provide information as at what time, wherever and since when the patients have been infected or been a carrier[2,13,10]. Similarly it also doesn't exemplify that hepatitis viral infections are the "only" cause of hepatoma developments.[2,13,10] In our study presented here, patients were grouped according to their medical categorization and data collected. In brief cumulatively, males hepatoma patients with HCV and HBV were 15, 24, and with cirrhosis and CLD, 6 and 5 respectively, whereas female patients were grouped as 14, 17, 5 and 7, respectively, for HCV, HBV cirrhosis and chronic liver disease. Communal division among two genders is exhibited as; HCV = 30 % (n = 15) in males and 32.55% (n = 14) in females, HBV = 48.00% (n = 24) and 39.53% (n = 17), Cirrhosis = 12.00% (n = 6) and 11.66% (n = 5) and CLD = 10.00% (n = 5) and 16.27% (n = 7), respectively. Alpha feto-protein increments were found to be high and varies in males between 21.20 to 768.00 ng/ml (mean = 281.82 ± 96.11 ng/ml) and in females between 15.00 to 612 ng/ml (mean = 201.00 ± 78.10 ng/ml). Conversely, no noteworthy disparity was established amid the high array of alpha feto-protein in two categorized masculine and feminine groups at $P < 0.05$.

It was argued very strongly by [6] that the limitation to link AFP levels independently with increased mortality in HCV-related HCC patients was the non-availability of details of staging data, size, number, or spread of tumor. However, previously reported studies, including those used for the CLIP, GRETCH, and CUPI scoring systems, have suggested AFP to be an independent prognostic factor.[3,19,22,23,27,24,25]

As observed in our study as well and also reported earlier by,[6] though the focus of their study too was on HCV-related HCC, some patients may also have alcohol use or co-infection with HBV or human immunodeficiency virus. However, in comparison with previous studies that focused on AFP–HCC outcomes, including reported by our team,[2,13,10] majority of the cohort reported was infected with HCV[6]. The group also pointed out another potential limitation which was the fact that around 28% of the patients selected didn't have AFP level tested within the time period around HCC diagnosis. Other international studies also corroborated the inference that AFP level is an independent predictor of mortality in HCV-related HCC cases[6]. For that reason AFP levels were integrated into the CLIP, GRETCH, and CUPI scoring systems during the period 1998 and 2002.[6,26] As previously documented, the CLIP system being corroborated in several countries and thus depicts credibility, was recommended by the A.J.C.C (American joint committee on cancer) as the clinical staging system favored by many.[6,28,29,30,31] In recent past, total tumor volume was also proposed as a scoring system to predict HCC survival by group of researchers in Taiwan.[6,32] The study suggested that the predictive accuracy of total tumor volume was enhanced with the addition of an AFP level greater than 400, thus depicting decreased survival rate.[6,32]

Arguably, since the systematically conducted studies do demonstrate that serum AFP levels are predictive of mortality after HCC diagnosis,[2,6,10] it also recognized that incremental changes in AFP levels such as 10 to <100, 100 to <1000, and ≥1000, at the time of HCC diagnosis were associated significantly with increased mortality independent of several demographic factors namely age, sex, and race/ethnicity, clinical factors such as ascites, encephalopathy, CHF, and MELD, or treatment regiments such as transplantation, resection, TACE, and RFA.[6]

Hepatitis infections, especially those of C-virus and B-virus origins, which is presented in this study and those reported earlier[2,13,6,10] was linked to hepatoma (as known as hepatocellular carcinoma) cohort. It is a contagion which might results in ample number of untoward medical condition and transience. The progression of stated medical symptom and impediment were cirrhosis, hepatic collapse with ascites, hepatic encephalitis, oesophegeal anomalies and hepatoma.[2,13,6,10] Certain facts documented earlier showed that populous infected with hepatitis viruses (and might have received blood transfusion) and then evolved to develop cirrhosis in progressing two decades diverge between 2-4% in children and young and 20-30% or greater in older populous.[7,15] Moreover, certain minimal percentage of cohort of cirrhotic patients might develop hepatoma annually.[17,18] Likewise, studies conducted earlier evaluated and then ascertain that between previous two years and the proceeding decade, around 165 thousands deaths from chronic hepatic anomalies/ diseases and approximately 27 thousands mortality from hepatoma might take place.[2,7,15,17,18] Assessments and conclusion drawn during the course of presented study also concurs similar inference as assumed in previous studies. Thus the cumulated data and inference gathered in current researched cohort stipulated following supposition. Hepatoma or hepato-cellular carcinoma, as it is commonly known, is primarily suspects from the subjects chronically laden and continually exposed to B-virus and C-virus infections, in addition to hepatic cirrhosis. Clearly, documentation showed that preponderance of male-gender with hepatoma, varying from 8:1 in countries with elevated frequency with 2:1 to 3:1 in populous trailing lower rate. Additionally, factors such as maturity, gender, carcinogenic substance, hormonal imbalance, alcohol and transmutation also sometimes instigate or

propel progression of hepatic carcinoma. Furthermore, broader investigation discovered that recurring sequence of cell bereavement and renaissance, as seen in chronic hepatitis, are imperative collaborators in pathogenesis of viral hepatitis-coupled liver cancer and cellular malignancy.[7,15,17,18] Recent survey revealed that nuclear polymorphism and transmutation are allied with hepatoma in populous infected with hepatitis viruses and/or suffering from chronic hepatic disorders such as chronic liver diseases.

CONCLUSION

Following inference can be drawn from our presented study that although hepatocellular carcinoma patients were found infected with hepatitis viruses such as C-virus and B-virus, it doesn't entail that these infections were the only contributory component for the occurrence of HCC. However, AFP levels did provide helpful facilitation in evaluating the occurrence and extent of hepatitis related HCC in both male and female patients. Moreover in cohorts, who were noted to be devoid of any viral infections, alpha feto-protein concentrations were comparatively lower, thus further corroborating the fact that AFP levels were more distinctively correlated with hepatitis related HCC. Variable cohorts are being investigated currently in our department to further convolute the etiology, pathogenesis, clinical and biochemical distinctiveness of hepatitis associated hepatoma.

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Table 1. Gender wise distribution of disease and infection variable and elevated AFP values in HCC patients

	Males	
Disease/infection variables	**Number of patients (n = 50)**	**AFP Ranges (ng/ml)**
HCV	10	>60.00 ± 11.60 to <150.00 ± 73.10
	5	>300.00 ± 89.45 to 768.00 ± 110.20
HBV	20	>150.00 ± 54.80 to <300.00 ± 95.25
	4	>300.00 ± 81.60 to <520.00 ± 105.15
Cirrhosis	3	> 21.20 ± 5.75 to <150.00 ± 70.30
	3	>150.00 ± 55.35 to <300.00 ± 110.40
CLD	5	>21.20 ± 4.50 to <60.00 ± 10.80
	Females (n = 43)	
HCV	9	>300.00 ± 76.90 to <612.00 ± 125.75
	5	>60.00 ± 10.15 to <150.00 ± 45.30
HBV	11	>60.00 ± 14.10 to <150.00 ± 39.60
	6	>150.00 ± 22.50 to <300.00 ± 60.15
Cirrhosis	2	> 15.00 ± 2.25 to <60.00 ± 12.35
	3	>60.00 ± 9.65 to <150.00 ± 25.80
CLD	2	<150.00 ± 44.60 to >300.00 ± 75.30
	5	>15.00 ± 1.90 to <150.00 ± 15.25

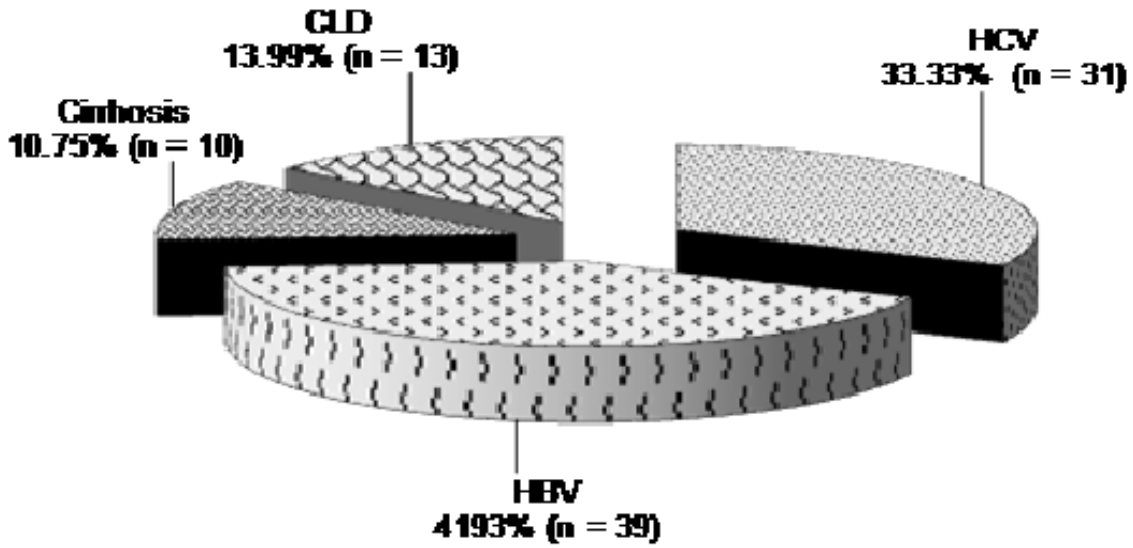

Figure 1. Cumulative distribution of clinical conditions in HCC patients (n=93)

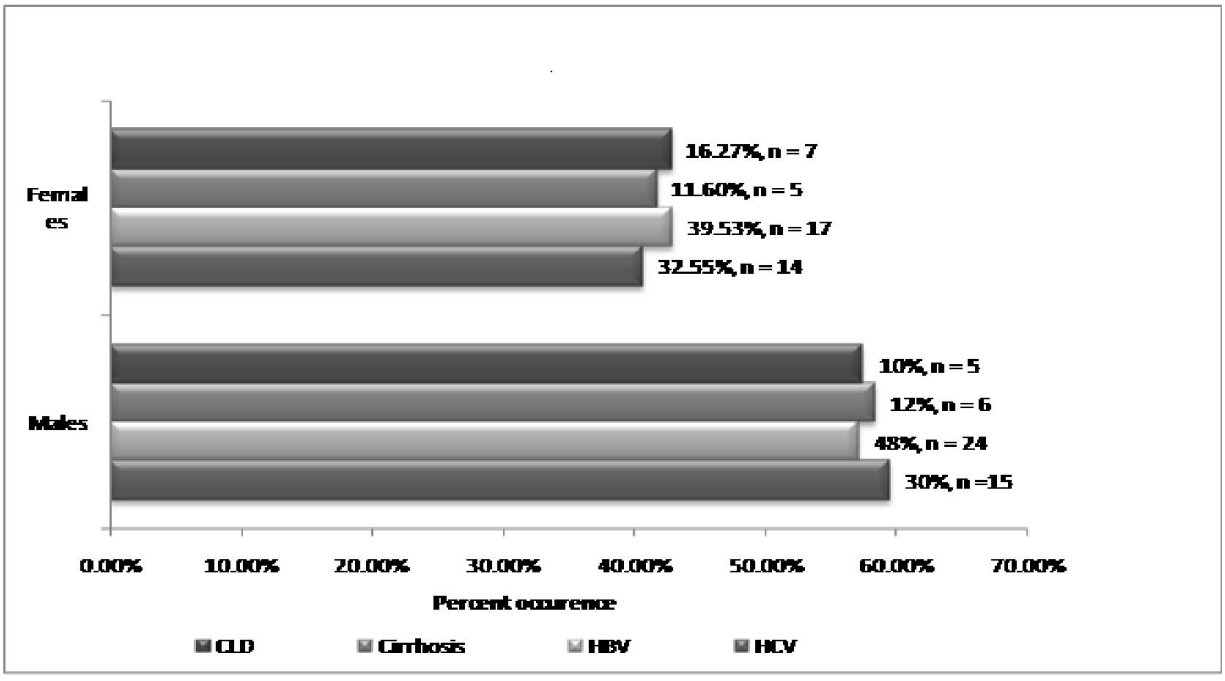

Figure 2. Gender wise percent distribution of clinical conditions in HCC patients