Diagnostic Accuracy of Serological Markers in Viral Hepatitis and Non Alcoholic Fatty Liver Disease. A Comparative Study in Tertiary Care Hospital of Western Nepal

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Original Article

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Abstract

Background
Liver diseases is apparently increasing and emerging as a major public health problem. Worldwide, chronic hepatitis B has become the tenth leading cause of death and persons infected with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV), are about 350 million and 125 million respectively. The aim of current retrospective comparative study was concerned primarily to evaluate the significance of non invasive serological markers for diagnosing liver diseases and their predictive implications in Pokhara valley.

Materials and Methods
It was a hospital based retrospective study carried out using the data maintained in the Department of Biochemistry of the Manipal Teaching Hospital, Pokhara, Nepal between 1st June 2009 and 31st October 2010. The variables collected were total protein, albumin, AST, ALT, total bilirubin, direct bilirubin. Descriptive statistics and testing of hypothesis were used for the analysis. Data was analyzed using EPI INFO and SPSS 16 software.

Results
Of 515 subjects, 120 were suffering from viral hepatitis and 88 had non alcoholic fatty liver disease. In cases of viral hepatitis, mean values of AST (CI 730.65 to 902.68) and ALT (CI 648.14 to 847.59) were markedly increased as compared to controls. Mild to moderate elevations in serum levels of aspartate aminotransferase (CI 43.42 to 49.49), alanine aminotransferase (CI 43.90 to 53.92) were the most common laboratory abnormalities found in patients with nonalcoholic fatty liver disease.

Conclusion
Non invasive tests have demonstrated a reasonable ability to identify significant fibrosis, cirrhosis in particular, nor is it surprising that liver disease specialists and patients favour a non invasive approach

Key Words
Viral hepatitis, Nonalcoholic fatty liver disease, Nepal

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Background

Liver diseases is apparently increasing and emerging as a major public health problem. Worldwide, chronic hepatitis B has become the tenth leading cause of death and persons infected with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV), are about 350 million and 125 million respectively[1]. High-risk sexual and drug use behaviour is most commonly associated with wild spread of hepatitis B and C particularly and about 2.7 million persons are chronically infected with HCV in USA. HCV accounts for 20% of cases of acute hepatitis[2]. The important factors responsible for development of cirrhosis and hepatocellular carcinoma with chronic hepatitis are age, extent, severity, duration, frequency and etiology of the hepatic lobular alterations[3]. young adults are more prone to infection of viral hepatitis[4]. In a typical year, the seroprevalence of HBV (0.82%) was much higher than seroprevalence of HCV(0.47%) in Nepal[5]. A large proportion of the world's population is affected with non alcoholic fatty liver disease. The prevalence in general population of NALFD is about 10 to 24 % in various countries and it rises to 57.5% to 74% in obese persons. Non alcoholic fatty liver disease is generally associated with coexisting conditions like hyperlipidemia, type 2 (non-insulin-dependent) diabetes mellitus and obesity and further , it is almost universally allied with diabetic patients who are morbidly obese[6]. Insulin resistance with its multifactorial molecular pathogenesis is the most reproducible factor for the progression of NALFD[7]. Men are affected earlier than women being the prevalence peaks in the fourth and sixth decade for same in NALFD. It appears to be more common in men and it increases with increasing age and after menopause[8]. Aminotrasferase are released into circulation during hepatocyte damage or injury. The aim of current retrospective comparative study was concerned primarily to evaluate the significance of non invasive serological markers for diagnosing liver diseases and their predictive implications in Pokhara valley.

Materials and Methods

It was a hospital based retrospective study carried out using the data maintained in the Department of Biochemistry of the Manipal Teaching Hospital, Pokhara, Nepal between 1st June 2009 and 31st October 2010. The variables collected were total protein, albumin, AST, ALT, total bilirubin, direct bilirubin. Approval for the study was obtained from the institutional research ethical committee. Total proteins were determined by Biuret method[9]. The albumin was measured by BCG method[10]. The total and direct bilirubin was estimated by Jendrassik/Grof method[11]. The transaminases (AST and ALT) were estimated by liqui uv test[12]. All these laboratory parameters were analysed using Human reagent kits and with the help of semi autoanalyser (Human, Germany). Analysis was done using descriptive statistics and testing of hypothesis. The data was analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version. The Chi-square test was used to examine the association between different variables. Z-test was used to compare the significance difference between two variables. A p-value of < 0.05 (two-tailed) was used to establish statistical significance.

Inclusion criteria: Patients with increased aminotransferase levels (greater than 1.5 times normal) for at least six months, presence of anti-HCV and anti-HBV in serum, low to absent alcohol consumption (less than 30 g/day for men and less than 20 g/day for women) with chief complaints related to vomiting, hepatomegaly, jaundice or ascites were included in our study.

Exclusion criteria: Patients with history of an alcohol intake of more than 40 g per week , severe cardiac or renal disease, active intravenous drug abuse.patients receiving previous treatment with interferon or immunosuppressive agents or were taking medication that could cause steatosis (i.e. salicylates, nonsteroidal anti-inflammatory drugs, corticosteroids, valproic acid, amiodarone, perhexiline maleate) were excluded from the study.

Controls: Healthy males and females with normal liver profile.

Results

Of 515 subjects, 120 were suffering from viral hepatitis and 88 had non alcoholic fatty liver disease.

Table 1: Comparison of biochemical parameters in viral hepatitis and controls

Variables	Viral hepatitis (120)	Normal (317)	p value
Total proteins	7.2 ± 0.46	6.96 ± 0.69	0.002*
Albumin	4.04 ± 0.27	3.81 ± 0.44	0.001*
AST	816 ± 475.85	27.1 ± 12.21	0.001*
ALT	747.8 ± 551.69	26.6 ± 10.67	0.001*
Ratio AST/ALT	1.31 ± 0.63	1.04 ± 0.20	0.001*
Total bilirubin	4.36 ± 1.45	0.85 ± 0.14	0.001*
Direct bilirubin	2.39 ± 1.17	0.23 ± 0.08	0.001*

* Statistically significant (p<0.05)

Table 1 displays that mean value of each variable in cases was statistically significant as compared to controls (p=0.001). In cases, mean values of AST (CI 730.65 to 902.68) and ALT (CI 648.14 to 847.59) were markedly increased as compared to controls. The mean values of total (CI 3.15 to 5.16) and direct (CI 2.00 to 2.78) bilirubin were markedly raised in cases of viral hepatitis and showed statistical significance as compared to the mean values of total (CI 0.83to 0.86) and direct (CI 0.23to 0.24) bilirubin in

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controls respectively.

Table 2: Comparison of biochemical parameters in non alcoholic fatty liver disease and controls

Variables	NAFLD (88)	Normal (317)	p value
Total proteins	7.03 ± 0.80	6.96 ±0.69	0.002*
Albumin	3.96 ± 0.45	3.81 ±0.44	0.001*
AST	46.45 ± 14.34	27.07± 12.21	0.001*
ALT	48.91 ± 23.65	26.6 ± 10.67	0.001*
Ratio AST/ALT	1.03 ± 0.26	1.04 ± 0.20	0.001*
Total bilirubin	1.56 ± 0.58	0.85 ±0.14	0.001*
Direct bilirubin	0.67 ± 0.27	0.23 ± 0.08	0.001*

* Statistically significant (p<0.05)

Table 2 shows that mean value of each variable in cases except total proteins and AST/ALT ratio was statistically significant as compared to controls. Mild to moderate elevations in serum levels of aspartate aminotransferase (CI 43.42 to 49.49), alanine aminotransferase (CI 43.90 to 53.92) were the most common laboratory abnormalities found in patients with nonalcoholic fatty liver disease. The mean values of total (1.56 ± SD0.58) and direct (0.67 ± SD 0.27) bilirubin were mildly raised in cases of NALFD and showed statistical significance as compared to the mean values of total (0.85 ± SD0.14) and direct (0.23 ± SD0.08) bilirubin in controls respectively.

Discussion

In light of dramatic increase in prevalence of liver diseases, a noninvasive, simple, reproducible and reliable biomarkers which can allow identifying patients with liver injury at early stages are greatly needed. Early detection of fibrosis and staging of spectrum of liver diseases can be done with the help of serological markers such as ALT, AST, total bilirubin, direct bilirubin, total proteins would in turn prevent progression to hepatic cirrhosis and also, they can be useful before therapeutic decisions or predicting outcomes of disease. Biochemical markers such as AST and ALT are released from damaged hepatocytes into the circulation. ALT is derived mainly from cytosol of hepatocytes. AST is derived both from cytosol and mitochondria of hepatocytes. Magnitude and relative elevation of ALT and AST are important for differential diagnosis, implies liver injury or death of hepatocytes. The stage of disease can change over time and most therapeutic interventions are focused on ameliorating severity of disease. Viral hepatitis is caused by filterable infectious agents and is primarily hepatotropic with outstanding clinical manifestations and evidences of liver injury[13]. The various forms of chronic viral hepatitis are alike in serological abnormalities, clinical signs and symptoms, and histologic changes[14]. The liver associated enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl peptidyl transferase (GGT) are indirect measures of liver homeostasis. Our present study typically showed marked elevations in mean values of ALT (CI 648.14 to 847.59) and AST (CI 730.65 to 902.68) as compared to controls in cases of viral hepatitis. The mean values of total (CI 3.15 to 5.16) and direct (CI 2.00 to 2.78) bilirubin were also raised in cases and showed statistical significance as compared to mean values of total (CI 0.83to 0.86) and direct (CI 0.23to 0.24)bilirubin in controls respectively. The previous studies also showed elevations of ALT upto 95.4% in HCV antibody positive patients[15]. Fibrosis, with early stages being confined to the portal tracts, and late stages being frank cirrhosis characterized by architectural disruption of the liver and was vitally important to be diagnosed at this stage. Hepatocyte necrosis and regenerative hyperplasia in viral hepatitis is immune mediated and spread from virally infected to uninfected cells via soluble cytotoxic mediators[16]. The histological changes in hepatitis includes large droplet steatosis, ballooning of malignant hepatocytes, Mallory-Denk bodies, inflammation and pericellular fibrosis[17]. Nonalcoholic fatty liver disease encompasses a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis. The changes in histological pattern for NAFLD includes steatosis (fatty change), lobular inflammation, periportal fibrosis, mallory bodies, nuclear vacuolation, bile ductal proliferation, perivenular and perisinusoidal fibrosis. Our current study revealed that there was mild elevation in serum levels of AST (CI 43.42 to 49.49) and ALT (CI 43.90 to 53.92) in cases of nonalcoholic fatty liver disease and the ratio of AST/ALT was < 1. The mean values of total proteins (7.08 ± SD0.80) and total bilirubin(1.56 ± SD0.58) did not show much variation as compared to controls. The above results concurred with findings of Angulo et al[6]. The primary metabolic abnormalities leading to lipid accumulation due to alterations in the pathways of uptake, synthesis, degradation, or secretion in hepatic lipid metabolism resulting from insulin resistance. Increased intrahepatic levels of fatty acids, adipocytokines, tumour necrosis factor-α mitochondrial dysfunction, vascular disturbance provide a source of oxidative stress with subsequent lipid peroxidation, cytokine induction. All these factors promote hepatocellular damage, inflammation and progressive liver disease[18]. Liver biopsy remains the gold standard for the evaluation of liver diseases. However, its

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invasiveness, the observations of significant side effect profile and susceptibility of this technique to sampling error ultimately make it a suboptimal technique. The current study emphasize that variations in levels of serum biomarkers has tremendous potential to radically alter the diagnostic and monitoring strategies through the reduction in need for liver biopsy.

Conclusion

Non invasive tests have demonstrated a reasonable ability to identify significant fibrosis, cirrhosis in particular, nor is it surprising that liver disease specialists and patients favour a non invasive approach. However, only those tests with the highest diagnostic accuracy, cost-effectiveness, and availability should be implemented. Indeed, the use of a standardized system to evaluate the utility of biomarkers would facilitate their implementation in clinical practice.

Conflict of Interests

The authors do not have any conflict of interest arising from the study.

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