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Original Research Article

Evaluation of Significance of Liver Enzymes as Screening Tests for the Early Detection of Clinically Asymptomatic Non Alcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus Patients

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Abstract

Background: Type 2 diabetes mellitus has been linked with abnormal liver function tests. Increased activities of liver enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT) are markers of liver cell injury. Increased activity of these markers is associated with metabolic syndrome, insulin resistance and type 2 diabetes mellitus. This study was aimed to evaluate the significance of liver enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT) in type 2 diabetes mellitus patients.

Materials & Methods: Total of 108 cases of type 2 diabetes mellitus patients, comprising of 68 males (62.9%) and 40 females (37%) were included in the study. 110 healthy subjects were includes as controls. Age of the subjects ranged between 27 to 75 years. Serum sample was used for the estimation of study parameters such as FBS, PPBS, AST, ALT, ALP, GGT, Total bilirubin, Direct bilirubin, Total proteins and Albumin.

Results: In the present study, mean values of AST, ALT, ALP and GGT were statistically significantly increased in type 2 diabetes mellitus patients when compared to controls (P value 0.001).

Conclusion: In this study, we found an association between the increased levels of liver enzymes such as AST, ALT, ALP and GGT in type 2 diabetes mellitus patients. Increased levels of ALT and AST are the surrogate markers for associated non alcoholic fatty liver disease in type 2 diabetes mellitus patients. Hence, testing for AST, ALT, ALP, and GGT should be carried out to screen for underlying non alcoholic fatty liver disease (NAFLD) in type 2 diabetes mellitus patients.

Keywords: Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), γ-Glutamyl transferase (GGT).

1. Introduction

Diabetes mellitus is a growing concern and it is one of the world's major diseases. India, the world's second most populous country, presently has more than 50 million diabetic patients, more than any other nation, making India "the diabetes capital of the world" [1,2]. It has been estimated that the global burden of type 2 diabetes mellitus (T2DM) will increase to 438 million in 2030. Similarly, for India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030 [2].

T2DM, a devastating disease is multifactorial involving multiple genes and environmental factors to varying extents. Genetic factors are related to impaired insulin secretion and insulin resistance. Environmental factors such as obesity, overeating, lack of exercise, stress, as well as aging contribute to disease progression [3].

Liver is the main organ involved in the metabolism of glucose and energy homeostasis. The carbohydrates absorbed from the gastrointestinal tract undergo hepatic processing and subsequent storage as glycogen in the liver or metabolised into amino acids or fatty acids [4]. Type 2 diabetes mellitus is associated with number of liver disorders including elevated liver enzymes, fatty liver, cirrhosis, hepatocellular carcinoma and acute liver failure [5]. Emerging evidence suggests that a strong link exists between liver enzymes and diabetes mellitus [6].

Glycogen accumulation in hepatocytes leads to hepatomegaly and liver enzyme abnormalities in poorly controlled diabetes patients. The stored glycogen in

hepatocytes cause typical biochemical findings like elevated transaminases, with or without mild elevations of alkaline phosphatase (ALP) and normal liver synthetic function. The increased activities of liver enzymes are markers of hepatic injury. Increased activity of these markers is associated with metabolic syndrome, insulin resistance and type 2 diabetes mellitus [7]. The elevated transaminases and hepatomegaly are found to be reversible with good glycemic control.

Non-alcoholic fatty liver disease (NAFLD) is a well-recognized complication of diabetes mellitus with frequency of 70-80%. Associated obesity is a confounding variable for fatty liver and the most common clinical finding is hepatomegaly. Changes in the liver function tests are considered as surrogate marker of hepatic injury in NAFLD [8]. NAFLD was first reported in 1980 in obese females with diabetes. It has been shown to be a predisposing factor for insulin resistance and hyperinsulinemia.

Genetics and environment play a role in the pathogenesis of NAFLD. A "two - hit" model of pathogenesis has been proposed, encompassing hepatic fat accumulation and hepatic oxidative stress. Oxidant stress from reactive lipid peroxidation, peroxisomal beta-oxidation, recruited inflammatory cells are the other potential explanation for the elevated levels of transaminases in insulin-resistant state. The excess free fatty acids found in the insulin-resistance are known to be directly toxic to hepatocytes [8].

Without treatment, compensated steatosis in NAFLD will eventually lead to decompensate steatosis with necroinflammation and fibrosis i.e. stage of non-alcoholic steatohepatitis (NASH). NASH is a leading cause of end-stage liver disease and also a contributor of cardiovascular disease in type 2 diabetes mellitus. Elevated serum concentrations of transaminases indicate leakage of hepatic intracellular enzymes into the circulation, as a result of hepatocellular injury and are used for primary screening of NASH.

The present study was aimed to evaluate the significance of the liver enzymes such as AST, ALT, ALP and GGT in type 2 diabetes mellitus patients compared to non-diabetic healthy control group.

2. Materials & Methods

This study is a cross sectional case-control study, conducted at Akash Institute of Medical Sciences & Research Centre, Devanahalli, Bangalore Rural, 108 type 2 ambulatory diabetes mellitus patients were taken as cases and 110 healthy subjects served as controls. The study population was chosen following ADA diagnostic criteria. Patients with type 1 diabetes mellitus, chronic liver disease, patients on hepatotoxic drugs, alcoholism, type 2 diabetes mellitus patients on insulin treatment, autoimmune diseases, congestive cardiac failure and renal diseases were excluded from the study. The clinical history and other necessary details were obtained from the patients records.

Venous blood samples were collected after taking aseptic precautions from the study subjects. 5 ml of blood was collected in plain vaccum tubes and 2 ml of blood was collected in EDTA vaccum tubes. Samples were left for 20 minutes at room temperature, and centrifuged at 3000 rpm for 4 to 5 minutes. Serum was used for the estimation of fasting blood sugar (FBS), PPBS, total bilirubin, direct bilirubin, AST, ALT, ALP, GGT, total protein and albumin by automated chemistry analyzer cobas c111. The study was approved by institutional ethical committee.

2.1 Statistical Analysis

Data were expressed as mean ±SD. P value <0.05 is considered as statistically significant. Statistical analysis was performed using SPSS 20.0

3. Results and analysis

Total of 108 type 2 diabetes mellitus patients were selected for the study. Among them 68 were males (62.9%) and 40 were females (37%) with average age of (50.1±11.4) years, ranging between 27 to 75 years. 110 healthy subjects with average age of (43.3 ±13.3) years, ranging between 25 to 75 years were selected as control group.

In this study, liver enzymes such as AST, ALT, ALP and GGT were found to be statistically significantly increased in type 2 diabetes mellitus patients when compared to controls. The liver enzyme measurements in T2DM patients were AST (42.2±12.8), ALT (50.38±19.7), ALP (93.6±35.6) and GGT (42.8±5.4). as illustrated in table 1.

As shown in table 2, statistically significant correlation was observed between FBS and AST, ALT and ALP in cases, while it was insignificant between FBS and GGT in cases. ALP is significantly correlated with PPBS in cases, whereas as correlation was insignificant between PPBS and AST, ALT and GGT in cases. Total bilirubin, Direct bilirubin, Total Protein and Albumin values did not correlate significantly with FBS and PPBS values.

Table 1: Comparison of age, liver function test parameters between type 2 diabetes mellitus patients and healthy controls

Parameters	Controls (n=110) Mean ± SD	Cases (n= 108) Mean ± SD	P-value
Age	43.3±13.3	50.1±11.4	0.001*
FBS (mg/dl)	94.7±9.1	173.4±73.7	0.001*
PPBS (mg/dl)	115.6±25.4	260.7±112.8	0.001*
Total Bilirubin (mg/dl)	0.61±0.30	0.7±0.55	0.021
Direct Bilirubin (mg/dl)	0.25±0.13	0.3±0.29	0.106
AST (IU/L)	24.2±14.5	42.2±12.8	0.001*
ALT(IU/L)	22.5±13.7	50.38±19.7	0.001*
ALP(IU/L)	76.5±21.8	93.6±35.6	0.001*
GGT(IU/L)	35.07±8.1	42.8±5.4	0.001*
Total protein (gm/dl)	7.3±0.6	7.4±0.5	0.53
Albumin(gm/dl)	4.5±0.43	4.4±0.46	0.062

***** Statistically Significant

Table 2: Correlation between FBS and Liver Enzymes in controls and cases

Parameters	Controls	Cases
AST	0.037	0.223*
ALT	0.113	0.244*
ALP	-0.026	0.248**
GGT	0.023	0.114

*. Correlation is statistically significant at 0.05 level (2-tailed).
**. Correlation is statistically significant at 0.01 level (2-tailed).

Table 3: Correlation between PPBS and Liver Enzymes in controls and cases

Parameters	Controls	Cases
AST	0.059	0.098
ALT	0.151	0.112
ALP	-0.047	0.245*
GGT	-0.066	0.063

*. Correlation is statistically significant at 0.05 level (2-tailed).

4. Discussion

Liver dysfunction associated with elevated liver enzymes is frequently encountered in type 2 diabetes mellitus. Chronic liver disease is an important cause of death in type 2 diabetes patients [9]. Increased levels of liver enzymes are frequently encountered in type 2 diabetes mellitus. The aetiology for abnormal liver enzymes in type 2 diabetes mellitus may be varied, but the most common cause is assumed to be non alcoholic fatty liver disease. The prevalence of non alcoholic fatty liver disease is more in type 2 diabetes mellitus patients and is almost universal in morbidly obese patients [10-12]. In the present study, it was observed that the mean values of AST, ALT, ALP and GGT are significantly increased in T2DM patients when compared to controls. A few available reports have highlighted ALP as the most frequent abnormality in type 2 diabetes mellitus patients [13-14]. However, this is not a universal finding and transaminases abnormalities have been shown to be the most common abnormality in some studies. Higher transaminases would be more suggestive that NAFLD is the probable cause in such patients [13].

NAFLD is a well recognized complication of diabetes mellitus and affects both males and females. Fat is stored in the form of triglyceride and may be a manifestation of increased fat transport to the liver, enhanced hepatic fat synthesis and decreased oxidation or removal of fat from the liver. The steatosis may be micro- or macrovesicular and may progress to NASH, fibrosis, cirrhosis and hepatocellular carcinoma. In few studies, increased liver enzyme levels are found to be predictors of future cardiovascular disease [15]. In the Firenze Bango a Ripoli (FIBAR) study done in Italy, increased GGT and AST were associated with increased incidence of cardiovascular disease [16]. Because of the association between NASH and metabolic syndrome, cardiovascular disease is frequent cause of death in patients with NASH. The insulin resistant state is also characterized by an increase in proinflammatory cytokines such as tumor necrosis factor – α (TNF-α) which may play a role in the pathogenesis of the inflammation in NASH, thus interfering with insulin signaling, thereby favoring steatosis.

In the present study, out of 108 cases, 20 subjects (18.5%) had increased AST, 26 subjects (24%) had increased ALT, 10 subjects (9.2%) had increased ALP and 37 subjects (34.2%) had increased GGT. 51 patients (47%) out of 108 T2DM patients had at least one abnormal liver enzyme. The main limitations of our study include small study population, lack of information regarding duration of type 2 diabetes mellitus, lack of invasive procedures like liver biopsy for better assessment of liver structural changes, radiological findings and follow up of the patients to know further progression. Further studies are recommended to find out the association between biochemical, haematological, histological and radiological changes of liver in type 2 diabetes mellitus patients.

5. Conclusion

The results of this study are in accordance with previously reported high prevalence of abnormal liver enzymes in Type 2 Diabetes Mellitus patients in other populations. 47% of the patients had at least one or more elevated liver enzyme levels. Therefore, abnormal liver enzymes in Type 2 Diabetes Mellitus patients can be an indicator of associated non alcoholic fatty liver disease (NAFLD). Despite the enzyme elevations, patients may be asymptomatic. Others may have general symptoms fatigue and right sided abdominal discomfort caused by hepatomegaly. Thus, assay for serum levels of AST, ALT, ALP, GGT should be carried out to screen the possibility of underlying fatty liver, which might need further evaluation and early intervention to prevent progression into cirrhosis and chronic liver disease in patients with Type 2 Diabetes Mellitus.

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