

## Liver enzymes and glycemic control markers in uncontrolled type 2 diabetes mellitus- A case control study

Sangappa Virupaxappa Kashinakunti<sup>1</sup>, Manjula Rangappa<sup>2,\*</sup>, Gurupadappa Kallaganada<sup>3</sup>

<sup>1</sup>Professor, Dept. of Biochemistry, <sup>2</sup>Associate Professor, Dept. of Community Medicine, S. Nijalingappa Medical College, Bagalkot, Karnataka, <sup>3</sup>Professor & HOD, Dept. of Biochemistry, Shimoga Institute of Medical Sciences, Shimoga, Karnataka

**\*Corresponding Author:**

Email: manjupushya2000@yahoo.com

### Abstract

**Introduction:** Mild increase in liver enzymes, may be a marker for significant liver injury. The increased transaminases and hepatomegaly are found to be reversible with glycemic control.

**Objectives:** 1. To compare the liver enzyme levels between type 2 DM with healthy controls 2. To study the correlation between FBS, HbA1c and Liver enzymes 3. To find the best cut-off values of AST and ALT, to suspect the non-alcoholic fatty liver disease (NAFLD) in DM.

**Materials and Method:** The study was conducted at HSK hospital, Bagalkot. Fifty subjects participated in Uncontrolled type 2 DM (HbA1c >7) group and Controls. Biochemical parameters like FBS, urea, creatinine, liver function tests and HbA1c were estimated. All the subjects underwent ultrasonography of abdomen to detect the fatty liver.

**Results:** The biochemical parameters FBS, HbA1c, blood urea, serum bilirubin, liver enzymes namely AST, ALT and GGT were raised significantly in T2DM patients compared to controls. There was positive correlation between FBS and HbA1c and the liver enzymes AST, ALT and GGT (P=0.0001). Best cut-off value of liver enzymes was calculated using ROC curve and the values for AST, ALT and GGT were >34 IU/L, 28 IU/L and 24.3 IU/L respectively.

**Conclusion:** Increased liver enzymes in uncontrolled type 2 DM, positively correlated with glycemic markers, when AST >34 u/l and ALT >28U/L, can act as a predictor NAFLD and may be useful for further evaluation of patients.

**Keywords:** Diabetes mellitus, Non-alcoholic liver disease, Liver enzymes.

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### Introduction

Presently, India has more than 50 million diabetes mellitus cases, making India “the diabetes capital of the world”. Type 2 diabetes (T2DM), obesity and dyslipidemia are predisposing factors of non – alcoholic fatty liver disease (NAFLD).<sup>(1)</sup> NAFLD was first reported in 1980 in obese females with diabetes.<sup>(2)</sup> The overall prevalence of NAFLD in western countries varies from 15-40% and in Asian countries from 9-40%.<sup>(3,4)</sup> Approximately 70% of T2DM patients have a fatty liver and they also appear to have more severe forms of the disease including Non-alcoholic steatohepatitis (NASH) and fibrosis,<sup>(3,5)</sup> the prevalence of NAFLD in T2DM patients in India is reported to be in range of 12.5-87.5%.<sup>(6,7)</sup>

NAFLD is abnormal fat accumulation in liver cells that resembles alcohol induced liver damage and is a clinico-histopathological diagnosis characterized by hepatocellular steatosis, without treatment which can progress to steatohepatitis, fibrosis, and ultimately cirrhosis<sup>(8)</sup> and even hepatocellular carcinoma (HCC), even in the absence of excessive alcohol intake. NASH is a leading cause of end-stage liver disease and also a contributor of cardiovascular disease (CVD) in T2DM.<sup>(2,9)</sup> The studies showed that hepatic enzyme elevation along with T2DM leads to a greater risk of CVD and kidney disease.<sup>(1)</sup>

Liver is the main organ involved in the metabolism of glucose and energy metabolism. The carbohydrates absorbed from the gastrointestinal tract undergo hepatic processing and subsequently stored as glycogen in the liver or metabolised into amino acids or fatty acids. A “two - hit” model for pathogenesis of NAFLD has been proposed, consisting of 1) hepatic fat accumulation and 2) hepatic oxidative stress. In T2DM, the loss of a direct effect of insulin to suppress liver glucose production and glycogenolysis in the liver causes an increase in liver glucose production. Free fatty acid oxidation and efflux of lipids from the liver are impaired. The increased availability of FFA, glucose, and hyperinsulinemia contribute to the fatty acids overload the hepatic mitochondrial-oxidation system, leading to accumulation of fatty acids in the liver. These mechanisms ultimately lead to non-alcoholic fatty liver disease (NAFLD) in T2DM patients.<sup>(10)</sup> Oxidative stress from reactive lipid peroxidation, peroxisomal beta-oxidation, recruited inflammatory cells are the other causes for the increased levels of transaminases in T2DM.<sup>(11)</sup>

Mild increase in levels of these enzymes, may be a marker for significant liver injury,<sup>(12)</sup> indicate leakage of liver intracellular enzymes into the circulation. Increased activity of these markers is associated with metabolic syndrome, insulin resistance and type 2 diabetes mellitus. The elevated transaminases and liver

enlargement are found to be reversible with good glycemic control.<sup>(2)</sup>

There is a growing evidence that elevated liver enzymes have the potential to serve as strong risk factors for the development of type 2 DM and this will help in prediction of T2DM in future along with other well known risk factors. Studies have shown that NAFLD is a predictor of pre-diabetes or T2DM.<sup>(13,14)</sup> Only few studies have shown that markers of liver injury can independently predict T2DM.<sup>(15)</sup>

In the majority of cases, NAFLD causes asymptomatic abnormality of liver enzyme levels. ALT is most closely related to liver fat accumulation and consequently ALT has been used as a marker of NAFLD.<sup>(10)</sup> Westerbacka J et al,<sup>(16)</sup> have demonstrated that ALT was closely associated with liver fat unlike Aspartate transaminase (AST) and gamma glutamyl transferase (GGT). ALT is used as a surrogate marker for many epidemiological studies,<sup>(8)</sup> there no much studies done about AST, ALP and GGT in this aspect. Hence, the present study was undertaken

1. To compare the liver enzymes between T2DM with healthy controls
2. To study the correlation between FBS, HbA1c and Liver enzymes
3. To find the best cut-off value for AST and ALT to suspect the NAFLD in DM.

## Materials and Method

The study was conducted at Hanagal Shri Kumareshwara hospital, Bagalkot from Jan 2016 to Jun 2016. The study was approved by Institutional ethics committee. Informed consent was obtained. 50 subjects participated in each group, Uncontrolled type 2 DM

(HbA1c >7) and Controls. Alcoholics, smokers, patients with diabetic complications, liver disease, other systemic conditions were excluded. Under aseptic precautions 5 ml of fasting sample was collected following biochemical parameters were estimated: FBS, blood urea, creatinine and liver function tests were estimated using Biosystems. A 25 fully automated biochemistry analyzer; and HbA1c was estimated by HPLC method.

All the subjects underwent ultrasonography of abdomen to detect the fatty liver, based on ultrasonography findings, diffuse increase in echogenicity as compared to that of the spleen or renal content.

Power of the study was calculated (100%) retrospectively based on the mean ALT values in cases and controls (95% CI). SPSS for window version; SPSS, 11.0 Inc, Chicago IL was used for statistical analysis. All the values were expressed in mean±SD. P value less than 0.05 was considered as statistically significant. Student t test was used for comparison; Pearson's correlation was used to find the correlation between the liver enzymes and glycemic control markers, receiver operator characteristic (ROC) curve was used to get best cut-off values for AST and ALT, GGT.

## Results

Base line characteristics and biochemical parameters in T2DM and controls are shown in Table 1. There was a statistically significant rise in BMI, waist circumference, systolic and diastolic blood pressure except age (P=0.060).

**Table 1: Baseline characteristics of cases and controls**

Parameters	Cases	Controls	t	p
Age years	51.4±15.7	46.3±10.1	-1.901	0.060
BMI (kg/m <sup>2</sup> )	25.7±3.4	22.2±3.4	-5.013	0.000
WC (cm)	97.2±10.0	84.4±11.7	-5.884	0.000
SBP (mm Hg)	151.0±13.8	115.4±5.9	-16.044	0.000
DBP (mm Hg)	93.3±3.6	76.4±4.4	-20.739	0.000

BMI: Body mass index

WC: Waist circumference

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

The biochemical parameters FBS, HbA1c, blood urea, serum bilirubin, liver enzymes namely AST, ALT and GGT were raised significantly in T2DM patients compared to controls (Table 2). Serum creatinine (P=0.5280) and ALP (p=0.236) also increased in T2DM compared to controls but were not statistically significant.

Serum albumin was decreased significantly in T2DM patients compared to controls (P=0.033), serum total protein was also decreased in T2DM than controls, but was not statistically significant (P=0.115).

**Table 2: Biochemical parameters in cases and controls**

Parameters	Cases	Controls	t	p
FBS (mg/dl)	182.9±68.5	87.0±17.4	-9.139	0.000
HbA1c %	8.5±1.3	5.356±0.6	-14.926	0.000
Urea (mg/dl)	25.8±9.4	20.0±6.7	-3.449	0.001
Creatinine (mg/dl)	1.4±2.9	1.1±0.3	.633	0.528
Total Bilirubin (mg/dl)	1.2±1.1	0.7±0.2	-3.313	0.001
Direct Bilirubin (mg/dl)	0.5±0.6	0.3±0.1	-2.014	0.047
Indirect Bilirubin (mg/dl)	0.7±0.5	0.3±0.2	-3.993	0.000
Total Protein (gm/dl)	5.7±0.8	5.9±0.8	-1.592	0.115
Albumin (gm/dl)	3.1±0.5	2.8±0.5	-2.164	0.033
Globulin (gm/dl)	2.7±0.7	3.9±1.0	-6.260	0.000
AST (U/L)	44.9±11.9	23.6±8.9	-9.864	0.000
ALT (U/L)	38.8±10.0	20.6±12.8	-7.895	0.000
ALP (U/L)	112.5±65.1	100.0±45.4	1.125	0.263
GGT (U/L)	33.7±12.2	20.6±12.8	-5.217	0.000

FBS: Fasting blood sugar  
 HbA1c: Glycosylated haemoglobin  
 AST: Aspartate transaminase  
 ALT: Alanine transaminase  
 ALP: Alkaline phosphatase  
 GGT: Gamma glutamy transferase

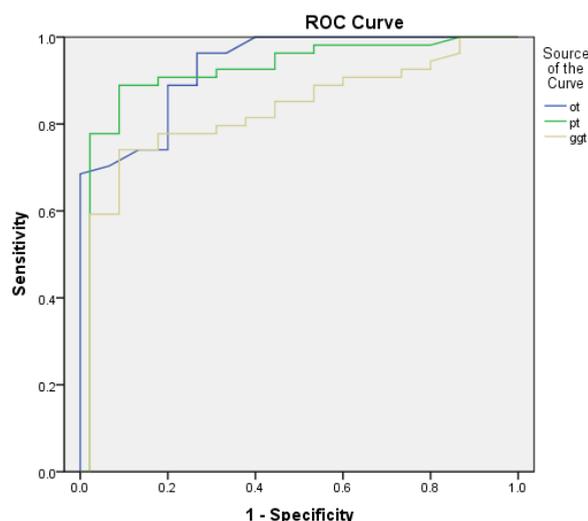
There was a positive correlation between FBS and HbA1c, and the liver enzymes AST, ALT and GGT (P=0.0001). There was a negative correlation with ALP and FBS and HbA1c, though it was not statistically significant (Table 3).

**Table 3: Correlation between FBS, HbA1c and liver enzymes**

		FBS	HbA1c
AST	r	0.580	0.676
	p	0.000	0.000
ALT	r	0.442	0.549
	p	0.000	0.000
ALP	r	-0.158	-0.145
	p	0.119	0.151
GGT	r	0.317	0.374
	p	0.001	0.000

FBS: Fasting blood sugar  
 HbA1c: Glycosylated haemoglobin  
 AST: Aspartate transaminase  
 ALT: Alanine transaminase  
 ALP: Alkaline phosphatase  
 GGT: Gamma glutamy transferase

Best cut-off value of liver enzymes was calculated using ROC curve and the values were for AST, ALT and GGT were >34 IU/L, 28 IU/L and 24.3 IU/L respectively (Fig. 1, Table 4).



**Fig. 1: Best cut-off value of Liver enzymes**

**Table 4: Cut off values of Liver enzymes**

	Area under the curve	Sensitivity %	Specificity %	Best cut off U/L
AST	0.933	66.7	86.7	>34
ALT	0.922	96.3	73.3	>28
GGT	0.831	88.9	91.1	>24.3

AST: Aspartate transaminase  
 ALT: Alanine transaminase  
 GGT: Gamma glutamy transferase

## Discussion

In the current study, FBS, HbA1c, blood urea, serum bilirubin, AST, ALT and GGT were raised significantly in T2DM patients compared to controls. Serum creatinine ( $P=0.5280$ ) and ALP ( $p=0.236$ ) also increased in T2DM compared to controls but it was not statistically significant.

Serum albumin was decreased significantly in T2DM patients compared to controls ( $P=0.033$ ), serum total protein was decreased in T2DM than controls, but was not statistically significant ( $P=0.115$ ).

Belay Z et al<sup>(17)</sup> showed that the mean values of, ALT, AST, ALP, total bilirubin, direct bilirubin and serum glucose were significantly higher in T2DM patients as compared with non-diabetic controls ( $P<0.05$ ). But, serum total protein concentration in T2DM patients were significantly lower in comparison to the control group at 95.0% significance level. In the present study also showed similar findings, but we did not find significant rise in ALP levels. Idris AS et al<sup>(18)</sup> reported that the mean values of ALT, AST and GGT hepatic enzymes were significantly higher in T2DM patients than in controls ( $P<0.001$ ). But, values of total protein and albumin concentrations were significantly lower in diabetes than in controls at 99.0% significance level.<sup>(18)</sup>

The present study found consensus with a study in Sudan, where 50 diabetic patients and 30 control subjects were tested for liver function tests, the mean values of ALT, AST,  $\gamma$ GT, total protein and albumin were reported to be significantly decreased among diabetes compared to the control. However, the mean values were within the normal limits.<sup>(18)</sup>

In a UK cohort study of 959 diabetic patients, 15.7% had raised ALT, 10.4% had increased ALP and only 3.9% had elevated bilirubin levels.<sup>(19)</sup> A similar finding was present in a study by Foster et al., in which the mean values of ALT, AST, ALP,  $\gamma$ -GT, bilirubin and albumin of 60 study subjects with diabetes were within the normal range.<sup>(20)</sup> Likewise in the present study, the mean values of ALT, AST, bilirubin and total protein were within the normal range among 80 diabetes patients. According to a study in Myanmar, the mean values of ALT, AST, ALP,  $\gamma$ GT, bilirubin were within normal limit among 81 diabetics studied. Raised ALT and AST were noted in 18.5% and 14.8%, respectively, and 4.9% had hyperbilirubinemia.<sup>(21)</sup>

Minor elevation of these enzymes level may be a good predictor of mortality from liver disease. Elevation of levels of ALT and AST or both to mild and moderate levels is a very common finding in NAFLD. Similarly, in T2DM patients, chronic mild elevations of liver enzymes are frequently encountered; emphasizing the already known fact that T2DM has a strong association with NAFLD, including its severe form NASH.<sup>(1)</sup>

Studies have also shown beneficial effect of dietary modification, weight loss and exercise in reducing

insulin resistance and in normalization of ALT in patients with NAFLD.<sup>(22)</sup>

Sunita S et al, in their study, AST and ALT were showed significant positive correlation with FBS, whereas GGT did not show significant correlation. Jayaram N et al. showed significant ( $p<0.001$ ) positive correlation ( $r=0.8451$ ) between FBS and ALT.<sup>(23)</sup> Al-Jameil N et al showed positive correlation between FBS with ALT ( $r=0.34$ ) and GGT ( $r=0.21$ ).<sup>(24)</sup> Bora K et al showed no correlation with ALT and AST with FBS.<sup>(25)</sup> In the current study also we found significant positive correlation between FBS with AST, ALT and GGT.

Al-Jameil N et al showed significant positive correlation between HbA1c with ALT( $r=0.32$ ) and GGT( $r=0.20$ ),<sup>(24)</sup> in the present study also HbA1c was positively correlated with AST, ALT and GGT.

Deepika G et al<sup>(26)</sup> and Sunita S et al<sup>(2)</sup> in their study, showed that FBS and HbA1c correlated significantly with ALP; similar findings were observed by Bora K et al<sup>(25)</sup> Current study did not show correlation between ALP and FBS or HbA1c.

In the present study, area under the ROC curve for AST at various cut-off was 0.95 in DM. A maximum sensitivity of 94.74% and specificity of 93.75% were achieved in diabetes at the best cut-off of AST greater than 34.0 mg/dl. Area under the ROC curve for ALT at various cut-off was 0.984. A sensitivity of 100% and specificity of 93.75% were achieved to detect NALD greater than 28.0 mg/dl. The area under the ROC curve for GGT at various cut-off was 0.95 in T2DM. A maximum sensitivity of 94.74% and specificity of 93.75% were achieved in diabetes at the best cut-off of GGT greater than 24.3mg/dl, this means that at the best cut-off value of 24.3mg/dl. We could not find any references in literature explaining such findings.

Limitations of the present study were small sample size, duration of diabetes was not taken in to consideration. Further, large sample size interventional cohort studies are required with radiological or histological correlation. In conclusion liver enzymes were increased in uncontrolled type 2 DM, positively correlated with glycemic markers, when AST  $>34$  u/l and ALT  $>28$ U/L, which can act as predictor for NALD and may be used for further evaluation of the patients

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