Research Article

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www.imedpub.com

Annals of Clinical and Laboratory Research
ISSN 2386-5180

2021
Vol.9 No.12:386

DOI: 10.36648/2386-5180.9.12.386

Association of Liver Function Tests, Lipid Profile and Glycemic Status in a Cohort of Patients with Type 2 Diabetes Mellitus in Sri Lanka

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Citation: Kariyawasan CC, Balasuriya BLT, Ranatunga SACD, Dissanayaka DMC, Herath SRGP (2021) Association of Liver Function Tests, Lipid Profile and Glycemic Status in a Cohort of Patients with Type 2 Diabetes Mellitus in Sri Lanka. Vol.9 No.12:386

Abstract

Type 2 Diabetes Mellitus (T2DM) an endocrine disorder causing chronic hyperglycaemia due to insulin resistance and deficiency leading to multi-organ damage and disturbances in carbohydrate, lipid, and protein metabolism. The objectives were:

To determine and compare the association between glycated haemoglobin (HbA1c) LP and LFT in T2DM patients.

To analyze the association between LFT and LP in T2DM patients and compare within sexes.

A retrospective analytical study performed at the department of pathology. Data of 201 patients ≥ 30 years with T2DM was obtained and statistically analyzed using (SPSS) version 20, descriptive statistical methods, and Pearson's Correlation.

In the total population (136 females, 65 males) between 31 to 83yrs, the LFTs and LP were high. Bilirubin was normal. In patients with HbA1c <7, serum ALP and TG remained normal but serum LDL was high. In those with HbA1c>7 liver enzymes and lipid profile were high. LFTs and LP of both groups revealed a significant positive correlation for ALP (p=0.000), TG (p=0.026) and VLDL (p=0.026) with a negative co-relation for bilirubin (p=0.020). HbA1c of both groups were statistically significant (p-value 0.000). Pearson's co-relation revealed a positive co-relation between HbA1C and VLDL and a negative co-relation between bilirubin and HbA1c. Serum ALT, bilirubin statistically higher in males (p=0.006). Serum ALP (p=0.018), HDL (p=0.040) higher in females.

Positive correlation between HbA1c, ALP, TG and VLDL indicates progression of disease and probability of cardiovascular complications. The negative correlation between HbA1c and bilirubin indicates good control and may be useful in monitoring control.

Keywords: Liver function tests, HbA1c, T2DM, Lipid profile, Serum bilirubin, Alkaline phosphatase

Received: November 03, 2021, **Accepted:** December 16, 2021, **Published:** December 23, 2021

Introduction

Type 2 Diabetes Mellitus (T2DM) is an endocrine disorder. It is characterized by high levels of glucose in the blood resulting from variable degrees of insulin resistance and deficiency. Chronic hyperglycemia can lead to multi-organ damage resulting in many complications on the renal, neurologic, and cardiovascular systems and many disturbances in carbohydrate, lipid, protein metabolism [1-3].

The liver plays an important role in the regulation of glucose homeostasis, during fasting and postprandial periods [4]. Patients with T2DM have a high prevalence of abnormal liver enzymes than non-diabetics. Mild and chronically elevated transaminases

are surrogate markers of insulin resistance [5]. Many studies have shown liver disease plays a vital role in the morbidity and mortality of T2DM patients [6]. Insulin resistance of T2DM patients is responsible for lipid abnormalities [7]. Diabetic dyslipidemia is initiated by the elevation of Triglyceride (TG) rich Very Low-Density Lipoprotein (VLDL-C) from hepatic overproduction [8]. Dyslipidemia is a condition that is characterized by significantly higher serum levels of triglycerides, low-density lipoprotein cholesterol (LDL-C) and total cholesterol (T.CHO) and lower levels of high-density lipoprotein (HDL-C) than normal healthy subjects [9,10]. Alterations of metabolism of cholesterol and liver enzymes have been considered as independent risk factors for the development of Cardiovascular Disease (CVD) [5]. Bilirubin recognized for its antioxidant properties have found to be high in well controlled T2DM.

As there were no published studies done locally on liver enzymes and lipid profiles in T2DM patients, this study was conducted to determine the association of Liver Function Tests (LFT) {Alanine transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphotase (ALP), Serum Bilirubin}, lipid profile, and glycemic status in a cohort of Sri Lankan patients with T2DM.

Materials and Methods

1. To determine and compare the association between glycated haemoglobin (HbA1c) with LP and LFT in T2DM patients.
2. To analyse any association between LFT and LP in T2DM patients and compare with in sexes.

Methodology

Study design

A retrospective analytical study.

Center of the study

Department of Haematology of the Sri Jayewardenepura General Hospital, Sri Lanka..

Study Design

Inclusion Criteria

Adult patients, both males and females diagnosed with T2DM in the age group 30 years and above were included.

Exclusion Criteria

The patients diagnosed with the Type 1 Diabetes Mellitus, hypothyroidism, Cushing's syndrome, chronic systemic illness, acute and chronic liver disease (including acute hepatitis of any cause, viral hepatitis, autoimmune hepatitis, and any illness with jaundice in the past), renal disorders, heart failure, pregnancy, and cancer were excluded from the study [11,12].

Data Collection

A random sampling technique was used, from June 2019 to June 2020, to obtain the laboratory records of 201 patients through the Laboratory Information System (LIS) and patients' records. Investigations of HbA1c, lipid profile, and LFT done on same day were recorded.

Our study population was grouped into two, according to the level of HbA1c. Subjects with HbA1c levels over 7% were grouped as poorly controlled and 7% or lower were grouped as well-controlled T2DM. The normal ranges of liver enzymes and bilirubin in our laboratory were, ALT (7 -35) U/L, AST (<31) U/L, ALP (30-120) U/L and (0.3-1.2) mg/dl respectively.

Normal ranges of Total Cholesterol, Triglycerides, LDL - cholesterol, Cholesterol: HDL-C ratio were (<200) mg/dl, (<150) mg/dl, (<100) mg/dl, (<4) respectively. Normal ranges of HDL-Cholesterol for males and females were (>40) mg/dl and (>60) mg/dl respectively.

Study Tool

A data extraction sheet was used to enter the socio-demographic, medical, and laboratory details.

Ethical Consideration

Ethical approval was obtained from the Ethics Review Committee of Sri Jayewardenepura General Hospital and Post Graduate Traning Centre, Thalapathpitiya, Nugegoda, Sri Lanka.

Statistical Analysis

All numerical and coded data derived from Laboratory Information System (LIS) and patients' records were introduced in a database using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistical methods were used to calculate the median, mean, and the ± standard deviation of age, HBA1c, liver enzymes, and serum lipids. Correlations between study variables were done with Pearson's Correlation method. The *p-value*, lower than 0.05 was considered statistically significant. Coefficient of determination (R Sq) was used as a statistical measure of how close the data are to the fitted regression line. Two diabetes groups were compared by independent sample t-test. Chi Sqaure test was applied for categorical variables.

Results

Of the total of 201 patients, 136 were female and 65 were males. The age range was 31 to 83 years and the mean age ± Standard Deviation (SD) was 59.3 ± 10.56 years.

The descriptive data of the diabetes population is depicted in **Table 1**. The mean ± SD values of serum AST, serum ALT and serum ALP were 26.4 ± 12 U/L, 34.5 ± 18.4 U/L, 90.28 ± 34.0 U/L and showed mild elevation. T. Bil was 0.61 ± 0.34 mg/dl. (Within the normal range).

The mean ± SD values of serum T.CHO, serum TG, serum HDL-C, serum LDL-C, and serum VLDL-C were 173.9 ± 42.9 mg/dl, 115.7 ± 41.7 mg/dl, 48.0 ± 11.6 mg/dl, 102.3 ± 38.7 mg/dl, 23.1 ± 8.3 mg/dl respectively and showed mild increment from the normal range. The T.CHO/HDL-C ratio was 3.7 ± 1.17. The mean ± SD value of HbA1c was 7.85 ± 1.84%.

The descriptive data of the two diabetes groups are mentioned in **Table 2.** The mean ± SD values of serum AST, serum ALT, and serum ALP of the T2DM controlled group were 25.5 ± 11.5 U/L, 32.8 ± 19.2 U/L, 78.3 ± 19.6 U/L respectively. In this group, AST and ALT were higher than the normal but the serum ALP remained

Table 1: Showing the Mean Values and Standard Deviation of Various Hematological Parameters.

Variable	Descriptive data (N= 201)			
	Minimum	Maximum	Mean	Std. Deviation
Age	31	83	59.32	10.56
HbA1C	4.7	14.5	7.85	1.84
Liver function tests				
AST	12.6	69.4	26.4	12.0
ALT	8.0	116.1	34.5	18.4
ALP	36.3	347.0	90.28	34.0
T. Bil	0.2	2.6	0.61	0.34
Lipid profile				
T.CHO	90.0	357.0	173.9	42.9
TRI	41.0	268.0	115.7	41.7
HDL-C	23.0	94.1	48.0	11.6
LDL-C	33.7	246.4	102.3	38.7
VLDL-C	8.2	53.6	23.1	8.3
Ratio	1.7	8.7	3.7	1.17

Table 2: Cases Selected For Alkaline Hemoglobin Electrophoresis On The Basis Of Discrimination Index / Indices.

Variable	T2DM controlled (N= 80)				T2DM poorly controlled (N=121)			
	Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
Age								
HbA1C	4.7	7.0	6.28	0.48	7.1	14.5	8.89	1.67
Liver function tests								
AST	13.0	69.4	25.5	11.5	12.6	67.0	26.9	12.3
ALT	8.0	116.1	32.8	19.2	11.0	113.0	35.6	17.9
ALP	36.3	151.0	78.3	19.6	50.9	347.0	98.1	38.9
T.Bil	0.2	2.6	0.68	0.44	0.2	1.3	0.57	0.23
Lipid profile								
T.CHO	102.0	257.0	176.3	43.3	90.0	357.0	172.3	42.8
TG	45.0	219.7	107.6	38.1	41.0	268.0	121.0	43.2
HDL-C	29.0	94.1	49.3	12.0	23.0	81.0	47.1	11.2
LDL-C	33.7	186.0	104.8	39.1	36.0	246.4	100.6	38.6
VLDL-C	9.0	43.9	21.5	7.6	8.2	53.6	24.2	8.6
Ratio	1.7	6.9	3.7	1.11	2.0	8.7	3.8	1.2

within the normal range. The mean ± SD values of serum T.CHO, serum TG, serum HDL-C, serum LDL-C and serum VLDL-C of this group were described in **Table 2**. Even though the mean ± SD value of the serum TG was within the normal range, the serum LDL-C showed mean ± SD values of 104.8 ± 39.1 U/L which was higher than the upper limit of normal.

There was no statistical significance for the serum AST (p=0.405) and ALT (p=.306) within the groups. However, T.Bil levels of both diabetic groups were compared and revealed a statistically significant relationship (p=0.020) which indicated a higher value in the well-controlled group **Table 3.**

Lipid profile parameters were also compared by independent sample t-test and revealed a significant relationship between the mean ± SD values of serum TG (p=0.026) and VLDL-C (p=0.026) in the diabetic groups but there was no significant relationship for T.CHO (p=0.526), HDL-C (p=0.2), LDL-C (p=0.456) and T.CHO/HDL-C ratio (p=0.537).

Pearson's correlation was done to evaluate the significance of HbA1c with AST, ALT, and ALP and lipid parameters of the diabetes population **Table 4.** There was a significant positive correlation between HbA1C and ALP (r=0.307, *p-value*=0.000) with the R Sq of 0.094 and showing a low linear relationship **Figure 1.** There was a significant positive correlation between HbA1C and TG (r=0.178, *p-value* =0.011) and VLDL-C (r=0.178, *p-value*=0.011) with the R Sq of 0.032 and showing a low linear relationship **Figure 1.**

There was a significant negative correlation between total bilirubin and HbA1c (r=-0.234, *p-value*=0.000) which also revealed a low linear relationship (R Sq of 0.055).

The correlation between the lipid parameters and liver enzymes is depicted in **Table 5**. ALT showed a significant positive correlation with TG and VLDL-C (p=0.014). There was no significant correlation among the other parameters. T.Bil level did not show any significant correlation with lipid profile parameters (p>0.05).

The comparison between male and female diabetes groups is in **Table 06**, revealed, there was a statistically significant relationship for serum ALT (p=0.006), higher in males as opposed to serum ALP (p=0.018), and HDL-C (p=0.040) higher in females.

Table 3: Distribution of total cases.

Variables	T2DM controlled	T2DM poorly controlled	P-value
Number	80	121	
Gender			
Male	30 (37.5%)	35 (28.9%)	0.203
Female	50 (62.5%)	86 (71.1%)	
Age (yrs)	59.5 ± 11.5	59.2 ± 9.8	0.119
HbA1C %	6.28 ± 0.48	8.89 ± 1.67	0
Liver function tests (Mean values)			
AST	25.5 ± 11.5	26.9 ± 12.3	0.405
ALT	32.8 ± 19.2	35.6 ± 17.9	0.306
ALP	78.3 ± 19.6	98.1 ± 38.9	0
T.Bil	0.68 ± 0.44	0.57 ± 0.23	0.02
Lipid profile (Mean values)			
T.CHO	176.3 ± 43.3	172.3 ± 42.8	0.526
TRI	107.6 ± 38.1	121.0 ± 43.2	0.026
HDL-C	49.3 ± 12.0	47.1 ± 11.2	0.2
LDL-C	104.8 ± 39.1	100.6 ± 38.6	0.456
VLDL-C	21.5 ± 7.6	24.2 ± 8.6	**0.026**
Ratio	3.7 ± 1.11	3.82 ± 1.2	0.537

Table 4: Differential Values of Discrimination Indices and the Correctly Identified Cases.

Variables	Correlation (r-value)	R Squared value	p-value
Liver function tests			
AST	-0.066	0.004	0.351
ALT	-0.046	0.002	0.520
ALP	0.307	0.094	**0.000**
T. Bil	-0.234	0.055	**0.000**
Lipid profile			
T.CHO	0.015	-	0.833
TRI	0.178	0.032	**0.011**
HDL-C	-0.093	0.009	0.187
LDL-C	0.01	-	0.887
VLDL-C	0.178	0.032	**0.011**
Ratio	0.091	0.008	0.198

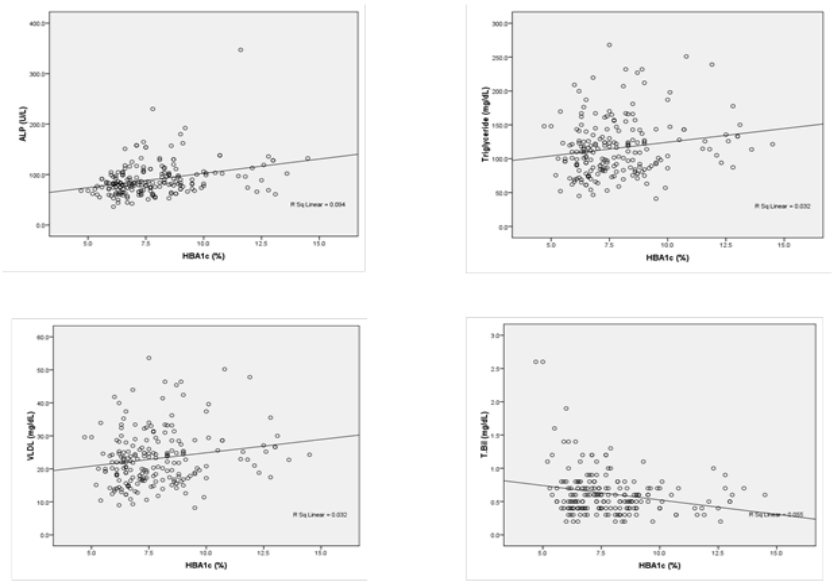

Figure 1 Scatter diagram of ALP, TRI, and VLDL-C with HBA1c.

Table 5: Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) And Youdens Index of Each Discrimination Index.

Variables	r and p values	AST	ALT	ALP	T. Bil
T.CHO	r	-0.109	0.015	-0.006	-0.096
	p	0.125	0.836	0.928	0.175
TRI	r	0.005	0.172	0.131	-0.013
	p	0.948	0.014	0.064	0.850
HDL-C	r	0.023	-0.08	0.058	0.035
	p	0.742	0.261	0.417	0.618
LDL-C	r	-0.128	0.000	-0.047	-0.126
	p	0.069	0.999	0.504	0.074
VLDL-C	r	0.005	0.172	-0.030	-0.013
	p	0.948	0.014	0.675	0.850

Table 6: Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) And Youdens Index of Each Discrimination Index.

Variables	Male	Female	P-value
N	65	136	-
Age (yrs)	58.83 ± 11.7	59.55 ± 10.0	0.177
HbA1C %	7.58 ± 1.8	7.98 ± 1.8	0.965
Liver function tests			
AST	28.56 ± 13.4	25.39 ± 11.1	0.190
ALT	40.08 ± 40.0	31.85 ± 31.8	0.006
ALP	81.48 ± 22.1	94.48 ± 37.8	0.018
T.Bil	0.722 ± 0.05	0.568 ± 0.021	0.006
Lipid profile			
T.CHO	166.33 ± 45.7	177.55 ± 41.2	0.122
TRI	115.89 ± 39.9	115.64 ± 42.6	0.903
HDL-C	43.83 ± 9.2	50.06 ± 12.1	0.040
LDL-C	99.01 ± 40.4	103.89 ± 38.0	0.775
VLDL	23.17 ± 7.9	23.12 ± 8.5	0.903
Ratio	3.88 ± 1.1	3.72 ± 1.1	0.573

There was a significant relationship for T.Bil among male and female diabetes groups (p=0.006) being higher in males.

Discussion

The World Health Organization (WHO) has reported the prevalence of T2DM estimated by the year 2025 would be 380 million people worldwide [13]. A large study on diabetes prevalence in Sri Lanka was published in 2005, which indicated a prevalence of 14.2% among males and 13.5% among females [14,15].

The liver is a very important site for carbohydrates and lipid metabolism. Many studies have shown T2DM patients to have hyperlipoproteinemia and altered liver enzyme levels. Type 2 DM raises the risk of developing Non Alcoholic Fattly Liver Disease (NAFLD) also known as Nonalcoholic Steatohepatitis (NASH). In this condition, liver fat triggers harmful inflammation that creates scar tissue there. This can lead to cirrhosis and end-stage liver disease. Unfortunately, NAFLD has few symptoms, making it tough to diagnose. An unexplained spike in liver enzymes, ultrasound used by liver specialists are the only means to detect this condition. Therefore assessing the liver functions in T2DM patients is a useful prerequisite in assessing the progress or development of NAFLD.

In our study, there was a statistically significant positive correlation between HbA1c with serum ALP, serum TG and serum VLDL-C of all diabetic patients. Other liver enzymes (serum ALT and AST) did not show a statistically significant relationship with HbA1c. A study done in North India revealed serum levels of AST, ALT, and ALP were significantly elevated in T2DM patients as compared to controls($p<0.05$) [16]. This study revealed serum AST elevation was also significant among men which was not revealed in our study. However, our study has shown higher levels of serum ALP in females which was similar to a study done by Deepika et al. [17]. A study done by Kashinakunti et al found that there was no significant rise in ALP in T2DM patients as compared to the healthy control group [10]. In respect of S. Bilirubin a study done in Turkey revealed similar results to ours revealing a statistically negative correlation between serum Bilirubin and HbA1c ($p<0.001$) [18].

Regards to association of liver function tests and the lipid prifile in T2DM patients, ALT showed a significant positive correlation with TG and VLDL-C (p=0.014) in our study with similar results seen in the studies done by Balaji A S et al. [5]. Al-Jamel et al. their study showed ALT having a significant positive correlation with TG, T.CHO, and LDL-C and negative correlation with HDL-C, [4]. ALT in our study revealed a negative correlation with HDL-C, but was not statistically significant. With regards to ALP, a study done

by Rajeshwari et al. [] revealed a positive correlation with TG (r= 0.393), VLDL-C (r= 0.192) and negative correlation with HDL-C (r=- 0.379)(9) which differed in our study, revealing a positive correlation with TG (r=0.131), HDL-C (r= 0.0.058) and negative correlation with VLDL-C (r= - 0.03) and this correlation was not statistically significant. Nigerian study reported a significant positive correlation between ALP, T.CHO and LDL-C [19] and a statistically significant negative correlation with HDL-C. They did not reveal a correlation between other liver enzymes and lipid profile parameters.

A cross sectional study done on Bungladeshi adults has shown higher activity of liver enzymes in subjects having diabetes than subjects who do not have T2DM. The most common abnormality of hepatic enzymes was found for AST, ALP and Gamma-Glutamyl Transferase (GGT). The prevalence of increased liver enzymes was higher in females than in the males in the diabetes group [20-22].

Conclusion

Even though the liver enzymes are higher than the normal range as expected in all the diabetic patients of this study, the positive correlation of ALP with HbA1c level can be used as a marker of the progression of the disease. This could assist in implementing proper glycaemic control in these patients.

The lipid profile too deranged in all T2DM patients of this study, the positive correlation of TG and VLDL with HbA1c is a good indicator of disease progression and increased probability of cardiovascular complications. This can be utilized in the clinical setting to enforce proper glycaemic control measures.

The negative correlation between total bilirubin and HbA1c shown in this study can be utilized in monitoring patients, as the protective antioxidant effect of bilirubin which inhibits the inflammatory damage on organs and tissues by high blood glucose levels can be used as a marker of good glycaemic control.

Strength and Limitations

The use of limited number of biochemical parameters in LFT and the absence of imaging studies of liver to assess liver histopathological changes are limitations of our work. Further, an island wide multi-center study is necessary in Sri Lanka to exactly illustrate a comprehensive conclusion of liver function tests and lipid profile parameters in T2DM population.

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