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Evaluation the Predictors of Non-Alcoholic Fatty Liver Disease (NAFLD) in Type 2 Diabetes Mellitus (T2DM) Patients

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ABSTRACT

Background: NAFLD is the chronic form of liver fatty disease, which is vastly common over all worldwide. It is highly connected with T2DM that is defined by hyperglycemia, insulin resistance (IR), and hepatic malfunctions. Our study was design to determine the predictors for NAFLD' incidence in T2DM patients.

Methods: We conducted study of 299 subjects that were classified into two groups, (110) Non-Diabetic subjects and (189) T2DM patients, then the T2DM patients divided into two groups: T2DM (99) and DM+pre-NAFLD (90) patients. We measured hyperglycemia tests, Total Cholesterol, Triglycerides (Tg), Low-Density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), AST/ALT ratio, and γ-Glulamyltransferase for all groups. We determined the Triglyceride Glucose index, Interleukin-6, Alkaline Phosphate (ALKP), and C Reactive Protein (CRP) levels as biomarkers for IR and NAFLD respectively.

Results: Our data demonstrated that DM+pre-NAFLD patients exhibited a significant increased in TC, TG, and LDL-C, and dramatic decreased in HDL-C compared to DM patients and Non-DM subjects. - AST, ALT, AST/ALT ratio and GGT levels showed a significant elevation in DM+pre-NAFLD and DM patients compared to the Non DM subjects. The data showed that TyG Index, IL-6, CRP and ALPK levels were increased in DM+pre-NAFLD patients only. The data showed a positive correlation between dyslipidemia, and liver function tests with IR and NAFLD biomarkers in DM+pre-NAFLD patients only.

Conclusions: In T2DM, dyslipidemia deters liver enzymes functions, promotes the IR and increases the fatty liver accumulation. Liver enzymes, TyG Index, IL-6, CRP and ALPK could be non-invasive biomarkers for NAFLD in T2DM patients.

Keywords: Predictors, non-alcoholic fatty liver disease, 2 Diabetes Mellitus.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) and T2DM are two majoring health disorders among people, and are greatly coincided because they have share the same risk factor of fat accumulation and IR [1-4]. Recently, many studies demonstrated that liver diseases are the major public health problems in diabetic patients, which include malfunction of hepatic enzymes, NAFLD, non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma (HCC) [4-6]. NAFLD is a metabolic disease has wide range of hepatic dysfunctions starts from simple fat accumulation to complete liver failure [5,6]. Moreover, The incidence NAFLD is increased up to 70% in diabetic and obese patients [7]. Furthermore, the patients with NAFLD are without clear clinical symptoms and can be diagnosis from abnormal level liver enzymes during routine lab tests [8,9]. Additionally, several studies have confirmed there is a close association between NAFLD and T2DM [9-11]. Pathogenesis of NAFLD is still unclear and IR is thought to be the main player in ectopic fat accumulation specially triglycerides [12].

Furthermore, IR induces an ectopic fat accumulation in liver, which promotes dyslipidemia and hyperglycemia and the alteration in triglycerides metabolism is essential for dyslipidemia in T2DM patients [12,13]. IR plays a major role in progress dyslipidemia in T2DM patients through inducing several factors because insulin is responsible for lipolysis of adipose tissue [14-16]. IR in T2DM patients enhance passing the fatty acid from fatty tissue and deteriorate insulin roles in taken of free fatty acid by skeletal muscles [15-17]. Thus, increasing ectopic flux of free fatty acid (FAA) to liver [17]. In fact, the levels of FAAs are increased in subjects with impaired FBG tolerance, which proposes that IR associated with FAA elevation happens before the incidence of hyperglycemia [16-18]. Besides, previous studies demonstrated that decline in glucose consumption in skeletal muscles was correlated with constant elevation of FAA in patients without diabetes.

In addition, various epidemiological researches revealed a clear link between IR and FAA elevation [18-20]. Therefore, in presence of IR, FAAs are accumulated in liver, pancreas and heart and muscle in the form of triglycerides [19-21]. Notably, drug like thiazolidinedione that lowers FAAs level have been shown to decrease IR in liver, pancreas, heart and adipose tissue [21]. Moreover, IR promotes lipase activity that is the main enzyme for phospholipids hydrolysis, which is present in HDL and LDL particles [22]. In addition to that, liver has the control the human body metabolism through many metabolic processes that control glucose and lipids metabolism [23]. Moreover, liver dysfunctions cause massive eruption in glucose and lipid metabolisms [22-24].

Therefore, liver enzymes are biomarkers for multiple metabolic diseases like DM, NAFLD, NASH and obesity [24]. Furthermore, these biomarkers are considering as non-invasive indicators for multiple liver dysfunction. Moreover, liver malfunction is an essential player in the metabolic diseases. Liver's malfunction is the main factor for all the metabolic disorder [25]. The production of glucose inside the liver is complicated process and is well controlled via different mechanisms; insulin controls fat tissue lipolysis, and the FFA in turn adjust hepatic glucose production. Glucose itself is an essential regulator of liver metabolism

("glucose effectiveness") [26,27]. The incidence of NAFLD and its main risk factors in T2DM patients is poorly studied in Southern part of Iraq. This project was design to determine the non-invasive predictors of NAFLD and how the dyslipidemia can enhance NAFLD incidences and progression in T2DM patients.

MATERIAL AND METHODS

Participants

All subjects were selected from the outpatient of two health centers at Southern part of Iraq. In this study, a total of 299 subjects divided into two groups (110) Non-Diabetic subjects and (189) T2DM patients. An exclusion criteria was applied to all subjects and patients with type 1 DM, hepatitis C or B, liver cirrhosis, jaundice, liver tumors, or under specific treatment that affects liver functions, chronic illness and alcoholic drinking were not included in this study. Prior to the study, an informed written consent was acquired from each participant and the project approved thru Institutional Ethical Committee. The age of the subjects was 35-65 years old. All subjects were considered as T2DM patients in accordance with world health organization criteria of fasting blood glucose ≥ 7mmmo/l or diagnosed by physician

Samples collection and biochemical test

After 8hrs overnight fasting, blood samples were collected into clean tube and 1 ml of blood sample was kept in EDTA coated tube to measure HAb1c level. For All other biochemical tests, serum samples were collected by blood centrifugation at 3000 rpm for 10 minute and stored at -70C° for further analysis. Fasting Blood Glucose (FBG), Total cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-Glulamyltransferase Alkaline Phosphate (ALP), and C-reactive protein were measured using enzymatic kit procedures. Low Density Lipoprotein Cholesterol (LDL-C) level was measured using Friedewald formula [28].. Triglycerides glucose (TyG) index was measured using the following formula: ln [fasting TGs (mg/dL)×fasting glucose (mg/dL)/2] [29,30]. IL-6 levels in plasma were detected with an enzyme-linked immunosorbent test.

Statistical analysis

Data were denoted as mean ± SD (standard deviation) values. Statistical analyses were done using one-way analysis of variance (ANOVA) to determine the difference between the groups at *$P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ significant levels. Furthermore, the correlation coefficient (r) value used to find the association between the parameters at level of 0.05 values. Pearson's correlation was conducted to identify the correlation, and the values of correlation coefficient (r) were denoted at 0.05 significant level.

RESULTS

The total 299 subjects were participated in this study, which were classified into two groups, 110 as non-diabetic subjects group and 189 T2DM patients group. The baseline features for all subjects showed in Table 1.

The hallmark of T2DM is chronic hyperglycemia that result due to disturbance in insulin secretion and function [1-4].. We measured the FBG for all subjects using enzymatic kit analysis; our data showed that patients with DM and DM+Pre-NAFLD had a significant elevation ($p<0.001$) in FBG compared to non-diabetic subjects as shown in [Figure1. A]. Next, in order to confirm the hyperglycemic status of DM and DM+Pre-NAFLD patients, we determined the level of blood glucose over long time using glycated hemoglobin or hemoglobin A1c (HbA1c) test. Our data in [Figure1 B] indicated that all DM and DM+Pre-NAFLD patients exhibited a significant increase in glycated hemoglobin level compared to non- diabetic individuals. Next we sought to measure the albumin and globulin levels for all groups. Our results demonstrated that the albumin and globulin levels significantly ($p<0.001$) increased in DM+Pre-NAFLD patients compared to DM and Non-DM groups [Figure1. C and D]. Together, our data showed all patients with DM and DM+Pre-NAFLD exhibit high blood glucose in short and long period of time compared to non-diabetic subjects.

Next, dyslipidemia is the main cause for fatty liver diseases mainly NAFLD and NASH[4-6]. To investigate the connection between dyslipidemia and NAFLD incidence and progression in the subjects suffering from T2DM, we measured the TC, Tg, LDL-C, and HDL-C levels for all subjects groups. Our results in [Figure2. A-C] displayed that about (90) T2DM patients have significant elevation ($p<0.001$) in TC, Tg, and LDL-C compared to (99) T2DM patients and non-diabetic subjects. Additionally, the data exhibited significant decline ($p<0.001$) in HDL-C in these (90) T2DM patients compared to (99) T2DM patients and non-diabetic subjects as shown in [Figure2. D]. Therefore, the data suggest that these (90) patients are more probably ready to develop NAFLD than the other (99) patients and we called them as DM+Pre-NAFLD patients.

Moreover, Liver function tests are useful method to screen and monitor the hepatic malfunctions such as jaundice, different types of hepatitis, NAFLD, NASH, and liver cirrhosis[23]. The routine screen of liver function tests can also be used as a diagnostic tool for patients with T2DM to monitor the liver roles through maintaining the hemostasis balance of the blood glucose inside human body [23.25]. Therefore, we measured ALT, AST, ALT/AST ratio and GGT levels for all subjects using the enzymatic kits procedure. Our results exhibited a significant increase ($p<0.001$) in ALT, AST, ALT/AST ratio, and GGT in DM+Pre-NAFLD patients compared to T2DM patients and non-diabetic subjects as shown in [Figure3. A-D]. Overall, the chronic elevation of liver function tests in T2DM patients could be non-invasive bimolecular markers to monitor and predict the prevalence and development of NAFLD in diabetic patients.

In addition, we tested the consequence effects of hyperglycemia, dyslipidemia, IR and malfunction of liver in NAFLD incidence and progression in T2DM patients. For IR development and incidence in T2DM patients, we used TyG Index and Interleukin-6 (IL-6) level as biomarkers for

IR as previously done [30-32]. In addition, IL-6 is a cytokine that regulate immune system and acute phase hemostasis reaction [31,32]. Our results demonstrated that the patients with DM+Pre-NFLAD have greater TyG Index (4.9) compared to T2DM patients and non-diabetic subject. Moreover, the data showed the level of IL-6 significantly elevated in DM+Pre-NFLAD patients only as shown in [Figure4. A and B]. Then, we measured the level of CRP and ALKP as biomarkers for NAFLD, steatohepatistis and hepatic fibrosis respectively [33-35]. The data showed that the DM+Pre-NFLAD patients display a significant increment in CRP and ALKP levels compared to T2DM patients and non-diabetic subject respectively as shown in [Figure4. C and D]. In summary, our results suggest there are clear associations among hyperglycemia, dyslipidemia, IR and NAFLD occurrence and development in T2DM patients.

Moreover, we asked if there is any relationship between hyperglycemia, dyslipidemia, malfunction of liver tests and IR and NAFLD biomarkers, we used the correlation coefficient test (r) at level ($p<0.05$) to study this relationship. Our data revealed there is a positive correlation among hyperglycemia, elevation of lipid profiles, liver function tests and IR and NAFLD biomarkers specifically in patients with DM+Pre-NFLAD as shown in Table 1.

DISCUSSION

The incidences of NAFLD in T2DM in adult subjects have been estimated more than 70%. Also, NAFLD and NASH can be associated with other liver diseases like the different types of hepatitis (A,B,&C), autoimmune hepatitis, hemochromatosis, and hypothyroidism. Spectrum of NAFLD includes NAFL, NASH, liver cirrhosis ,and hepatocellular carcinoma (HCC) [25,26]. NALFD is to become the main and initial cause for liver diseases in all over worldwide because the large scale of disease development and decreasing the weight is the effective way to treat it [25]. Furthermore, T2DM and obesity are epidemiologically and pathologically associated with fatty liver diseases such as NASH and NAFLD [2,3]. Furthermore, the prevalence and incidence of NAFLD are increasing in developed countries because the changing of life style, obesity, and lack of physical exercise [30-32]. Many studies had showed that the people are suffered from NAFLD at age between 30-60 years old in developing countries. Liver biopsy is the typical technique to diagnosis all liver diseases, but it is not possible, an invasive, and high cost procedure to perform to each patient [30]. Therefore, it is important to use simple tests and molecular biomarkers to monitor and identify all subjects that are highly risk for fatty liver diseases.

Moreover, NAFLD is coupled with hyperglycemia, dyslipidemia, and IR and all these features are described in T2DM patients. Our results revealed a significant elevation of FBG and HAb1c in DM and DM+Pre-NFLAD patients compared to non-diabetic subjects. These results likely are reasonable because insulin's loss impact on the hepatic glucose drive to glycogenolysis and enhance the hepatic production of glucose [33-35]. In addition to the hyperglycemia, our data exhibited an obvious increase in TC, TG and LDL-C levels and significant decline in HDL-C level in DM+Pre-NFLAD patients compared to DM patients and non-diabetic subjects respectively. The increase of lipid profile in DM+Pre-NFLAD patients due to insulin' malfunctions and this lead to ectopic accumulation of triglycerides and enhance the lipolysis process in hepatic tissue. As a result the level of lipids will enhance in the blood of diabetic patients.

Furthermore, the data confirmed a clear elevation of liver function tests DM and DM+Pre-NFLAD patients compared the non-diabetic subjects. Chronic elevation of ALT and AST in blood circulation is a reflection of IR and hepatic tissue injury. In addition, our data showed GGT level was also increased in patients with DM and many studies suggested that GGT as biomarker for IR in diabetic patients [36-39]. Furthermore, several studies proposed that the elevation of liver function tests abnormalities is a hallmark of NAFLD in DM patients. In addition, our data showed that IR and NAFLD biomarkers were significantly elevated in DM+pre-NAFLD patient compared to DM patients and Non-diabetic subject. These results are reasonable because all these markers reflect the hepatic damage and inflammation due to ectopic fat accumulation and dysfunction of liver enzymes.

CONCLUSIONS

Altogether our data have confirmed that hyperglycemia and dyslipidemia in T2DM patients deter liver enzymes functions, raise IR incidence and enhance the fatty liver accumulation and progression. In addition, liver functions tests, TyG index, CRP, and ALKP show a progressive correlation with hyperglycemia and lipid profile tests and adverse correlation with HDL-C in DM+Pre-NAFLD patients. Therefore, routine screening of lipid profile parameters and liver enzymes tests and IR analysis could be non-invasive biomarkers for all fatty liver diseases particularly NAFLD in T2DM patients and to prevent its incidence and progression.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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Table 1: The basic characteristics of the study subjects

The characteristic	Non-diabetic subjects	T2DM patients
Male Female	75 35	131 58
Age (Y)	46±10.1	45±12.2
Body weight (Kg)	82.1±10.3	84.3±13.5
BMI	28±2.4	29± 3.3
Periods of T2DM (Y)	-	8-15
Smoking status Smoker Non -Smoker	 19 91	 32 157

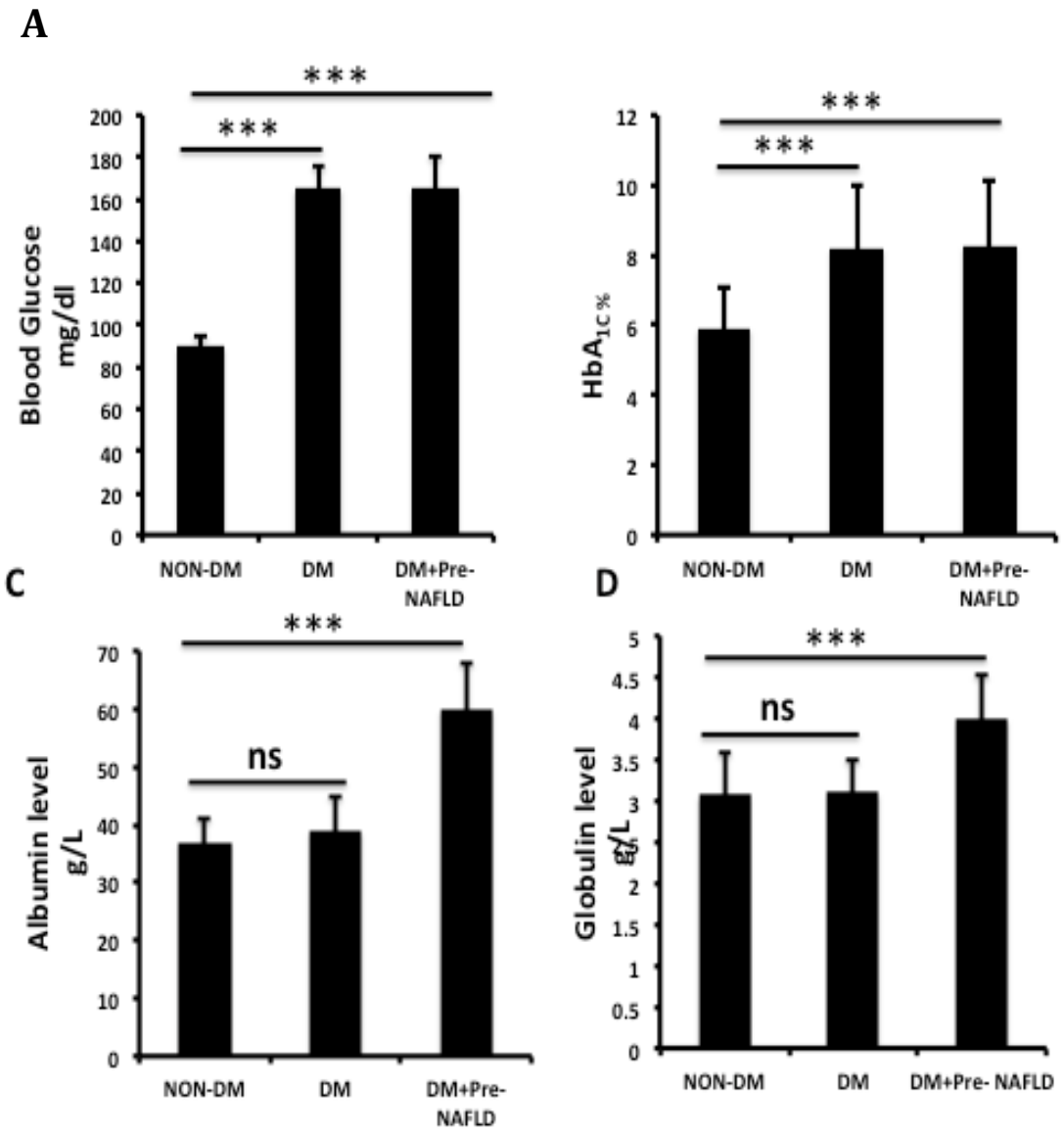

Figure 1: DM and DM+pre-NAFLD patients exhibit hyperglycemia compared to non–DM subjects.

A- The level of FBG. B- The level of HbA1c. C-D The level of Albumin and globulin respectively elevate in DM+pre-NAFLD compared to DM patients and Non-DM subjects. The data are representative results from 110 Non-Diabetic subjects and (99) DM and (90) DM+pre-NAFLD patients respectively. Results are showed as means ± SD values and statistical significance (*P*) was determined by one-way analysis of variance (ANOVA) at $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$

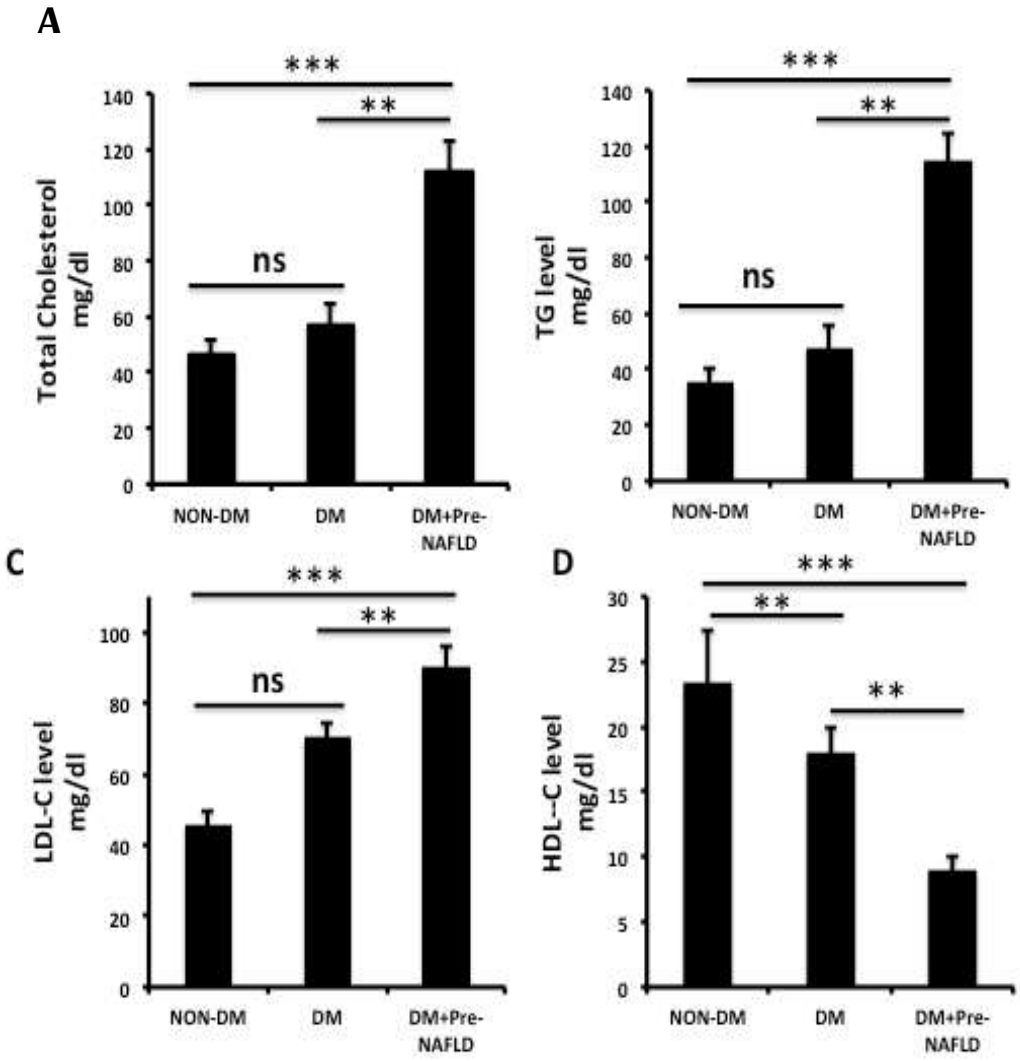

Figure 2. DM and DM+pre-NAFLD patients show dyslipidemia compared to non–DM subjects.

A- The level of Total Cholesterol (TC). B- The level of TG. C- The level of LDL-C. D- level of HDL-C. The data are representative results from 110 Non-Diabetic subjects and (99) DM and (90) DM+pre-NAFLD patients respectively. Results are showed as means ± SD values and statistical significance (*P*) was determined by one-way analysis of variance (ANOVA) at $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

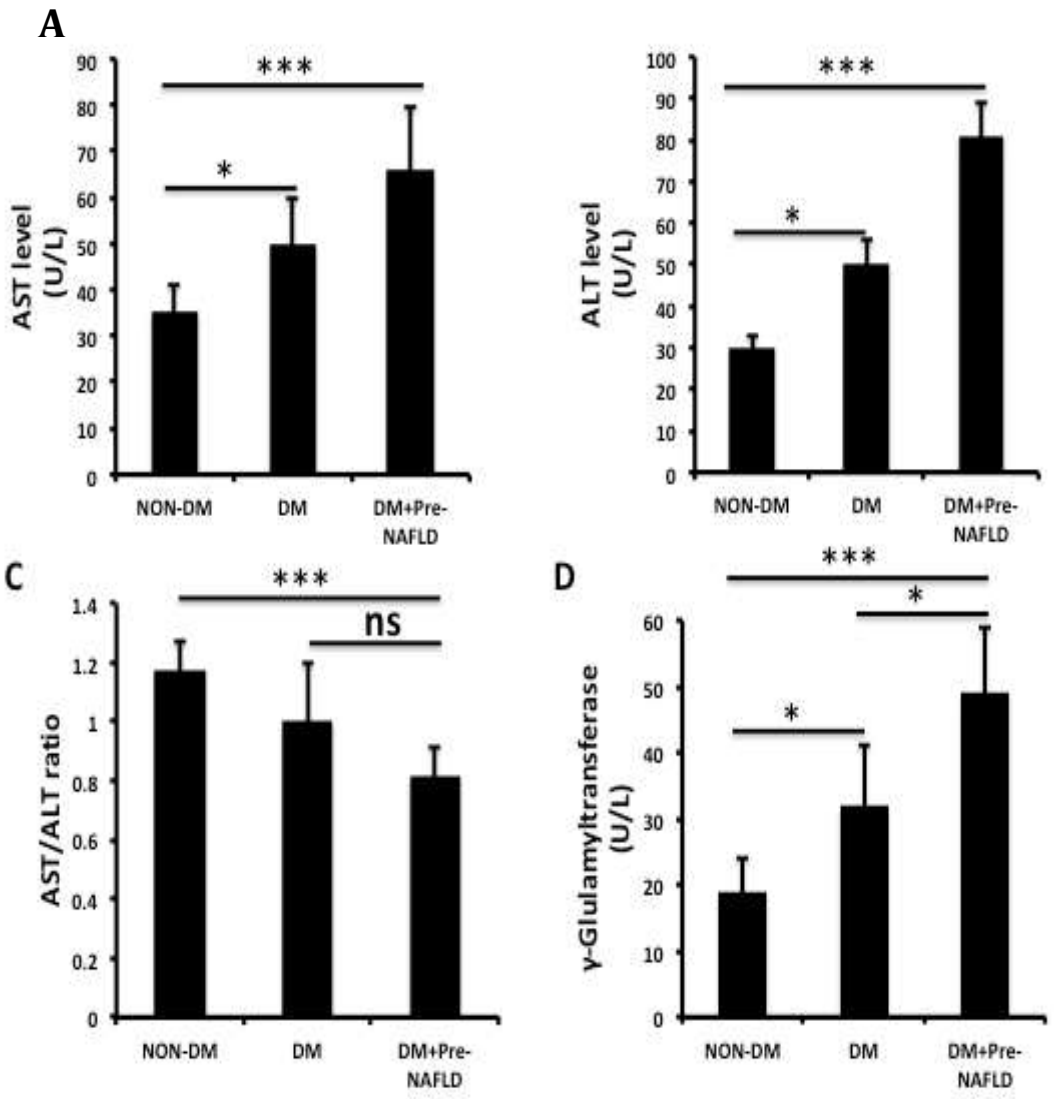

Figure 3: DM and DM+pre-NAFLD patients exhibit an elevation of liver function tests levels compared to non–DM subjects.

A- AST. B- The level of ALT. C- AST/ALT ratio. D- The level of GGT. The data are representative results from 110 Non-Diabetic subjects and (99) DM and (90) DM+pre-NAFLD patients respectively. Results are showed as means ± SD values and statistical significance (*P*) was determined by one-way analysis of variance (ANOVA) at $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$

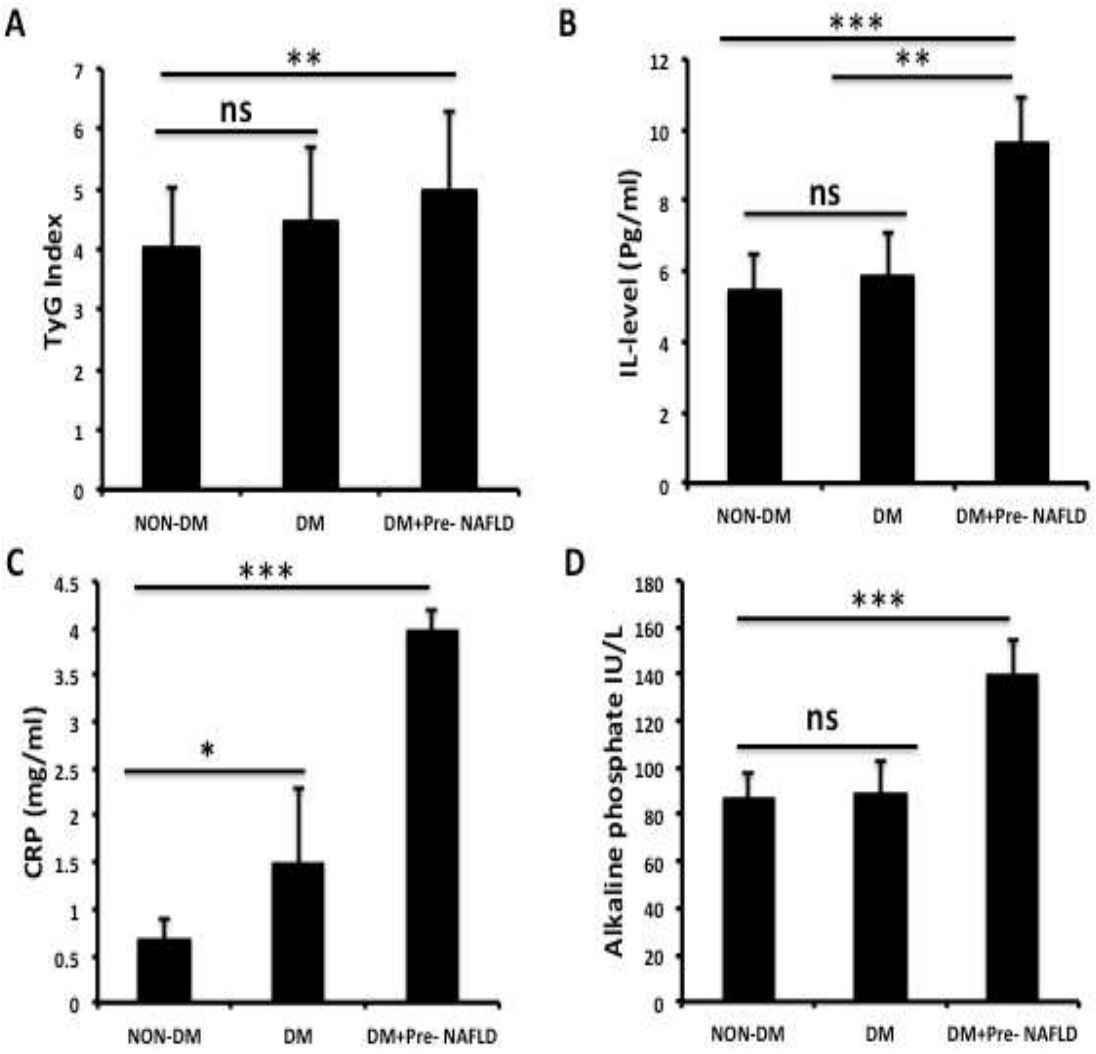

Figure 4: DM+pre-NAFLD patients exhibit an elevation in TyG index, IL-6, CRP and ALKP levels compared to DM patients and non–DM subjects.

A- TyG index. B- The level of IL-6 . C- The level of CRP D- The level of ALKP. The data are representative results from (110) Non-Diabetic subjects , (99) DM and (90) DM+pre-NAFLD patients respectively. Results are showed as means ± SD values and statistical significance (*P*) was determined by one-way analysis of variance (ANOVA) at $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

Table 2: The correlation between hyperglycemia, with dyslipidemia and risk factors of NAFLD in DM patients

Test	DM patients							DM+Pre-NAFLD Patients						
	CRP	ALT	AST	ALKP	GGT	TyG Index	IL-6	CRP	ALT	AST	ALKP	GGT	TyG Index	IL-6
FBG	0.15	0.16	0.23*	0.18	0.20*	0.29*	0.13	0.34*	0.33*	0.28*	0.29*	0.22*	0.35*	0.38*
HbA1c	0.15	0.24*	0.16	0.15	0.22*	0.28*	0.11	0.24*	0.25*	0.19	0.32*	0.31*	0.29*	0.24*
TC	0.21*	0.12	0.14	0.13	0.26*	0.14	0.16	0.35*	0.11	0.08	0.09	0.33*	0.29*	0.22*
TG	0.23*	0.26*	0.17	0.22*	0.32*	0.26*	0.14	0.21*	0.12	0.23*	0.19*	0.25*	0.31*	0.30*
LDL-C	0.22*	0.30*	0.16	0.15	0.31*	0.12	0.11	0.19*	0.10	0.07	0.31*	0.24*	0.22*	0.21*
HDL-C	0.11	0.14	0.13	0.12	-0.32*	-0.29*	-0.27*	-0.28*	-0.31*	-0.46*	0.15	-0.30*	-0.18*	0.13
ALT	0.17	1	0.21*	0.16	0.22*	0.24*	0.11	0.29*	1	0.22*	0.29*	0.22*	0.25*	0.19*
AST	0.16	0.15	1	0.15	0.16	0.21*	0.13	0.24*	0.13	1	0.29*	0.18*	0.22*	0.27*
GGT	0.14	0.19*	0.16	0.14	1	0.22*	0.12	0.22*	0.20*	0.19*	0.29*	1	0.23*	0.31*

Table 2: The Correlation between hyperglycemia with dyslipidemia and risk factors in DM patients using correlation coefficient (r) at *P* value *(<0.05*)* significant level.

FPG= Fasting plasma glucose. HbA1C. ALT= Alanine aminotransferase. AST= Aspartate aminotransferase. GGT= Gamma glutamyl transferase. TC= Total cholesterol. LDL-C = Low density lipoprotein cholesterol. HDL-C = High density lipoprotein cholesterol. TG= Triglycerides. CRP=C-Reactive Protein. IL-6=Interleukin 6. Triglyceride Glucose Index=TyG index, and Alkaline Phosphate =ALKP.