Relation between Serum Calcium Level and Non Alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus

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Abstract: **Background*:*** Calcium homeostasis has been shown to affect insulin resistance and secretion. Moreover, several studies have reported that elevated serum calcium is associated with an increased risk of developing metabolic abnormalities, including type 2 diabetes mellitus (DM). However, it remains unclear whether serum calcium level can affect the presence of nonalcoholic fatty liver disease (NAFLD), which has been considered as the hepatic expression of metabolic syndrome. **Aim of the present study** was to investigate the relationship between serum calcium level and NAFLD in patients with type 2 DM. **Subjects and Methods*:*** This study was conducted on 100 subjects subdivided into 3 groups**:**20 apparently healthy persons with no history of diabetes mellitus or non-alcoholic fatty liver disease (NAFLD) used as a control group (Group I), 40 Diabetic patients (T2DM) with non-alcoholic fatty liver disease (NAFLD) as (Group II) and 40 Diabetic Patients (T2DM) without non-alcoholic fatty liver disease (NAFLD) as (Group III). They were subjected to full history taking & thorough clinical examination, and they investigated for Total Serum Calcium, Fasting and 2 hours post prandial blood glucose level, Glycated haemoglobin (HA1c), Lipid profile (Total Serum Cholesterol, Serum Triglycerides, HDL-Cholesterol and LDL-Cholesterol), Liver enzymes (AST, ALT and GGT), Serum Creatinine & urea, Ultrasonography for NAFLD evaluation and calculation of fatty liver index. **Results:** The major findings made in the current study are the discovery of significant correlation between total serum calcium concentrations with fasting serum glucose. Diabetes mellitus is a condition in which cell calcium homeostasis is impaired. Association of NAFLD with diabetes causes more increase of serum calcium suggesting the possibility that increased serum calcium may in part be involved in developing NAFLD in subjects with type 2 DM. FLI, was suggested as a proxy for fatty liver. Also only GGT was an independent predictor of FL while AST and ALT level has a little diagnostic or prognostic value when assessing patients for NAFLD. In addition, total cholesterol, LDL cholesterol and plasma triglyceride levels were significantly higher in subjects with NAFLD. **Conclusion*:*** These findings suggest the possibility that increased Total serum calcium may in part be involved in developing NAFLD in subjects with type 2 DM.

[Sanaa Sayed Hafez Gazareen, Alaa-Eldin Abd-Elsalam Dawood, Ashraf Anas Zytoon, Yaser Abd-Elsatar Elghobashy[3]and Khalid Tahseen Ahmed Gabr **Relation between Serum Calcium Level and Non Alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus.** *Stem Cell* 2017;8(3):86-98]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 13. doi:10.7537/marsscj080317.13.

Keywords: Serum Calcium Level, Non Alcoholic Fatty Liver Disease, Type 2 Diabetes Mellitus.

1. Introduction

Worldwide, 382 million people, or 8.3% of adults, are estimated to have diabetes. About 80% live in low and middleincome countries. If these trends continue, by 2035, 592 million people, or one adult in 10, will have diabetes. This equates to approximately three new cases every 10 seconds or almost 10 million per year. **Type 2 diabetes,** which is characterized by hyperglycemia, accounts for 90% of all diabetes cases worldwide ***(IDF Diabetes Atlas 2013).***

Calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue, with a very narrow range of $Ca^{2}i$ (ionized calcium) needed for optimal insulin-mediated functions. Changes in $Ca^{2}i$ (ionized calcium) in primary insulin target tissues may contribute to peripheral insulin resistance via impaired insulin signal transduction, leading to decreased glucose transporter-4 activity. Results from randomized trials on the effect of calcium supplementation on insulin resistance show either no effect or improvement of insulin action with supplementation ***(Pittas et al., 2007).***

The Resnick ionic hypothesis suggested that metabolic disorders, such as metabolic syndrome, and diabetes, share a common, altered intracellular condition, characterized by elevated free intracellular Ca^{+2} (calcium) level ***(Wang et al., 2013)***. Increasing intracellular calcium levels have been shown to decrease the effect of insulin in adipocytes due to reduced number of glucose transporters (GLUT4) and decreased insulin receptor activity. The dietary intake

of calcium did not seem to influence insulin sensitivity ***(Hagstrom et al., 2007).***

Non Alcoholic Fatty Liver Disease (NAFLD) is defined by fat accumulation in the liver exceeding 5% of its weight. Insulin resistance is related to obesity and is central to the pathogenesis of NAFLD. In addition, oxidative stress and cytokines are important contributing factors, together resulting in steatosis and progressive liver damage. The disease can remain asymptomatic for years, or can progress to cirrhosis. Triggers for considering a diagnosis of Non Alcoholic steatohepatitis (NASH) and starting testing of liver enzymes are: hypertension, type 2 diabetes, sleep apnea, a positive family history, non-black ethnicity, obesity, hyperlipidemia, and a sedentary lifestyle. Proper control of diabetes, hyperlipidemia, and cardiovascular risks is recommended. Studies with atorvastatin and pravastatin have shown improvement in patients with NASH. NAFLD patients with dyslipidemia should be treated with statins ***(La Brecque et al., 2014).***

Non Alcoholic Fatty Liver Disease (NAFLD) is a pathological condition consisting of a spectrum of liver diseases due to macro vesicular accumulation of triglycerides within hepatocytes (hepatic steatosis). NAFLD is also strongly associated with overweight/obesity, insulin resistance (IR), and type 2 diabetes (T2DM) ***(Ratziu et al., 2010).*** In developed countries, NAFLD is observed in 20-30% of the general populationand in 75% of type 2 diabetic patients; necro-inflammatory activity and fibrosis coexist in the 2-3% of cases (nonalcoholic steatohepatitis, NASH) and mayevolve in cirrhosis and liver failure in 20-25% of affected subjects. Currently, NAFLD is considered one of the leading causes of cryptogenetic cirrhosis ***(Leite et al., 2009).***

Changes in $Ca^{2}i$ (ionized calcium) modulate adipocyte metabolism, which may promote triglyceride accumulation via increased de novo lipogenesis and inability to suppress insulin-mediated lipolysis leading to fat accumulation. Patients with type 2 DM exhibit impaired cellular calcium homeostasis including defects in adipocytes, and liver ***(McCarty & Thomas 2003).***

2. Patients and Methods

A total of **100** subjects were included in our study and were categorized into 3 groups:

Group I:20apparently healthy persons with no history of diabetes mellitus or non-alcoholic fatty liver disease (NAFLD), aged from 42 - 60 years, 14 males (70 %) and 6 female (30 %), used as a control group.

Group II:40 Diabetic patients (T2DM) with non-alcoholic fatty liver disease (NAFLD), aged from 45 - 66 years. They were 27 males (67.5 %) and 13 females (32.5 %).

Inclusion Criteria:

All the patients attending diabetic (T2DM) were considered for the study.

Exclusion Criteria:

Patients giving a history of alcohol intake, pregnancy, known hepatic diseases, hepatotoxic drug intake, and patients with type 1 diabetes mellitus were excluded.

Group III:40 Diabetic Patients (T2DM) without non-alcoholic fatty liver disease (NAFLD), aged from 43 - 64 years. They were 29 males (72.5 %) and 11 female (27.5 %).

Inclusion Criteria:

All the patients attending diabetic (T2DM) were considered for the study.

Exclusion Criteria: Patients giving a history of alcohol intake, pregnancy, known hepatic diseases, hepatotoxic drug intake, and patients with type 1 diabetes mellitus were excluded.

- The patients were recruited for the study on the basis of standard clinical and laboratory criteria for diagnosis of Type 2 DM. They were selected from EL-Menoufia University Hospital, Internal Medicine Department.
- All the study subjects answered a questionnaire, which contains details of age, gender, alcohol intake and medical history. The study subjects had to provide detailed information on the height, weight and waist circumference were noted down for all the patients and recorded in a structured protocol format.

Blood sampling:

- About 5ml of blood was taken from all subjects included in this study (*After a 12 hour overnight fast*) and centrifuged for serum separation for estimation of [**Fasting blood glucose**, **Total Serum calcium** (mg/dl), **lipid profile** (Total Serum Cholesterol, HDL Cholesterol, LDL Cholesterol and serum Triglycerides) and **Liver enzymes**, AST (aspartate transaminase), ALT, (alanine transaminase) and GGT, (Gamma-Glutamyl Transferase) levels].
- About 3ml of blood was taken from all subjects included in this study (while they were post prandial state 2 hour after estimation of fasting blood glucose) and centrifuged for serum separation for estimation of [**2 hours post prandial blood glucose level**, **Serum Creatinine & Urea**].
- About 2ml of blood was taken from all subjects included in this study (while they were in post prandial state) on ethylene diamine tetra-acetic acid (EDTA) tube for estimation of **Glycated haemoglobin (HA1c)**.

All patients and controls were subjected to the following investigations:

1- Determination of Fasting and 2 hours post prandial Serum Glucoselevel: by *AUTOMATED TC-*

MATRIX CHEMISTRY ANALYZER (USA), based on *Colorimetric method* ***(Caraway & Watts 1987)***.

2- Determination of Glycated haemoglobin (HA1c): by *Spectro-photometer RA50* ***(Bry et al, 2001).***

3- Determination of Total Serum Calcium: by *Spectro-photometer RA50*based on *Colorimetric method* ***(Friedman et al., 1980).***

4- Determination of Lipidprofile: ***Total Serum Cholesterol, Serum Triglycerides****and* ***HDL-Cholesterol*** were determined by *AUTOMATED TC-MATRIX CHEMISTRY ANALYZER (USA)* based on *Colorimetric method* ***(Jalali et al., 2013, Tarchalski et al., 2003 and Matsuzaki et al., 1996***respectively***).*** ***While LDL-Cholesterol*** was determined by using *Friedwald's formula:* LDL-C = Total Cholesterol – (Triglycerides/5 + HDL-Cholesterol).

5- **Determination of Serum Creatinine and Urea:** by *AUTOMATED TC-MATRIX CHEMISTRY ANALYZER (USA),* based on *Colorimetric-kinetic modified jaffe's method* ***(Tietz 1995****for Creatinine assay* and ***Kaplan et al., 1984****for Urea assay).*

6- Determination of Liver Enzymes *(AST, ALT* and *GGT):* by *AUTOMATED TC-MATRIX CHEMISTRY ANALYZER (USA),* based on *kinetic method* ***(Thefeld et al., 1994****for AST* ***& ALT*** and ***Tholen et. al., 2004****for GGT).*

7- Fatty liver assessment, by the following**:**

(A) Ultrasonography (Imaging modality used in this study). Using*a sonoscape apparatus*equipped with a convex 3,5 MHz probe. Steatosis was graded according to ***(Saverymuttu et al., 1986 and Kojima et al., 2003)*** into**0**, absent; **1**, mild; **2**, moderate; **3**, severe.

(B) Calculation of fatty liver index. Using ***Bedogni et al., 2006*** formula as follows:

FLI = {exp 0.953 × log (triglycerides) + 0.139×BMI + 0.718 × log

(GGT) + 0.053 × waist circumference - 15.745)/ (1 + exp 0.953 × log (triglycerides) + 0.139 × BMI + 0.718 × log (GGT) + 0.053 × waist circumference - 15.745)} × 100.

Statistical analysis

In addition to the descriptive data, statistical analysis was done using IBM SPSS STATISTC VERSION 20 PROGRAM. Data were expressed as mean ± SD and analyzedusing the ***Chi square (x2) test*** and the ***Anova Test***to assess the significance of difference in the levels between different parameters.

$P < 0.05$ was accepted as significant. Coefficient (r) of two variables was also done by using ***Pearson Correlation Coefficient (r)*** *with* ***P Value*** *Calculation.*

3. Results

Laboratory assessments of the measured parameters in the different submitted groups are presented in the following tables and figures:

TABLE (1): Comparison between Patient Groups (Group II – Group III) and control group (Group I), as regard sex distribution.

Sex	Group I (No=20)	Group II (No=40)	Group III (No=40)	Chi square (x2) test	P value
Male	14 (70%)	27 (67.5%)	29 (72.5%)	0.238	0.888
Female	6 (30%)	13 (32.5%)	11 (27.5%)		

*Calculation was done by using ***Chi square (x2) test.***

TABLE (2): The Range, MEAN ±Standard Deviation (SD) as well as Mean Difference and P Value of The Age (Years), BMI, WC (cm), determined **Total S. Calcium Level (Ca^{+2})** (mg/L), Fatty Liver Index (**FLI**), **Blood glucose level parameters** (mg/dl) [Fasting blood sugar, 2 h. post-prandial blood sugar and Glycated hemoglobin levels], **Liver Enzymes** (IU/ml) [AST, ALT and GGT], **Kidney Function Tests** (mg/dl) [Serum Creatinine and Urea] and **Lipid profile** (mg/dl) [Total Serum Cholesterol, HDL- Cholesterol, LDL Cholesterol and Serum Triglycerides] of patient groups (Group II –III) and control group (Group I**).**

	Anova Test				
		Range	Mean ±SD	F	Pvalue
▪ **Age (Years)**	**Group I**	42-60	50.2±6.24	0.45	0.639
	Group II	45-66	51.55±5.79		
	Group III	43-64	50.45±6.48		
▪ **BMI**	**Group I**	22.4-25.6	24.28±0.996	281.67	<0.0001*
	Group II	29.7-34.9	32.86±1.60		
	Group III	25-30	27.55±1.4		
▪ **WC (cm)**	**Group I**	70-83	77.85±3.79	313.26	<0.0001*
	Group II	94-114	101.25±5.33		

	Anova Test				
		Range	Mean ±SD	F	Pvalue
	Group III	72-87	79.98±3.30		
▪ **Total S. Calcium Level (Ca^{+2})**	**Group I**	7.5-11	9.29±1.06	90.71	<0.0001*
	Group II	10.8-12.5	11.62±0.36		
	Group III	9.3-11.8	11.02±0.57		
▪ **FLI**	**Group I**	11-35	24.05±5.51	1556.08	<0.0001*
	Group II	83-97	92.03±3.56		
	Group III	38-59	53.28±5.71		
▪ **Blood glucose level parameters**					
• **Fasting bl. Sugar**	**Group I**	70-99	88.25±8.71	204.71	<0.0001*
	Group II	128-202	159.4±20.27		
	Group III	145-212	178.25±15.05		
• **2 h. pp. bl. Sugar**	**Group I**	112-140	127.85±8.49	138.11	<0.001*
	Group II	182-277	221±27.75		
	Group III	173-298	240.7±28.61		
• **Glycated Hemoglobin**	**Group I**	2.5-4.4	3.64±0.55	487.45	<0.0001*
	Group II	7.1-8	7.70±0.28		
	Group III	5.3-7	6.28±0.59		
▪ **Liver Enzymes**					
• **ALT**	**Group I**	18-42	32.7±7	21.39	<0.0001*
	Group II	30-88	45.58±12.48		
	Group III	18-42	32.75±7.10		
• **AST**	**Group I**	10-34	24.55±7.72	30.45	<0.0001*
	Group II	25-68	39.68±10.74		
	Group III	10-35	27.48±5.51		
• **GGT**	**Group I**	18-38	31.3±5.37	344.73	<0.0001*
	Group II	60-85	70.38±5.92		
	Group III	35-56	46.48±5.88		
▪ **Kidney Function Tests**					
• **Urea**	**Group I**	22-40	33.4±4.88	0.32	0.727
	Group II	25-44	34.38±5.19		
	Group III	28-44	34.35±4.31		
• **Creatinine**	**Group I**	0.7-1.4	0.93±0.91	2.81	0.065
	Group II	0.75-1.5	1.05±0.21		
	Group III	0.7-1.4	0.98±0.17		
▪ **Lipid profile**					
• **Total Serum Cholesterol**	**Group I**	167-200	185.45±8.95	216.7	<0.0001*
	Group II	235-274	241.83±9.17		
	Group III	195-250	216.5±15.43		
• **HDL-Cholesterol**	**Group I**	62-92	75.05±8.62	243.45	<0.0001*
	Group II	10-38	24.39±8.51		
	Group III	33-64	50.23±8.46		
• **LDL-Cholesterol**	**Group I**	77-107	90.7±10.1	93.09	<0.0001*
	Group II	175-235	196.63±9.85		
	Group III	100-250	182.18±44.62		
• **Serum Triglycerides**	**Group I**	118-148	133.6±9.22	137.06	<0.0001*
	Group II	218-350	286.65±41.90		
	Group III	170-305	217.2±33.33		

*** Range, Mean and Standard Deviation** were calculated by using ***Anova Test***.

TABLE (3): Comparison between Patient Groups (Group II – Group III) and control group (Group I). Mean Difference t and P Value of The Age (Years), BMI, WC (cm), determined **Total S. Calcium Level (Ca^{+2})** (mg/L), Fatty Liver Index (**FLI**), **Blood glucose level parameters** (mg/dl) [Fasting blood sugar, 2 h. post-prandial blood sugar and Glycated hemoglobin levels], **Liver Enzymes** (IU/ml) [AST, ALT and GGT], **Kidney Function Tests** (mg/dl) [Serum Creatinine and Urea] and **Lipid profile** (mg/dl) [Total Serum Cholesterol, HDL- Cholesterol, LDL Cholesterol and Serum Triglycerides].

	Tukey HSD Test		
	M1 vs M2	**M1 vs M3**	**M2 vs M3**
■ **BMI**	<0.01*	<0.01*	<0.01*
■ **WC (cm)**	<0.01*	NS	<0.01*
■ **Total S. Calcium Level (Ca^{+2})**	<0.01*	<0.01*	<0.01*
■ **FLI**	<0.01*	<0.01*	<0.01*
■ **Blood glucose level parameters**			
• **F. bl. Sugar**	<0.01*	<0.01*	<0.01*
• **2 h. pp. bl. Sugar**	<0.01*	<0.01*	<0.01*
• **G. Hb**	<0.01*	<0.01*	<0.01*
➢ **Liver Function Tests**			
• **ALT**	<0.01*	NS	<0.01*
• **AST**	<0.01*	NS	<0.01*
• **GGT**	<0.01*	<0.01*	<0.01*
➢ **Kidney Function Tests**			
• **Urea**	NS	NS	NS
• **Creatinine**	NS	NS	NS
➢ **Lipid profile**			
• **Total Serum Cholesterol**	<0.01*	<0.01*	<0.01*
• **HDL-Cholesterol**	<0.01*	<0.01*	<0.01*
• **LDL-Cholesterol**	<0.01*	<0.01*	<0.01*
• **Serum Triglycerides**	<0.01*	<0.01*	<0.01*

* Comparison was done between each study group and others by ***Tukey HSD Test.***

TABLE (4): Outcome of Ultrasound Evaluation of the Patient Group with NAFLD (group II).

	Grade 1.(Mild)	**Grade 2.(Moderate)**	**Grade 3.(Severe)**
Group II	4 (10 %)	20 (50 %)	16 (40 %)

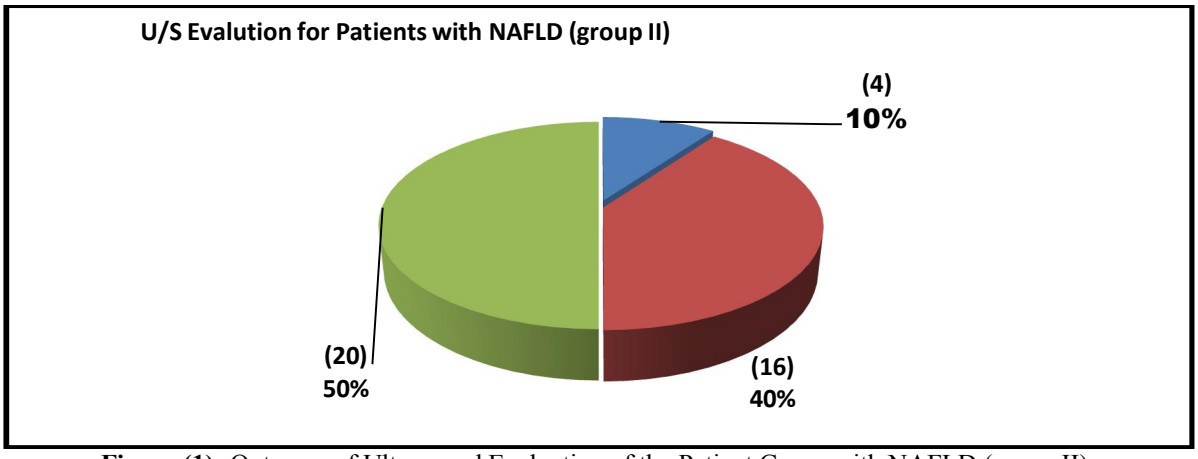


Figure (1): Outcome of Ultrasound Evaluation of the Patient Group with NAFLD (group II).

TABLE (5): Correlation between **Total Serum Calcium Level (Ca^{+2})** and different parameters used in this study.

	Group I		Group II		Group III	
	Correlation coefficient (r)	P Value	Correlation coefficient (r)	P Value	Correlation coefficient (r)	P Value
■ FLI	0.267	0.096	0.331	0.037	0.223	0.167
■ BMI	-0.154	0.346	0.205	0.210	-0.094	0.564
■ Blood glucose level parameters						
• F. bl. Sugar	-0.080	0.628	0.323	0.042	0.318	0.046
• 2 h. pp. bl. Sugar	-0.05	0.759	0.213	0.187	0.202	0.214
• G. Hb	0.084	0.607	0.402	0.010	0.328	0.039
➢ Liver Enzymes						
• ALT	0.106	0.514	0.182	0.262	-0.147	0.369
• AST	0.035	0.831	0.267	0.096	-0.137	0.403
• GGT	0.279	0.081	0.220	0.174	0.193	0.233
➢ Kidney Functon Tests						
• Urea	0.137	0.398	0.023	0.890	0.070	0.667
• Creatinine	0.283	0.077	0.256	0.111	0.080	0.626
➢ Lipid profile						
• Total S. Cholesterol	-0.152	0.349	0.009	0.954	0.006	0.971
• HDL-Cholesterol	-0.130	0.424	0.268	- 0.095	-0.045	0.783
• LDL-Cholesterol	0.020	0.901	0.291	0.068	0.051	0.757
• Serum Triglycerides	-0.027	0.869	0.213	0.187	0.034	0.834

* Correlation was done by **Pearson Correlation Coefficient (r) and P Value Calculators.**

TABLE (6): Correlation between **Fatty Liver Index (FLI)** and different parameters used in this study.

	Group I		Group II		Group III	
	Correlation coefficient (r)	P Value	Correlation coefficient (r)	P Value	Correlation coefficient (r)	P Value
■ BMI	0.653	< 0.00001	0.875	< 0.00001	0.392	0.013
■ Blood glucose level parameters						
• F. bl. Sugar	-0.272	0.090	0.049	0.764	0.429	0.066
• 2 h. pp. bl. Sugar	-0.120	0.461	0.066	0.687	0.082	0.615
• G. Hb	0.056	0.731	0.066	0.688	0.018	0.913
➢ Liver Enzymes						
• ALT	-0.078	0.637	0.321	0.043	0.211	0.192
• AST	0.20	0.216	0.210	0.194	-0.075	0.650
• GGT	0.323	0.042	0.374	0.018	0.259	0.106
➢ Kidney Functon Tests						
• Urea	-0.034	0.835	0.168	0.301	0.133	0.415
• Creatinine	-0.117	0.476	0.147	0.365	0.245	0.127
➢ Lipid profile						
• Total S. Cholesterol	0.329	0.038	0.217	0.178	-0.040	0.806
• HDL-Cholesterol	-0.317	0.046	-0.108	0.507	-0.281	0.080
• LDL-Cholesterol	0.069	0.670	0.365	0.021	0.177	0.278
• Serum Triglycerides	0.524	0.0005	0.454	0.003	0.295	0.064

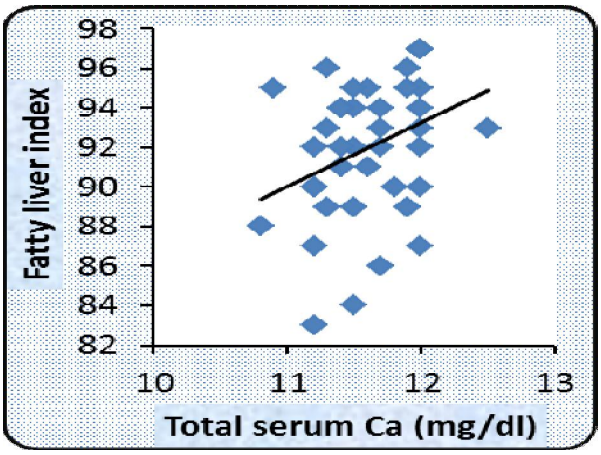
* Correlation was done by **Pearson Correlation Coefficient (r) and P Value Calculators.**

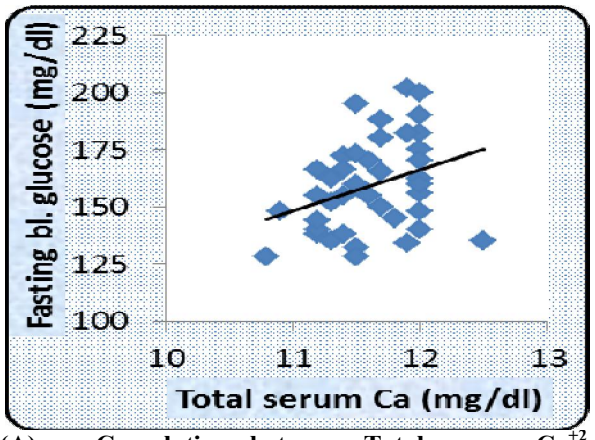
4. Discussion

Type 2 diabetes results from the failure of pancreatic beta cells to adequately compensate for obesity and insulin resistance. Both functional defects and reduced beta cell mass contribute to beta cell failure in type 2 diabetes, with apoptosis constituting the main form of beta cell death. Increased lipids and hyperglycaemia are likely the causes of beta cell apoptosis, but the mechanisms responsible remain unknown ***(Rhodes, 2005)***.

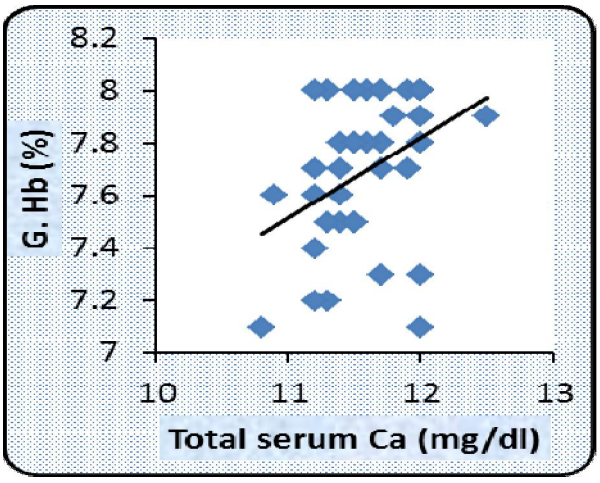
Growing evidence suggests that calcium homeostasis may be linked to glucose metabolism and development of diabetes. Calcium-sensing receptor (CaR) is expressed not only in tissues involved in calcium homeostasis (e.g., parathyroid gland), but also in other tissues, such as pancreatic islets of Langerhans ***(Gray et al., 2006).*** An in vitro study showed that activation of Ca^{2+}Receptors in pancreatic beta cells initiated an insulin secretory response and is involved in intra-islet communication between beta cells ***(Jones et al., 2007)***. The role of serum calcium in diabetes development is unclear but emerging evidence indicated that calcium may affect glucose metabolism. Calcium ions are involved in the regulation of insulin secretion and action at cellular level ***(Pittas et al., 2007)***, potentially through the activation of CaR ***(Jones et al., 2007)***.

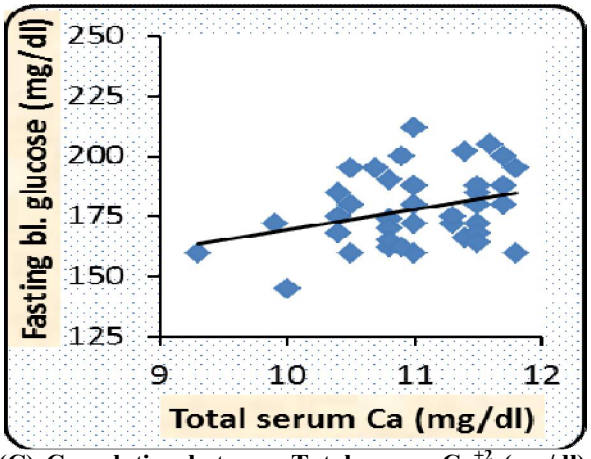
Insulin resistance plays a key rolein the pathogenesis of NAFLD and there is a strong association between this condition and the abnormalcomponents of metabolic syndrome (MetS) especially T2DM, where NAFLD is considered the hepatic manifestation of MetS ***(Tarantino & Finelli 2013).*** Because NAFLD is strongly associated with IR, patients with T2DM and NAFLD often have poor glycemic control compared to their counterparts without NAFLD (***Zoppini et al., 2009***). The intrahepatic triglyceride content is the major determinant in explaining the amount of insulin needed to achieve good glycemic control in T2DM patients. In fact, in insulin treated T2DM patients with stable glycemic control, it has been demonstrated that the intrahepatic triglyceride content was more closely correlated with the daily insulin dose and the ability of insulin to suppress hepatic glucose production and better explained the inter-individual variation in insulin requirements (***Ryysy et al., 2000***).



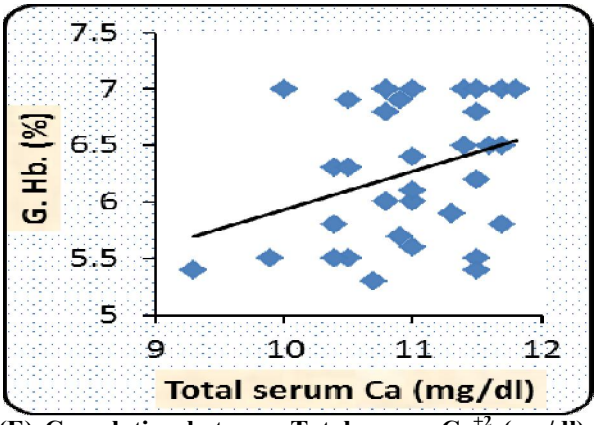


(A) Correlation between Total serum Ca^{+2} (mg/dl) and Fatty liver indexin Group II.
(B) Correlation between Total serum Ca^{+2} (mg/dl) and Fasting bl. Glucose (mg/dl) in Group II.

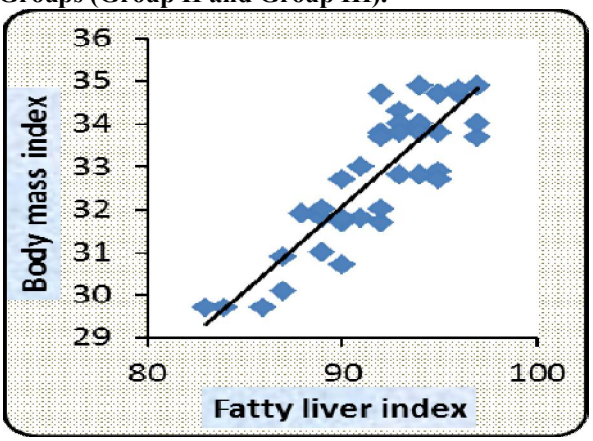




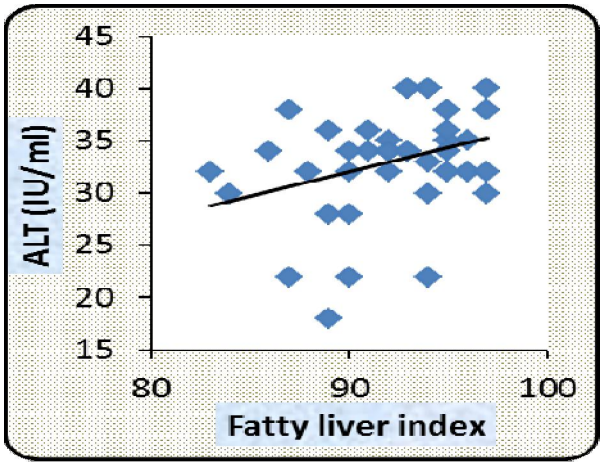
(C) Correlation between Total serum Ca^{+2} (mg/dl) and Glycated Hb. (%) in Group II.
(D) Correlation between Total serum Ca^{+2} (mg/dl) and Fasting bl. Glucose (mg/dl) in Group III.



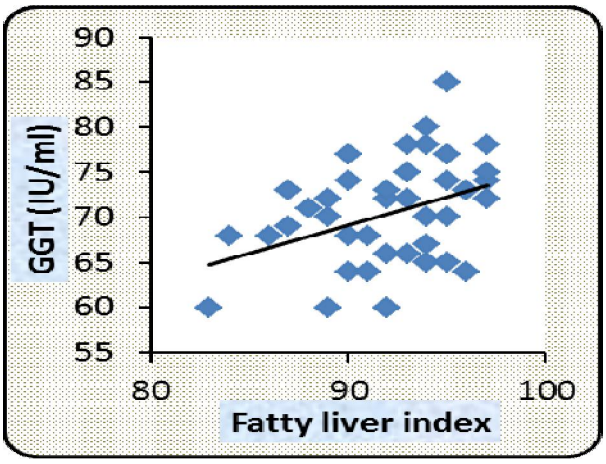
(E) Correlation between Total serum Ca^{+2} (mg/dl) and Glycated Hb (%) in Group III.
Figure 2 (A-B-C-D-E): Correlation between Total Serum Calcium Level (Ca^{+2}) and different laboratory parameters used in this study in Patient Groups (Group II and Group III).

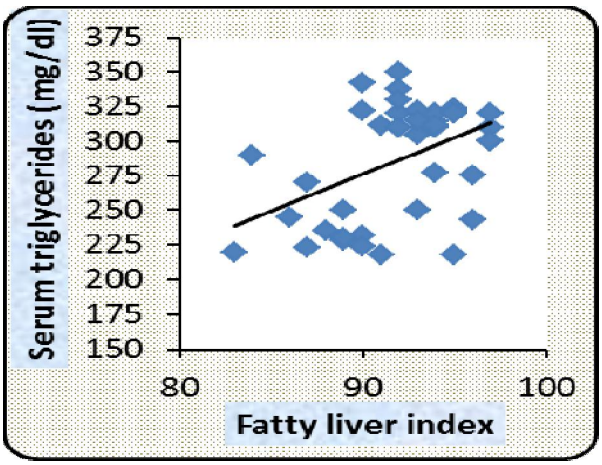


(A) Correlation between Fatty liver indexand body mass index in Group II.

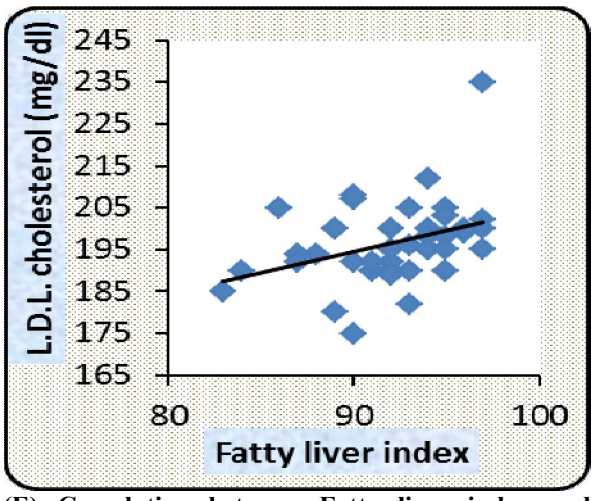


(B) Correlation between Fatty liver index and ALT (IU/L) in Group II.

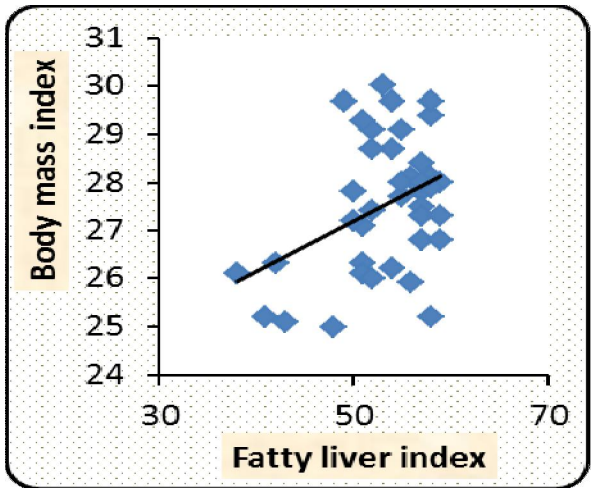




(C) Correlation between Fatty liver index and GGT (IU/L) in Group II.
(D) Correlation between Fatty liver index and serum Triglycerides (mg/dl) in Group II.



(E) Correlation between Fatty liver index and L.D.L. cholesterol (mg/dl) in Group II.



(F) Correlation between Fatty liver indexand body mass indexin Group III.

Figure 5 (A-B-C-D-E-F): Correlation between Fatty Liver Index (FLI) and different laboratory parameters used in this study in Patient Groups (Group II and Group III).

The current Study showed that high total serum calcium was associated with diabetes, suggesting that serum calcium is independently related to diabetes. There was significant difference in Total serum Calcium [Ca (mg/dl)] level between each patient Groups (Group II and Group III) and the control group (Group I), also there is a significant difference between patient Groups (Group I and Group II), [Calcium was higher in diabetic group with NAFLD (Group II)]. ***This Study also showed*** statistically significant positive correlation between total serum Calcium [Ca (mg/L)] level and blood glucose laboratory parameters [fasting blood glucose & glycated haemoglobin (G. Hb)] in both patient Groups (Group II and Group III), which means that diabetes is associated with high serum Calcium level compared with the control group. In contrast the correlation was statistically non-significant positive for 2h P.P. blood glucose estimates.

The current Study also showed a significant difference in Fatty liver index between each patient Groups (Group II and Group III) and the control group (Group I), also there is a significant difference between patient Groups (Group II and Group II), [Fatty liver index was higher in diabetic group with NAFLD (Group II)]. ***This Study also showed*** statistically significant positive correlation between Fatty liver index and liver enzymes [ALT & GGT] and serum Triglycerides and L.D.L. Cholesterol in diabetic group with NAFLD (Group II) compared with diabetic group without NAFLD (Group III) in which the correlation was statistically non-significant positive. ***Fatty liver indexalso showed*** statistically significant positive correlation with body mass index in all studied groups.

This Study is in agreement with the Lim et al., 2015 who suggested the possibility that increased serum calcium may in part be involved in developing NAFLD in subjects with type 2 DM. In addition, total cholesterol, LDL cholesterol and plasma triglyceride levels were significantly higher in subjects with NAFLD. ***It is also in agreement with (Ju et al., 2015)*** who stated Serum calcium level was significantly associated with NAFLD. ***Becerra-Tomas et al., 2014*** also stated that serum calcium is associated with an increased risk of diabetes.

This Study is in agreement with Tromso Study ***(Jorde et al., 2013) a study with higher statistical power (25,055 individuals and 705 new cases of diabetes)*** who stated that participants with the highest category of total serum calcium had a significant increased risk of diabetes when compared with those in the lowest category. ***Also it isin agreement with***the IRAS study which reported a non-linear association between calcium and diabetes ***(Lorenzo et al., 2014)***. By pooling the data from Tromso and IRAS studies together with the result of the current study in a meta-analysis, provided further evidence to support the association.

This Study is also in agreement with Yamaguchi et al., 2011 who stated that Serum Ca concentration was significantly and positively correlated with FPG in T2DM. These findings suggest that serum Ca is potentially involved in the aggravation of hyperglycemia and insulin resistance in T2DM. Thus, serum Ca may be more strongly related to glucose metabolism and may be linked to impaired glucose metabolism in T2DM. These findings seem to be in accordance with those of Sun et al. who showed that serum Ca was significantly and positively

correlated with glucose and insulin resistance in non-DM subjects after adjustment for 25-OH vitamin D and PTH ***(sun et al., 2005)***.

Guessous et al., 2011 stated that serum calcium was strongly associated with conventional MSy components, in particular T2DM, fasting serum glucose and serum triglycerides. Serum calcium showed a positive trend with the number of MSy components. ***Also this Study is in agreement with Mee et al., 2010*** who stated that there was a strong association between serum calcium levels and the prevalence of MetS (including NAFLD and T2DM). This association was independent of age, gender, BMI, or serum creatinine, phosphorus, PTH, 25(OH) D levels, smoking, alcohol drinking, exercise, total energy, calcium and sodium intake. They discovered a significant association between serum calcium levels and the risk of diabetes. They also suggested that altered calcium homeostasis is an important independent risk factor for metabolic disease. Fasting blood glucose, total cholesterol and triglyceride levels increased linearly, HDL-C levels decreased linearly in subjects with the lowest to the highest serum calcium. ***Ahlstrom et al., 2009 also reported that*** serum calcium was associated with the number of MSy components in a population-based study of 1000 elderly subjects. They confirmed this association in a larger population of subjects aged 35–75 years. They also suggest that serum calcium might be considered as an additional component of the MSy. In addition, serum calcium was associated with insulin resistance and markers of oxidative stress, independently of the conventional MSy risk factors.

This Study is also in agreement with the following cross-sectional studies: (a) ***Saltevo et al., 2011*** who stated thatincreased Total calcium concentration was associated with the metabolic syndrome in the general population. (b) ***Hagström et al., 2007*** also stated thatserum calcium was associated independently with insulin sensitivity measured with euglycemic / hyperinsulinemic clamping in a community based cohort. They also stated that endogenous Ca^{2+} might beinvolved early in the pathogenesis of diabetes, and they foundthat this effect was primarily mediated through effectson insulin sensitivity rather than defective insulin secretion. (c) ***Lind et al., 1988*** also stated that Total calcium concentration was correlated with fasting glucose concentration in a Swedish community. (d) In the study by Warehamet al ***(Wareham et al., 1997)***, impaired glucose tolerance was related to elevated calcium concentration, even after the effect of age, obesity or vitamin D concentration had been accounted for.

This Study is also in agreement with Aoki & Miyagawa 1990 who suggested that an increased serum Ca^{2+} level is linked to Ca^{2+} influx into arterial muscle and increased cytosolic Ca^{2+} because intravenous Ca infusion induced vasoconstriction and blood pressure elevation in normotensive men. Thus, the positive correlations between serum Ca^{2+} and FPG found in this study might occur because serum Ca^{2+} positively affects cytosolic Ca^{2+} in both pancreatic β-cells and muscle, partly via CaR, which results in hyper-insulinemia, reduced glucose uptake, and insulin resistance. Thus, it is also possible that Ca^{2+} levels could simply be associated with insulin resistance without any causality.

In contrast to the current study Entessar et al., 2008 stated that the mean serum calcium level was significantly lower in type 2 diabetes patients than the control group, yet the difference was statistically insignificant. They demonstrate that the reduction in serum calcium level is most probably due to several factors: reduction in insulin level which impairs bone formation due to its stimulatory action on osteoblast proliferation, and impairment of calcium homeostasis. These results agreed with others (***McCabe et al., 2011and Ma et al., 2012).***

The current study showed a strong positive association between the FLI, as a surrogate measure for fatty liver, and type 2 diabetes. The FLI provided a good diagnostic accuracy for fatty liver. Although ultrasonography is an accurate and reliable tool to detect moderate to severe fatty liver, the ability to detect lower percentages of liver fat by ultrasound might be limited ***(Jäger et al., 2015)***.

Krishnan et al., 2016 concluded that there was a high association of fatty liver disease in T2DM patients diagnosed either by ultrasound or by FLI. In the present study, a FLI < 30 ruled out and a FLI ≥ 60 ruled in hepatic steatosis as detected by ultrasonography. These resultswere in line with the findings of a previous study inwhich FLI showed good predictive performance in thediagnosis of NAFLD ***(Nima et al., 2016*** and ***Koehler et al., 2013).*** The present study revealed that FLI has a high discriminatory power in the diagnosis of NAFLD. This result could be somewhat anticipated due tothe fact that FLI is composed of four quantities relatedto NAFLD, including BMI, WC, GGT, and TG ***(Bedogni et al., 2006)***.

As shown in many other studies ***(e.g. Krishnan et al., 2016)***, the current study showed that the BMI of diabetic patients with NAFLD was significantly higher than those without NAFLD. Waist circumference was higher and statistically significant in subjects with NAFLD. The prevalence of obesity as shown by BMI and waist circumference is higher in NAFLD patients in many other studies (***Neuschwander & Caldwell 2003***). Obesity and T2DM share a "metabolic soil" that promotes hepatocyte lipotoxicity: adipose tissue

insulin resistance, subclinical inflammation, hyper-insulinemia, and abnormal glucose metabolism ***(Cusi, 2008).***

Milić et al., 2014 stated thata high BMI or WC, the main obesity indices, is considered anessential risk factor for NAFLD, and the prevalence of NAFLD substantially increases in obese individuals with T2DM**.** Waist circumference and BMI were the strongest predictors of FL; this strongly supports the hypothesis that obesity is the main responsible of the current epidemic of FL ***(Bedogni et al., 2006, and Bellentani et al., 2000)***. However, this study found nosignificant difference between the performance of FLI and BMI. An almost equal performance betweenobesity indices and FLI could be somewhat expected, as although obesity is strongly associated with NAFLD, it is also associated with other componentsinvolved in the calculation of FLI, including TG and liver enzymes ***(Lam & Mobarhan 2004*** and ***Liu et al., 2010).***

Conclusion and Recommendations

The data from current study ***support the notion that*** endogenous calcium may be involved early in the development of diabetes and that this effect is mediated mainly through effects on insulin sensitivity rather than defective insulin secretion. Also Serum calcium was significantly associated with NAFLD; FLI has a promising predictive powerin the diagnosis of NAFLD. So Further investigation is needed to verify that calcium level indicate a higher risk of NAFLD in Type 2 DM and to identify the mechanisms that link the mineral imbalance to the pathogenesis of NAFLD in those patients.

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