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Study of the Relationship between Chitotriosidase and Atherosclerosis in a Sample of Egyptian Patients with T2DM

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Abstract

Background: Diabetes mellitus type 2 (T2DM) is the leading cause of cardiovascular morbidity and mortality worldwide. Poor glucose control, hypertension, and dyslipidemia are the main factors that increase the risk of atherosclerotic disease in T2DM.

Aim: Study the relationship between Chitotriosidase and the development of atherosclerosis in a sample of Egyptian patients with T2DM.

Method: This case control study was conducted on 75 subjects, divided into 2 groups: 50 T2DM patients which were further divided into two groups (Ia) Non atherosclerotic, (Ib) Atherosclerotic patients, according to Carotid artery intimal thickness (CIMT>0.9 mm), 25 healthy subjects as control. Fasting plasma glucose, 2h post prandial plasma glucose, Carotid artery intimal thickness using carotid artery duplex, HbA1c, Lipid profile (cholesterol, triglycerides, LDL-C, HDL), Serum creatinine, AST, ALT and Serum Chitotriosidase were assessed.

Result: There was a statistical significant increase in serum Chitotriosidase in Atherosclerotic diabetic patients than Non atherosclerotic diabetic one (p value<0.001), (2.5-3.98) ng/ml) vs. (1.1 (1-1.25) ng/ml) respectively. Also Serum chitotriosidase was positively correlated with blood pressure, glycemic profile, lipid profile and CIMT.

Conclusion: There is increase in serum Chitotriosidase in atherosclerotic diabetic patients than non-atherosclerotic diabetic one.

Keywords: Chitotriosidase; Atherosclerosis; T2DM

Abbreviations: BMI: Body Mass Index; CHIT1: Chitotriosidase; COPD: Chronic Obstructive Pulmonary Disease; CIMT: Carotid Artery Intimal Thickness; DBP: Diastolic Blood Pressure; HDL: High DENSITY Lipoprotein; HbA1c: Hemoglobin A1c; FBS: Fasting Blood Sugar; LDL: Low Density Lipoprotein; T2DM: Type 2 Diabetes Mellitus; SBP: Systolic Blood Pressure; 2hpp: 2hour Post Prandial.

Introduction

Type 2 Diabetes mellitus is the leading cause of cardiovascular morbidity and mortality worldwide. Poor glucose control, hypertension, and dyslipidemia are the main factors that increase the risk of atherosclerotic disease in T2DM [1].

Inflammation and endothelial dysfunction initiate the pathogenesis of atherosclerosis, Being the most abundant cell type in atherosclerotic plaques, Macrophages are present in all phases of atherogenesis, and are markers of atherosclerotic plaque formation [2].

Chitotriosidase is produced, stored, and secreted by macrophages and neutrophils [3]. It implicated in the pathogenesis of many diseases such as bronchial asthma, COPD, Gaucher's disease, non-alcoholic fatty liver disease and neuro-degenerative disorders like Alzheimer's disease and amyotrophic lateral sclerosis [4].

The elevation of CHIT1 may reflect activation of macrophages. In a healthy population, CHIT1 activity is very low and Based on this data, some authors have postulated participation of chitotriosidase in the development of atherosclerosis, which creates a possible link to the course of T2D [5].

It has been reported that serum ChT activity is significantly increased in patients with established atherosclerosis in relation to the severity of the lesion, suggesting a possible role for ChT as a marker of advanced atherosclerosis [6].

ChT activity is increased up to 55-fold in extracts of atherosclerotic tissue, demonstrating a clear association between ChT expression and lipid-laden macrophages in the atherosclerotic vessel wall [7].

Since macrophages are present in all phases of atherosclerosis and increase the number and activity depending on the severity of those phases, CHIT1 activity in macrophages has been accepted as one of the biomarkers for atherosclerotic plaque formation. The average CHIT1 activity in serum remained constant after 6 months of cholesterol and triglyceride-lowering treatment with either atorvastatin or bezfibrte, suggesting that LDL-cholesterol and triglyceride reduction obtained with both drugs did not modify the macrophage CHIT1 expression/ activity in these subjects. The plasma lipid correction does not seem to interfere in the CHIT1 expression level in vivo, supporting the idea

that CHIT1 activity cannot be used to monitor progression of atherosclerosis [8].

ChT measurement is easy, reproducible, reliable, and cost effective. It is always possible to make routine ChT measurements on daily basis for each patient. Also, ChT is a very stable enzyme which can allow direct comparison of plasma and serum samples that have been stored under widely different conditions. Multiple cycles of freeze drying had no effect on chitotriosidase activity in plasma or serum [9].

Patients and Methods

This case control study was conducted on 75 subjects with their ages ranging from 35-65 with mean of 48.25 ± 5.90 years old, selected from internal medicine and endocrinology outpatient clinics of Ain shams University Hospitals during the period from February 2017 to June 2018 after signing an informed consent for all participant.

They were divided into 2 groups.

Group 1

50 T2DM patients which were further divided into two groups (Ia) Non atherosclerotic, (Ib) Atherosclerotic patients, according to Carotid artery intimal thickness (CIMT>0.9 mm).

Group 2

25 healthy subjects as control. With follow up of group (Ib) 6 months later after adjustment of their glycemic and lipid profiles near to target. Exclusion criteria: patients with any pulmonary, cerebral, recent myocardial infraction, infection and renal impairment. All participants subjected to full medical history was taken from all patients emphasizing on the duration of diabetes mellitus, treatment, diabetic complications (regular fundus examination, puffiness of eye lids, frothy urine, diabetic foot, any vascular interventions, tingling, and cerebrovascular accidents, claudication pain), co-morbid conditions, anti-dyslipidemia drugs and smoking. All patients subjected to full clinical examination including: Anthropometric measurements of weight, height, BMI, waist circumference and waist/hip ratio, measurement of blood pressure, carotid and femoral bruit, signs of heart failure, foot ulcers, atrophic changes and neurological examination.

Laboratory and imaging studies

Fasting plasma glucose, two hours post prandial plasma glucose, HbA1c, Lipid profile (cholesterol, triglycerides, LDL-C, HDL), Serum creatinine, AST, ALT, Serum Chitotriosidase by ELISA using kits (LifeSpan BioSciences company, Washington ,United States), and Carotid artery intimal thickness using carotid artery duplex.

Results

Regarding the glycemic profile (FBS, 2hPP, HbA1c) show high statistical significant difference between the studied groups (p value<0.01), being the highest in group Ib followed by group Ia then group II, as following fasting plasma glucose being the highest in group Ib (213.75 ± 47.55 mg/dl) followed by group Ia (156.65 ± 50.15 mg/dl) then group II (86.52 ± 10.93 mg/dl), 2 hours post prandial plasma glucose being the highest in group Ib (275.63 ± 44.40 mg/dl) followed by group Ia (225.41 ± 54.15mg/dl) while group II(126.48 ± 14.05 mg/dl), HBA1C being the highest in group Ib (9.21 ± 1.00%) followed by group Ia 8.31 ± 1.51)%while group II 5.08 ± 0.23%.

Regarding the lipid profile (total cholesterol, LDL, TG) show a high statistical significant difference between the studied groups (p value<0.01), being the highest in group Ib followed by group Ia, then group II as following total cholesterol being the highest in group Ib (243.94 ± 34.08 mg/dl) followed by group Ia (212.71 ± 23.05 mg/dl), then group II (168.52 ± 10.38 mg/dl), LDL-C, being the highest in group Ib (134.06 ± 33.29 mg/dl) followed by group Ia (126.18 ± 26.33 mg/dl) while group II (93.20 ± 12.22mg/dl), Regarding Triglycerides, being the highest in group Ib (226.94 ± 135.56 mg/dl) followed by group Ia (160.88 ± 28.97 mg/dl) then group II (88.68 ± 15.70 mg/dl). While HDL-C, show a high statistical significant difference between the studied groups (p value<0.01), being the lowest in group I b (33.94 ± 12.01 mg/dl) followed by group Ia (38.18 ± 8.07 mg/dl) while group II (50.56 ± 3.91 mg/dl).

Regarding serum Chitotriosidase, there was a high statistical significant difference between the studied groups (p value<0.01), being the highest in group Ib (Median (IQR)) (2.88 (2.5-3.98) ng/ml) followed by group Ia (1.1 (1-1.25) while group II (0.20 (0.10-0.25) ng/ml.

Regarding Carotid artery duplex IMT there was a high statistical significant difference between the studied groups (p value<0.01), being the highest in group Ib (1.04 ± 0.12 mm) followed by group Ia (0.66 ± 0.10 mm) while group II (0.56 ± 0.06 mm) (Table1).

		(Group Ia)	(Group Ib)	Group II	Test value	p value	Sig.
		No.=34	No.=16	No.=25			
FBG (mg/dl)	Mean ± SD	156.65 ± 50.15	213.75 ± 47.55	86.52 ± 10.93	49.806•	0	HS
	Range	80-334	140-310	67-120			
2HP.P (mg/dl)	Mean ± SD	225.41 ± 54.15	275.63 ± 44.40	126.48 ± 14.05	68.079•	0	HS
	Range	140-400	180-340	98-150			

HBA1c (%)	Mean ± SD	8.31 ± 1.51	9.21 ± 1.00	5.08 ± 0.23	84.217•	0	HS
	Range	6-14.2	7.7-11	4.7-5.7			
Cholesterol (mg/dl)	Mean ± SD	212.71 ± 23.05	243.94 ± 34.08	168.52 ± 10.38	57.088•	0	HS
	Range	178-270	170-290	154-188			
Triglyceride (mg/dl)	Mean ± SD	160.88 ± 28.97	226.94 ± 135.56	88.68 ± 15.70	22.429•	0	HS
	Range	72-210	115-575	64-124			
LDL (mg/dl)	Mean ± SD	126.18 ± 26.33	134.06 ± 33.29	93.20 ± 12.22	18.114•	0	HS
	Range	63-183	70-200	79-123			
HDL (mg/dl)	Mean ± SD	38.18 ± 8.07	33.94 ± 12.01	50.56 ± 3.91	25.718•	0	HS
	Range	22-54	17-56	42-55			
	Range	11-52	10-53	11-29			
Chitotriosidase (ng/ml)	Median(IQR)	1.1 (1 -1.25)	2.88 (2.5-3.98)	0.2 (0.1-0.25)	63.710 ≠	0	HS
	Range	0.4-2	2-6.5	0-0.4			
Carotid artery duplex IMT(mm)	Mean ± SD	0.66 ± 0.10	1.04 ± 0.12	0.56 ± 0.06	128.413	0	HS
	Range	0.45-0.80	0.70-1.20	0.45-0.70			

Table 1: Comparison between Group (Ia), Group (Ib) and Group (II) regarding different parameters.

There is a positive correlation between chitotriosidase and blood pressure as systolic blood pressure, (r=0.35) with (p value<0.05) and diastolic blood pressure (r=0.372) with (p value<0.01).

There is a positive correlation between chitotriosidase and glycemic profile as FBG (r=0.589) with (p value<0.01), 2HP.P (r=0.630) with (p value<0.01), and HBA1C(r=0.629) with (p value<0.01).

There is a positive correlation between chitotriosidase and lipid profile as cholesterol (r=0.431) with (p value<0.01), triglycerides (r=0.322) with (p value<0.05).

There is a positive correlation between chitotriosidase and CIMT with (r=0.735) and (p value<0.01) (Table 2).

	Chitotriosidase	
	R	**p value**
SBP (mmHg)	0.35	*0.013
DBP (mmHg)	0.372	**0.008
FBG (mg/dl)	0.589	**0.000
2HP.P (mg/dl)	0.63	**0.000
HBA1c (%)	0.629	**0.000
Cholesterol (mg/dl)	0.431	**0.002
Triglyceride (mg/dl)	0.322	*0.022
Carotid artery duplex IMT (mm)	0.735	**0.000

Table 2: Correlation between chitotriosidase and different variables in all groups.

After a period of follow up for group Ib for 6 months trying to adjust their glycemic and lipid profiles near to target, we found the following: Regarding fasting plasma glucose there was a high statistical significant difference (p value<0.01), being initially 213.75 ± 47.55 mg/dl while after 6 months 159.69 ± 37.10mg/dl, 2 hours post prandial plasma glucose there was a high statistical significant difference (p value<0.01), being initially 275.63 ± 44.40 mg/dl while after 6 months 218.94 ± 26.70mg/dl, HBA1C there was a high statistical significant difference (p value<0.01), being initially 9.21 ± 1.00% while after 6 months 8.71 ± 0.83% (Table 3).

		Initially	Follow up	Test value	p value	Sig.
			after 6 months			
		No.=16	No.=16			
FBG (mg/dl)	Mean ± SD	213.75 ± 47.55	159.69 ± 37.10	4.113	0.001	HS
	Range	140-310	60-220			
2HP.P (mg/dl)	Mean ± SD	275.63 ± 44.40	218.94 ± 26.70	7.19	0	HS
	Range	180-340	175-265			
HbA1c (%)	Mean ± SD	9.21 ± 1.00	8.71 ± 0.83	5.242	0	HS
	Range	7.7-11	7.5-10.1			
Chol (mg/dl)	Mean ± SD	243.94 ± 34.08	209.19 ± 19.07	5.612	0	HS
	Range	170-290	170-245			
Triglycerides (mg/dl)	Mean ± SD	226.94 ± 135.56	178.81 ± 66.11	2.588	0.021	S
	Range	115-575	100-330			
LDL (mg/dl)	Mean ± SD	134.06 ± 33.29	114.25 ± 27.25	4.231	0.001	HS
	Range	70-200	70-185			
HDL (mg/dl)	Mean ± SD	33.94 ± 12.01	34.45 ± 9.30	-0.759	0.466	NS
	Range	17-56	17-49			
Chitotriosidase (ng/ml)	Median(IQR	2.88 (2.5-3.98)	2.85 (2.5-3.9)	-1.083	0.279	NS
	Range	2-6.5	2.1-6.1			
Carotid artery duplex IMT (mm)	Mean ± SD	1.04 ± 0.12	1.04 ± 0.07	-0.1	0.921	NS
	Range	0.70-1.20	1-1.2			

Table 3: Comparison between Group Ib results regarding different parameters initially and after 6 months.

As regards total cholesterol, there was a high statistical significant difference (p value<0.01), being initially 243.94 ± 34.08mg/dl while after 6 months 209.19 ± 19.07 mg/dl, LDL-C, there was a high statistical significant difference (p value<0.01), being initially 134.06 ± 33.29 mg/dl while after 6 months 114.25 ± 27.25 mg/dl, Triglycerides, there was a high statistical significant difference (p value<0.01), being initially 226.94 ± 135.56 mg /dl while after 6 months 178.81 ± 66.11 mg/dl. HDL-C, there was no statistical significant difference, being initially 33.94 ± 12.01 mg /dl while after 6 months 34.45 ± 9.30 mg/dl.

Regarding Carotid artery duplex IMT there was no statistical significant difference, being initially 1.04 ± 0.12 mm while after 6 months 1.04 ± 0.07 mm.

Regrading serum Chitotriosidase, there was no statistical significant difference; being initially 2.88 (2.5-3.98) ng /ml while after 6 months 2.85 (2.5-3.9) ng/ml (Table 3).

Discussion

The current study showed that the serum Chitotriosidase levels (p value<0.01), being higher in diabetic subjects was 1.25 (1-2.5) ng/ml) while its level in healthy subjects was 0.20 (0.10-0.25) ng/ml), these results were in line with Żurawska-Płaksej et al. which revealed in 2015, that Chitotriosidase activity in the patients with ongoing type 2 diabetes was higher than in the control group [10]. Also these result came in parrel with Sonmez et al. who found in 2010, that serum ChT activity is increased in patients with newly diagnosed patients with T2DM [11].

The present study showed that there was a significant positive correlation between chitotriosidase and Carotid artery IMT with (r=0.735) and (p value<0.01), This result is in agreement with Artieda et al. who found in 2003, that Serum chitotriosidase activity is significantly increased in patients suffering from atherosclerosis disease and is related to the severity of the atherosclerotic lesion, suggesting a possible role as atherosclerotic extent marker [8].

The current study showed there is a highly significant positive correlation between chitotriosidase, and FBG (r=0.589) with (p value<0.01), and 2HP.P (r=0.630) with (p value<0.01), which is in agreement with Żurawska-Płaksej et al. that revealed in 2015, that plasma CHIT1 activity was independently correlated with glucose level in the total population and in diabetic patients, which may indicate its participation in the course of diabetes [10].

The present study showed a significant positive correlation between chitotriosidase and HBA1C (r=0.629) with (p value<0.01). These

results were in agreement with Turan et al. found in their study in 2017, that the chitotriosidase is closely related to HbA1c level being its activity increased in the patients having HbA1c>10% than those of the control group [12]. But this result is against Yildiz et al., who reported in his study in 2013, there is no significant correlation between chitotriosidase activity and HbA1C [13].

The current study found that there was a positive correlation between chitotriosidse, and cholesterol (r=0.431) with (p value<0.01), and triglycerides (r=0.322) with (p value<0.05). These results were distinct and against Żurawska-Płaksej et al., Guclu et al., Turan et al., Boot et al. who reported previously the lack of relationship between plasma CHIT1 activity and parameters of lipid metabolism in type 2 diabetic patients [10,12,14,15].

Boot et al. constructed his hypothesis based on data found that the average CHIT1 activity in serum remained constant after 6 months of lipid-lowering treatment with either atorvastatin or bezafibrate, suggesting that LDL-cholesterol and triglyceride reduction obtained with both drugs did not modify the macrophage CHIT1 expression in these subjects. Therefore, they said that plasma lipid correction does not seem to interfere in the CHIT1 expression level in vivo, supporting the idea that CHIT1 activity cannot be used as a biological marker of atherosclerotic plaque modification related to hypolipidemic treatment [15].

The current study showed significant positive correlation between CHIT1 activity and Systolic blood pressure, (r=0.35) with (p value<0.05), and diastolic blood pressure (r=0.372) with (p value<0.01). Which were similar to results found by Żurawska-Płaksej et al. who reported in 2016, that CHIT1 activity was additionally associated with the SBP, DBP value [10].

Our study showed ROC curve of chitotriosidase of cut off 1.6 ng/ml above it diabetic patients have high probability of having atherosclerosis with sensitivity 100% and specificity 96.06%.

And lastly, in the current study we found that there is no statistically significant difference regarding chitotriosidase in diabetic atherosclerotic patients after 6 months follow up with modification of their blood glucose and lipid profile (p value-1.083), being its level was 2.88 (2.5-3.98 ng/ml) initially and after follow up it level became 2.85 (2.5-3.9 ng/ml). So it can't be used for follow up at least for short duration and need more studies.

Conclusion

The present study revealed that the serum chitotriosidase can be used as predictable marker for diabetic vasculopathy, however it can't be used for follow up, at least for short duration.

Conflict of Interest

The authors declare no conflict of interests.

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