Taq1 B Polymorphism Of Cholestryl Ester Transfer Protein; A Potential Risk Factor For Atherosclerosis In Niddm Diabetes Mellitus

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Highlights: TAQ1 B polymorphism Hetero type (B1B2) is found more in diabetic patients with increased intima media thickness, however Wild type (B2B2), and Homo type (B1B1) were found in people with diabetes, with normal intima media thickness. The mutant, and wild type was not correlatedto lipid profile, which means that genetic background play a vital role in diabetic atherosclerosis which is not correlated only to dyslipidemia, and this may open a new window for research in contributing factors in this era. **Abstract:** Aim: In this study we assessed the role of TAQ1 B polymorphism of cholesterol ester transfer protein in diabetes atherosclerosis. Methods: 90 type II diabetic patients were enrolled in the study, full clinical evaluation, fasting blood sugar, lipid profile, and DNA analysis for the G277A of CETP gene polymorphism by PCR-RFLP technique was performed for all patients. Assessment of the carotid intima media thickness were done for all patients according to which patients were classified to those with normal or increased intima media thickness. Results: 95% mutant type (Homo,Hetero types) in the group with increased carotid intima media thickness compared to those of normal intima media thickness, in which the percent was 80%, and this was statistically significant. There was statistically significant difference in the right and left carotid intima media thickness between the wild type and the mutant type with higher values in the patients with the mutant type, however there was no statistically significant difference in lipid profile, between those patients with wild type and those with mutant type. Conclusion: Hetero type (B1B2) is found more in patients with diabetes with increased intima media thickness, however Wild type (B2B2), and Homo type (B1B1) were found in patients with diabetes with normal intima media thickness.

[Hazem S. A-Y,Marianne F. M,Rand s, Mary F, Fatma A. El S, Walaa A. R. **TAQ1 B Polymorphism Of Cholestryl Ester Transfer Protein; A Potential Risk Factor For Atherosclerosis In NIDDM Diabetes Mellitus.** *Life Sci J* 2015;12(9):25-30]. (ISSN:1097-8135). http://www.lifesciencesite.com. 4

Keywords: Taq1 B; Polymorphism; Cholestryl Ester; Transfer Protein; Atherosclerosis; Diabetes Mellitus

1. Introduction:

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by abnormalities in carbohydrate and fat metabolism. The causes of type 2 diabetes are multi-factorial and include both genetic and environmental elements that affect beta-cell function and tissue (muscle, liver, adipose tissue, and pancreas) insulin sensitivity (1).

Atherosclerosis is the cause of a majority of cardiovascular events, and atherosclerosis is accelerated by diabetes and the metabolic syndrome. Many risk factors are associated with the metabolic syndrome and help explain the increased cardiovascular disease (CVD) in that condition (2). Strong epidemiological evidence supports an association between glycemic control and CVD risk (3). Atherosclerosis is initiated by the adhesion of monocytes to arterial endothelial cells, followed by their transmigration into the subendothelial space along a chemotactic gradient. One mechanism by which high glucose conditions may enhance this process involves activation of NFκB (4).

Cholesteryl Ester Transfer Protein (CETP) is a hydrophobic glycoprotein that circulates in plasma bound mainly to HDL-C. (5) Cholesteryl ester transfer protein (CETP) is reportedly able to affect the amount of cholesterol available for deposition and/or removal from peripheral tissues, in its capacity to mediate the transfer of cholesterol from high density lipoprotein (HDL) to very low density lipoprotein, in exchange for triacylglycerols from the latter. The TAQ I B polymorphism of the human CETP gene has been associated with decreased CETP mass and an increase in HDL-cholesterol (6).

Recent genome-wide association studies have reported that CETP genotypes are associated with HDL-C levels more strongly than any other loci across the genome (7). Taq1B polymorphism (SNP:rs708272,c. G277A) is the most widely studied CETP polymorphisms resulting from G to A base pair change at nucleotide 277 in intron 1 of the CETP gene which disrupts Taq1 restriction site. The allele containing the Taq1 endonuclease site is called B1, while the allele without the restriction site is called B2***(8).*** The less common "B2" allele (absence of the

TAQ IB restriction site) has been associated with increased HDL-C levels and decreased CETP activity and levels (9).

Effects of CETP polymorphisms on lipids and lipoproteins profile and CETP activity have been reported. Ordovas et al., (10) showed that TAQ 1B polymorphism at the CETP gene locus was associated with changes in lipoprotein size, CETP activity and HDL-C levels. -971 G/A polymorphism has been shown to be significantly related to plasma HDL-C levels and CETP concentrations (11).

Ultrasound measurements of the intima media thickness (IMT) in the carotid arteries were used as an indicator of carotid atherosclerosis. (12)

2. Methods:

We included 90 type 2 diabetic patient, from internal medicine and diabetes outpatient clinics and internal medicine inpatient,KaserElAini hospital in our study.

All diabetic patientswere chosen according to WHO criteria for diabetic diagnosis,

We divided our patients into 2 groups basedon carotid intimal medial thickness (CIMT) which can be regarded as a marker of atherosclerosis and of increased cardiovascular risk. Group with increased intima media thickness and the other group with normal carotid intima media thickness.

Exclusion criteria

Patients on medications that could affect plasma glucose level (e.g. steroids, tamoxifen, Methotrexate, or oral contraceptive), malignancy, hypo/hyperthyroid disease, pregnancy and any other concomitant acute infection.

Methodology:

All our patients were subjected to full clinical history, and thorough clinical examination.

Imaging procedure:

All patients were subjected to imaging procedure in the form of carotid Doppler to measure intima media thickness of the distal common carotid as shown in fig (1), to detect any atherosclerotic plaques if present, and the proximal systolic volume (PSV), end diastolic volume (EDV), resistivity index (RI) of the internal carotid artery by carotid ultrasound. B (brightness)-mode grey scale, color, and spectral Doppler techniques were used to investigate the carotid arteries according to standardized protocol. The same operator interpreted all studies in a blind fashion, and the same ultrasound unit HD 5000 was used, using a linear probe (7.5 mega hertz) for scanning all participants.

Laboratory tests:

Sampling:

Ten milliliters venous blood samples were collected from 10-12 h fasting subjects under aseptic conditions and were divided into two vacutainer tubes, EDTA sterile vacutainer tubes used for DNA extraction and serum sterile vacutainer tubes used for biochemical assays. Serum was separated by centrifugation at 3000 rpm for 10 min at room temperature and stored at -20 °C until assay.

Biochemical analysis:

Glucose (fasting blood glucose) and lipid profile (serum cholesterol, serum triglyceride, LDLc, HDLc were measured using Modular analyzers by Roche diagnostics (Roche Applied Science, Germany) using instrument manufacturer reagents and recommendations

DNA extraction and genotyping:

DNA analysis for the G277A of CETP gene polymorphism by PCR-RFLP technique was performed.

Genomic DNA was extracted from peripheral blood leukocytesusing whole Blood Genomic DNA Purification Mini Kit #K0781 (ThermoScientific). Enzymatic amplificationof G277A of CETP gene, intron 1, was done as previouslymentioned (21) using one set of primers CETPint1forward primer: 5`-CACTAGCCCAGAGAGAGAGGAGTG CC-3`; CETPint1, reverse primer: 5`-CTGAGCCCAGCCGCACACTAA C-3`, (Operon Biotechnologies (GmbH/Biocampus, Germany).

The PCR amplification was performed in 25 µl reaction volume, containing 12.5 µl 2_PCR Master Mix (Thermo Scientific, www.thermoscientific.com); 2_PCR buffer, 3mM MgCl2, 0.5 units Taq DNA polymerase/ml, 400 mM of each dNTP, 1 µl of each primer (10 pmol), 2.5 µl of genomic DNA and 8 µl sterilized nuclease-free water. The reaction was performed in a Hybaid thermal cycler (Promega Corporation, Madison, WI), programmed as follows: initial denaturation at 94 °C for 5 min, 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 60 °C and 30 s extension at 72 °C and a 10-min final extension at 72 °C. The amplified products were detected in 1.5% agarose gel as a single band of 535 bp. Seven microliters of the amplified products were digested with 0.2U of TAQ 1B (Thermo Scientific, www.thermoscientific. com); which generated, in the presence of A allele, two fragments of 174bp & 361bp were detected by restriction at 65 °C for 5 minutes. Fragment size analysis was performed by 3.5% agarose gel/TAE 1x. (21)

Statistical Analysis:

The SPSS 10.0 for windows was used for data management and analysis and the Microsoft power point for charts. Quantitative data were presented as mean +SD. For comparison of the two groups means, the Student's t-test was used, while for the comparison of the three groups' means, one way analysis of variance (ANOVA) was used followed by

Post Hoc test. Qualitative data were expressed as frequency and percentage. Association between qualitative data was done using Chi- square test. Risk estimate was done by odds ratio. P value was considered significant at 0.05

3. Results:

We classify our patients to group I: increase carotid intima thickness, group II: normal carotid intima thickness.

In group I: there was 10 patients on oral hypoglycemic agents (22.2%), 8 patients were on insulin (17.8%), and 27 patients on combination therapy (60%).

In group II: there was 13 patients on oral hypoglycemic agents (28.9%), 11 patients were on insulin (24.4%), 21 patients on combination therapy (46.7%)

We found no statistically significant difference in sex, age, and duration of diabetes between group I with increase carotid intima thickness, and group II with normal carotid intima thickness.

There was statistically significant difference in waist circumference between group I with increase carotid intima thickness, and group II with normal carotid intima thickness, with higher values in group I, however there was no statistically significant difference as regards the BMI between the two groups. Table (1)

There was statistically significant difference in lipid profile between the two groups. Table (2)

There was statistically significant difference in wild type B2B2, Homo type B1B1, Hetero type B1B2 between the two groups table (3). In our study we found 95% mutant type (Homo+Hetero types) in the group with increased carotid intima media thickness compared to those of normal intima media thickness, in which the percent was 80%, and this was statistically significant. Table (4).

There was no statistically significant difference in the frequency of Allele between the two groupstable (5).

There was statistically significant difference in the right and left carotid intima media thickness between the wild type and the mutant type with higher values in the patients with the mutant type, however there was no statistically significant difference in lipid profile, BMI between those patients with wild type and those with mutant type. Table (6)

There was statistically significant difference between the right carotid intima media thickness in Wild type B2B2, Homo type B1B1, and Hetero type B1B2 with P value<0.05,with higher value in the last group, however there was no statistically significant correlation between serum cholesterol, triglyceride, LDLc, HDLc in the three groups. Table (7)

Table (1): Sex and age distribution, Demographic data of studied groups. In the studied groups.

		Group I (n=45) with increased carotid intima media thickness	Group II (n=45)with normal carotid intima media thickness	p-value
Sex	Male	13 (28.9%)	8 (17.8%)	0.106
	Female	32 (71.1%)	37 (82.2%)	
Age/Y		33-67 (51.2±7.9)	32-65 (49.3±8.2)	0.270
Duration of DM/Y		5-20(9.3±3.5)	5-20(9.5±4.2)	0.787
Waist/cm		97-180(113.2±14.3)	85-169(106.8±14.5)	0.037
BMI (Kg/m2)		23-45(33.8±4.7)	23-49(32.5±5.6)	0.239

Table (2): Laboratory data of studied groups.

Lab. Data	Group I (N=45)	Group II (N=45)	p value
*FBG (mg/dl)	57 -386 (184.8±86.9)	70-472 (209.1±87.8)	0.192
Total Cholesterol (mg/dl)	87-337 (201.4±56.1)	112-322 (186.3±47.5)	0.172
HDL-C(mg/dl)	28-69 (45±9.6)	22-78 (45.6±11.3)	0.788
*TG(mg/dl)	60-398 (161.7±85)	61-400 (139.0±101.2)	0.254
LDL-C(mg/dl)	17-228 (122.4±42.4)	50-254 (112.2±39.8)	0.243

FPG: fasting plasma glucose, TG: triglycerides, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol.

Table (3): Genotype distribution of studied groups:

Genotype	Group I (N=45)	Group II (N=45)	*p*-value
Wild type (B2B2)	2 (4.4%)	9 (20.0%)	0.021
Homo type (B1B1)	16 (35.6%)	20 (44.4%)	
Hetero type (B1B2)	27 (60.0%)	16 (35.6%)	

Table (4): Frequency of wild and mutant genotypes among the two groups.

Genotype	Group I (n=45)	Group II (n=45)	O.R.	95% C.I.	p -value
Wild type (B2B2)	2 (4.4%)	9 (20.0%)	5.375	(1.091-26.486)	0.049
Mutant type (Homo+Hetero types) (B1B1+ B1B2)	43 (95.6%)	36 (80.0%)			

Table (5): Allele frequency in the studied groups.

Allele	Group I (n=90)	Group II (n=90)	p -value
B1	59 (65.6%)	56 (62.2%)	0.32
B2	31 (34.4%)	34 (37.8%)	

Table (6): Relation- ship between different parameters according to wild or mutant genotypes.*

	Wild type (B2B2) n=11	Mutant type (B1B1+B1B2) n=79	p -value
Age/Y	51.9±4.1	50.0±8.4	0.240
Duration of DM/Y	8.6±4.1	9.5±3.8	0.476
Waist/cm	104.8±12.3	110.8±14.9	0.231
*** BMI(Kg/m^2)**	32.5±3.9	33.2±5.4	0.667
*** RT CIMT/cm**	0.077±0.010	0.087±0.016	0.006
*** LT CIMT/cm**	0.077±0.012	0.088±0.018	0.021
*** FPG(mg/dl)**	238.0±106	191.3±84.1	0.098
*** Total cholesterol (mg/dl)**	188.9±52.6	194.6±52.6	0.736
*** HDL-C(mg/dl)**	45.7±9.7	45.3±10.7	0.892
*** TG(mg/dl)**	146.5±71	150.9±96.8	0.885
*** LDL-C(mg/dl)**	110.5±38	118.3±41.8	0.558

Rt CIMT: right carotid intimal medial thickness, Lt CIMT: left carotid intimal medial thickness, BMI: body mass Index, FPG: fasting plasma glucose, TG: triglycerides, HDLC: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol.

Table (7): Relationship between different parameters according to different genotypes.*

	Wild type B2B2 (n=11)	Homo type B1B1 (n=36)	Hetero type B1B2 (n=43)	p-value
Age/Y	51.9±4.1	49.2±8.3	50.7±8.7	0.536
Duration of dm/Y	8.6±4.2	10.3±3.7	8.9±3.9	0.209
Waist/cm	104.8±12.3	111.2±14.6	110.4±15.5	0.449
*** BMI (Kg/m^2)**	32.5±3.9	34.8+5.4	32±5.1	0.061
***RT CIMT/cm**	0.07±0.01	0.08±0.02	0.09±0.01	0.025
*** LT CIMT/cm**	0.077±0.012	0.086±0.016	0.09±0.019	0.089
*** FPG(mg/dl)**	238±106	192.3±97.3	190.4±72.4	0.265
*** Total Cholesterol (mg/dl)**	188.9±52.6	188.9±42.2	199.4±59.9	0.638
*** HDL-C(mg/dl)**	45.7±9.7	43±11.1	47.1±10	0.231
*** TG(mg/dl)**	146.5±71	132.6±53.3	166.3±120.4	0.279
*** LDL-C(mg/dl)**	110.5±38	118.3±36.7	118.2±46.2	0.843

* Rt CIMT: right carotid intimal medial thickness, Lt CIMT: left carotid intimal medial thickness, BMI: body mass index, FPG: fasting plasma glucose, TG: triglycerides, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol.

4. Discussion:

The most common alterations in lipid and lipoprotein metabolism in type 2 diabetes involve an elevation in both plasma triglyceride and VLDL concentrations, a dense LDL phenotype and low levels of HDL cholesterol. The inverse relationship between the level of HDL cholesterol and the risk of cardiovascular disease is commonly explained by the crucial role of HDL in reverse cholesterol transport. Cholesterol ester transfer protein (CETP) has a central role in the metabolism of HDL and may therefore alter the susceptibility to atherosclerotic vascular disease. (13)

CETP deficiency is associated with hyperalphalipoproteinemia, which is primarily due to an increase of cholesteryl ester–enriched large-sized HDL. Conversely, the triglyceride-rich lipoproteins and LDL are smaller, reflecting the role of CETP in neutral lipid exchange. (14) The relationship between reduced CETP function and the susceptibility to atherosclerosis has proven complex and confusing: both longevity and increased CAD risk have been reported (15). Hirano and coworkers (15) have shown that reduced CETP function (in 201 individuals with HDL-c levels > 2.58 mmol/l) in conjunction with reduced hepatic lipase activity is associated with an increased risk for CAD. This indicates that the metabolic setting of the individual might, at least in part, determine the ultimate effect of CETP on atherosclerosis.

It has long been recognized that body mass index (BMI; in kg/m^2) is a predictor of the morbidity and mortality that are due to numerous chronic diseases, including type 2 diabetes, cardiovascular disease (CVD), and stroke (17). In addition, it has been established that abdominal obesity, assessed by waist circumference (WC), predicts obesity-related health risk (18), and the weighted evidence indicates that WC coupled with BMI predicts health risk better than does BMI alone. (19)In our study there was a significant difference in waist circumference between patients with normal carotid intima media thickness and those with increased carotid intima media thickness, with higher values, however there was no statistically significant difference in BMI between the two groups and this signify that waist circumference had a role in atherosclerosis, however BMI not.

The *TAQ*I polymorphism B1 allele of *CETP* has been shown to be an independent risk factor for development of cerebral vascular disease, in patients with T2DM.(20). There was no statistically difference in fasting blood sugar, and serum cholesterol, triglyceride, LDLc, HDLc between the two groups, those with normal, or increased intima media thickness, but there was statistically difference in number of patients with Mutant type (Homo+Hetero types) (B1B1+ B1B2) between patients with increased carotid media thickness (95.6%) and those with normal media thickness (80%), and this signify that not only the blood sugar level and lipid profile is the only predictor for atherosclerosis, but also Mutant type (Homo+Hetero types) (B1B1+ B1B2) had a great role.

Conclusion

Hetero type (B1B2) is found more in patients with diabetes with increased intima media thickness, however Wild type (B2B2), and Homo type (B1B1) were found in patients with diabetes with normal intima media thickness. The mutant, and wild type was not correlated to lipid profile, which means that genetic background play a vital role in diabetic atherosclerosis which is not correlated only to dyslipidemia, and this may open a new window for research in contributing factors in this era

No fund support
No conflict of interest

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9/8/2015