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Research Article

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Association of Catalase Gene rs769217 Polymorphism with Carotid Intima-media Thickness in Diabetic Patients

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Abstract

Diabetes mellitus is a risk factor for atherosclerosis/cardiovascular diseases. Atherosclerosis in diabetic patients has been linked to increased oxidative stress and intima-media thickness is used to detect its presence. Catalase is involved in hydrogen peroxide catabolism and is important in defense against oxidative stress, which contributes significantly to atherosclerotic processes.

There are no data on association of catalase gene genotypes of rs769217 polymorphism and carotid artery intima-media thickness. Therefore, this study investigated the potential association of catalase rs769217 polymorphism and intima media thickness in diabetic patients. Right and left carotid intima-media thickness increased ($P \leq 0.001$) in type 1 and type 2 diabetes when it was compared to those of controls. Blood catalase activities of CC genotypes of rs769217 polymorphism in catalase gene were associated with cITT ($P < 0.043$) in type 1 diabetics, type 2 diabetics and in controls.

This interesting new finding could suggest that diabetics and controls with CC genotype of rs769217 polymorphism may have higher carotid intima media thickness and higher risks for cardiovascular events when they blood catalase is high. This higher catalase could destroy hydrogen peroxide more effectively thus preventing the signaling action of hydrogen peroxide.

Introduction

Diabetes mellitus is the most prevalent and independent risk factor for atherosclerosis/cardiovascular diseases including coronary artery disease. Patients with type 2 diabetes mellitus are predisposed to accelerated atherosclerosis and cardiovascular diseases is the leading cause of mortality in type 2 diabetics. Atherosclerosis in type 2 diabetic patients has been linked to increased oxidative stress. Intima-media thickness is used to detect the presence of atherosclerotic disease in humans and to track the regression, arrest or progression of atherosclerosis. Ultrasound intima-media thickness measurements were first proposed and validated *in vitro* by Paolo Pignoli in 1984 and later publicized [1,2].

The use of intima-media thickness as a non-invasive tool to track changes in arterial walls has increased substantially since the mid-1990s. Rundek T. et al. recently reported that traditional vascular risk factors explain only a part of the variance in carotid Intima-media thickness [3].

While traditional cardio vascular diseases risk factors, including diabetes, hypertension, hyperlipidemia, obesity, physical inactivity, may be more prevalent among chronic kidney disease patients, these factors seem to underestimate the accelerated cardiovascular disease in the chronic kidney disease population. Search for additional biomarkers that could explain the enhanced cardio vascular diseases risk in chronic kidney disease patients has gained increasing importance. Although it is unlikely that any single nontraditional risk factor would fully account for the increased cardio vascular diseases risk in individuals with chronic kidney disease, oxidative stress appears to play a central role in the development and progression of cardio vascular diseases and its complications. Catalase is involved in hydrogen peroxide catabolism and is important in defense against oxidative stress, which contributes significantly to atherosclerotic processes [4].

Catalase reduces toxic concentrations of hydrogen peroxide. For the blood the catalase activity is very high in erythrocytes and very low in plasma and white blood cells. Deficiency of erythrocyte catalase may cause increased hydrogen peroxide concentration which may effect the catalase poor and oxidation sensitive pancreatic beta-cells [4]. Human inherited catalase deficiency (acatalasemia) is associated with increased risk of type 2 diabetes mellitus Carotid artery intima-media thickness (cITT) correlated with oxidative stress in chronic haemodialysis patients with accelerated atherosclerosis [5-7]. Dursum et al. detected a significant negative correlation was detected between

cITT and oxidative stress markers of serum superoxide dismutase and catalase in uremic patients (r:-0.65, P<0.001) and in diabetics (r: -0.41, P:0.03) [8,9].

Letonja M. et al. found that the T allele of -262C/T polymorphism of catalase gene is associated with lower risk for higher plaque score but it did not affect clinical parameters, cIMT and plaque stability [10].

Nivet-Antoine V. et al. examined catalase gene polymorphisms of -844GtoA, -89AtoT, -20TtoC and found that CAT2 haplotype including heterozygous carriers (-844GA, -89AT, -20TC) appeared as an independent risk factor of arterial aging, similarly to previously identified factors such as age, systolic blood pressure, male, sex, tobacco use, hs-CRP, BMI and diabetes [11].

Lucas et al. examined the oxidative stress markers in patients over 70 years and less than 70 years submitted to carotid enderactomy. They found a higher levels of ROS and NADPH oxidase activity in the older group and no change in the activities of antioxidant enzymes of catalase and superoxide dismutase [12].

Furthermore, elevated levels of oxidative stress have been reported in aneurysmal tissues [13]. Reactive oxygen species (ROS) and reactive nitrogen species are upregulated and decreased antioxidant enzyme activity, including decreased neutrophil catalase activity, and lower levels of α-tocopherol in the plasma of patients with abdominal aortic aneurysms have been described. Ramos-Mozo P. et al. suggested that catalase might be a novel biomarker in pathology of abdominal aortic aneurysms [14-18].

In their experiments Parastatidis et al. found that that a reduction in aortic wall catalase activity can predispose to abdominal aortic aneurysms formation. Furthermore, restoration of catalase activity in the vascular wall enhances aortic vascular smooth muscle cell survival and prevents abdominal aortic aneurysms formation in mouse [19].

The C111T silent polymorphism in exon 9 (+22348C→T, rs769217, Asp389Asp) of the catalase gene could be associated with several pathologic conditions such as vitiligo, hearing loss, and bone mineral density [20-22]. This nucleotide change may cause slower transcription from the mutant allele than from the wild allele. For several cases recent evidence has indicated that silent (synonymous) mutations may effect splicing and/or RNA stability [23,24]. There are no data on association of this polymorphism and carotid artery intima-media thickness in diabetic patients.

Aims of this study

There are no data on association of catalase gene genotypes of rs769217 polymorphism and carotid artery intima-media thickness. Therefore, this study investigated the potential association of catalase rs769217 polymorphism and intima media thickness in diabetic patients. B-mode ultrasonography mediated measurement of carotid artery intima-media thickness (cIMT) could contribute to the study of subclinical carotid atherosclerosis and patients at high risk for cardiovascular diseases can be identified.

Patients and Methods

Patients

Type 2 and 1 diabetic patients attending diabetes outpatient clinic at the Department of Internal Medicine, University of Debrecen were recruited for the study. For these patients an institutional ethical clearance and an informed consent were received. Diabetics with severe hypoglycemia, hyperglycemia, diabetic ketoacidosis within three months prior to sample collection, active infections, malignancy and other co-morbid illnesses, pregnant females were excluded from the study. Only Caucasians aged greater than/equal to 18 and less than/equal to 75 years were included in this study. Hypertensive patients (cases and controls) were included only if they did not have hypertensive crisis within three months prior to sample collection. 122 type 2 diabetics and 92 type 1 diabetics met our inclusion criteria and were involved in the study.

For comparison 36 controls were recruited from healthy employees of the University of Debrecen. An informed consent, detailed history and socio-demographic details were obtained from all participants. Laboratory analysis and measurement of cIMT was carried out by personnel blinded to clinical status of the subjects.

Biomarker and genetic testing

The blood samples were taken after overnight fasting. Blood catalase activity was measured by a spectrophotometric method based on the decrease in its hydrogen peroxide substrate [24]. Its mean and sd are 113±16 MU/L what results the reference range of 81-145 MU/L. Catalase gene rs769217 polymorphism was examined by a method of PCR-single strand conformation polymorphism [25].

Carotid Intima-Media Thickness (cIMT) measurement

Philips HD 11 XE ultrasound equipment with a 7.5 MHz linear transducer was used to measure IMT (mm). Online measurements of IMT were performed in the far artery wall of the common carotid arteries, 10 mm proximal to the carotid bulb. All measurements were performed on frozen, enlarged images at end-diastole, and the transducer was in the medio-lateral direction. IMT was measured on a 1-cm segment. In each of these 1-cm segments, 10 measurements of IMT were performed at 1-mm increments on both sides. The mean IMT of the 20 values in each patient was calculated. Carotid intima-media thickness above 0.7 mm was regarded as pathologic.

Statistical analysis

Means and standard errors (mean±sd) were calculated for carotid-intima-media thickness and blood catalase activities. The significance of differences in means was analyzed with Student's *t*-test.

For comparison of genotype frequencies we used the *chi*-squared test on 2x2 contingency table and 3x3 contingency table for comparison to controls.

P values <0.05 were regarded as significant. Genotype distribution was assessed for deviation from Hardy-Weinberg equilibrium by *chi*-squared test, its values bellow 5.991 suggested equilibrium. If the data were less than 5 the statistical analyses were not performed. It is denoted as A in the (Table 2).

Results

The means of blood catalase activities and either right or left carotid-intima-media thicknesses did not show significant (p>0.064) differences according to the genotypes of catalase gene rs769217 polymorphism (Table 1) either in both types of diabetes and in controls.

The genotypes frequencies (P>0.289) and the allele frequencies (P>0.337) of diabetics did not show differences when they were compared to those of controls.

The comparison of patients results to those of controls yielded significant (P<0.016) changes (Table 1). Blood catalase activities decreased in type 1 (101.6±24.3 MU/L, P:0.003) and type 2 (100.3±27.8 MU/L, P:0.013) compared to controls (116.4±21.4 MU/L).

Right and left carotid intima-media thickness increased in type 1 (right 0.605±0.144 mm, P:0.0163, left 0.614±0.166 mm, P:0.001) and type 2 (right 0.667±0.184 mm, P<0.001, left 0.691±0.173 mm, P:<0.001) diabetes when it was compared to those of controls (right 0.540±0.105 mm, left 0.525±0.09mm) (Table 1).

Table 1: Blood catalase activities and carotid intima media tickness (cINMT) in genotypes of rs 769217 polymorphism of catalase gene in diabetics and controls

Genotypes	CC	CT	TT	Significance[1]	All	Significance[2]
Type 1 diabetes	47	41	4	chi: 0.181	92	
Blood catalase (MU/L)	104.5±26.5	99.1±22.3	93.7±18.0	P>0.329	101.6±24.3	0.031*
Right cIMT (mm)	0.627±0.145	0.581±0.146	0.605±0.085	P>0.251	0.605±0.144	0.163
Left cIMT (mm)	0.650±0.177	0.584±0.153	0.523±0.043	P>0.064	0.614±0.166	0.001*
Type 2 diabetes	71	44	9	chi: 0.358	124	
Blood catalase (MU/L)	97.4±27.1	99.3±29.5	99.4±27.1	P>0.281	100.3±27.8	0.013*
Right cIMT (mm)	0.681±0.205	0.649±0.164	0.647±0.076	P>0.349	0.667±0.184	<0.001*
Left cIMT (mm)	0.697±0.172	0.682±0.189	0.691±0.101	P>0.674	0.691±0.173	<0.001*
Controls (35)	16	15	4	chi: 0.253		
Blood catalase (MU/L)	112.7±20.8	117.3±19.5	102.5±8.7	P>0.171	116.4±21.4	
Right cIMT (mm)	0.527±0.145	0.581±0.146	0.605±0.085	P>0.187	0.540±0.105	
Left cIMT (mm)	0.550±0.177	0.584±0.153	0.523±0.043	P>0.221	0.525±0.097	

[1]2X2 contingency table
[2]compared to controls

Blood catalase activities of CC genotypes of rs769217 polymorphism in catalase gene were associated with cITT (Table 2). For type 1 diabetics it was 0.0434 for the right cITT and 0.030 for the left cITT. Similar results were received for type 2 diabetics (right cITT: 0.021, left cITT: 0.042) and for controls (right cITT: 0.041, left cITT: 0.022).

For CT (P>0.1584) and TT (P>0.2456) genotypes of either diabetics or controls did not yield significant association with carotid intima-media thickness (Table 2).

Table 2: Association between genotypes of rs769217 catalase gene polymorphism and carotid intima-media thickness (cITT)

Catalase	vs right cITT			vs left cITT		
	CC	CT	TT	CC	CT	TT
Type 1						
n	46	40	4	46	40	4
P	0.043*	0.235	A	0.030*	0.178	A
Type 2						
n	71	49	10	71	49	10
P	0.021*	-0.371	0.245	0.042*	-0.257	0.301
Controls						
n	16	15	4	16	15	4
P	0.041*	-0.427	A	0.022*	-0.158	A

* P<0.05 and A: n<5

Discussion

Diabetes mellitus is an independent risk factor for atherosclerosis and these patients are predisposed to accelerated atherosclerosis and cardiovascular diseases. Intima-media thickness is used to detect the presence of atherosclerotic disease in humans.

Atherosclerosis has been linked to increased oxidative stress via overproduction of reactive oxygen species and hydrogen peroxide. Enzyme catalase plays a role in controlling hydrogen peroxide concentration which could contribute to the pathology of diabetes [4,5].

Blood catalase activities were decereased (p<0.031) in both types of diabetes (101.6±24.3 MU/L for type 1 and 100.3±27.8 MU/L for type 2) when they were compared to that of controls (116.4±21.4 MU/L). These results are similar to those of our earlier reports [6,7]. The decreased blood catalase may contribute to the increased concentration of hydrogen peroxide which could damage the oxidation sensitive beta cells of pancreas [5-7].

The genotypes of rs769217 catalase gene polymorphism did yield significant differences (p>0.171) in blood catalase activities neither in diabetes nor in controls. The papers on examination of this polymorphism did not report for blood catalase activities of these genotypes [11-13]. Our previous report on the blood catalase activities of these genotypes also yielded also no (p>0.2894) change [26-28].

The left form of carotid intima-media thickness in type 1 diabetes

(0.614±0.166 mm) and both forms in type 2 diabetes (right: 0.667±0.184 mm, left: 0.691±0.173 mm) yielded significant (0.001) increase when compared to that of the controls (right: 0.540±0.105 mm, left: 0.525±0.097 mm). Similar increases for carotid intima-media thickness were reported in both types of diabetes [29-31]. Rodriquez et al. and the Diabetes Control and Complication Trial suggested that diabetes is associated with higher cIMT. While the results from Lorenz et al. do not support the use of cIMT progression as a surrogate end point for diabetics [29-31].

Examination of other catalase gene polymorphisms of -844GtoA, -89AtoT, -20TtoC Nivet-Antoine V. et al. found that CAT2 haplotype seems to be an independent risk factor of arterial aging [11]. Letonja, et al. found an association of the -262C/T polymorphism in the catalase gene promoter with carotid atherosclerosis in Slovenian patients with type 2 diabetes [10].

The examination of rs769217 polymorphism and diseases was examined in several diseases. Some authors reported on its association with vitiligo, hearing loss, and bone mineral density [19-21]. Our earlier data showed a weak association of this polymorphism and blood catalase in female vitiligo patients [27]. Liu's findings suggested that the catalase rs769217 T allele is associated with increased risk of chronic hepatitis B, hepatitis B virus related liver cirrhosis and hepato cellular liver carcinoma in Guangxi population [32]. Other authors reported on no association of this polymorphism and vitiligo [33,34]. Dursum et al. found a negative correlation between cIMT and catalase in uremic ($r = -0.65$, $P < 0.001$) and in diabetic patients ($r=-0.41$, $P=.003$) while van Zyl et al. detected no association of catalase and carotid intima media thickness [8,9,35].

Contrary to these findings, our results showed a positive correlation of carotid intima media thickness and blood catalase for type 1 ($p<0.043$), type 2 diabetes ($p<0.042$) and for controls ($p<0.041$) in the CC genotypes of this polymorphism. For the mutant CT and TT genotypes there is no association blood catalase and carotid intima media thickness. This interesting new finding could suggest that diabetics and controls with CC genotype of rs769217 polymorphism may have higher carotid intima media thickness and higher risks for cardiovascular events when they blood catalase is high. The lower thickness was associated with lower blood catalase. For explanation of this finding we may suppose that the wild genotype of this polymorphism requires higher blood catalase. This higher catalase could destroy hydrogen peroxide more effectively thus preventing the signaling action of hydrogen peroxide. The low and physiologic concentration of H_2O_2 in mammalian cells has been shown to stimulate biological responses and to activate specific biochemical pathways in these cells [36, 37]. Furthermore, H_2O_2 modulates gene expression at all steps from transcription to protein synthesis [38]. The low concentration of hydrogen peroxide due to the high catalase may decrease those biochemical pathways which could prevent the increase in carotid intima media thickness. The factors and pathways which contribute to the increased carotid intima media thickness via hydrogen peroxide require further examinations.

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