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Original article

Study the Correlation of Diabetic Complications and Macrophage Erythroblast Attacher (MAEA) rs6815464 Gene Polymorphism

Hazem Samy Matar [a] , Esraa Adel Abd El-Azeim [a], Rania El-Sayed Sheir [a], Mona Zaky sherif Nasser [b] , Mahmoud Hassan Ahmed [a] .

[a] *Internal Medicine department, Faculty of Medicine, Beni-Suef University, Egypt*

[b] *clinical pathology department, Faculty of Medicine, Beni-Suef University, Egypt*

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Corresponding Author:

Esraa Adel Abd El-Azeim

soso7ossam@gmail.com

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Abstract

This study was designed to evaluate MAEA gene rs6815464 polymorphism as a risk factor of type 2 diabetes mellitus (T2DM) associated microangiopathic complications in the Egyptian population. Our patients were divided into two groups; diabetic patients with complications (30) recruited from Diabetes, endocrine and internal medicine clinic, Beni-Suef university hospital, compared with age and sex matched normal volunteers acting as a control group (61). Our study showed that diabetic retinopathy was the most common microangiopathic complication of T2DM (97%) followed by (90%) of the patients had diabetic nephropathy, while (70%) of the patients had diabetic neuropathy. Nonalcoholic fatty liver disease (NAFLD) was significantly higher in diabetic patients than in controls ($p < 0.0001$*). No significant correlation of MAEA rs6815464 polymorphism with the occurrence of diabetic microangiopathic complications was observed.

1. Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, which is caused by a deficiency in insulin secretion or its biological action or both. Long standing DM causes chronic damage and dysfunction of various organs, to eye, kidney, heart, blood vessels and nerves [1]. Type 2 diabetes occurs when body cells resist the normal effect of insulin to drive glucose in the blood into the cells, this is called insulin resistance. So, glucose starts to increase in the blood, then the pancreas responds by producing more insulin to maintain a normal blood sugar, but over time, the body's insulin resistance gets worse. Finally, the pancreas becomes exhausted, it cannot keep up with the need for more insulin and the blood glucose level starts to rise [2]. Diabetic retinopathy is a common diabetes-related microvascular complication, which is the leading cause of vision loss worldwide [3]. Patients with type 2 diabetes are at risk of developing neurovascular complications that can lead to diabetic retinopathy and/or diabetic macular edema (DME). Non proliferative diabetic retinopathy (NPDR) was present in 25% of patients 5 years after being diagnosed with diabetes, 60% at 10 years and 80% at 15 years. The incidence of proliferative diabetic retinopathy (PDR) varied from 2% in those who had diabetes for less than 5 years to 15.5% in those who had diabetes for 15 or more years [4]. Diabetic nephropathy also known as diabetic kidney disease, is the chronic loss of kidney function occurring in patients with diabetes mellitus, it is one of the leading causes of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide years [5]. Type 2 diabetes mellitus is one of the most important risk factors of CKD, around 30%–50% of ESRD patients worldwide come from a diabetic origin [6].

Type 2 diabetes mellitus is known to cause a wide variety of metabolic disturbances and affect both the peripheral nervous system and central nervous system, either directly or indirectly, which can lead to several complications collectively referred to as diabetic neuropathy over a long period of time [7]. Diabetic neuropathy affects about one-third of the diabetic patients, it causes a great impact on the patient’s quality of life and increases the rate of morbidity and mortality [8].

Macrophage erythroblast attacher (MAEA) was discovered in 1994 [9] as an integral membrane protein that mediates the attachment of the erythroid cells to macrophages and is essential for bone marrow hematopoiesis [10].

Subsequent studies have revealed that MAEA is a nuclear matrix component contributing to nuclear architecture and cell division [11].

MAEA shows ubiquitous expressions in different cells and tissues including osteoblasts and osteoclasts, however its function in bone

metabolism is still unknown. MAEA gene is located in chromosome 4p16.3 [12].

This study aimed to evaluate the clinical significance of MAEA gene rs6815464 polymorphism in T2DM with and without complications.

2. Patients and Methods:

Subjects:

The present work was performed within one year from May 2020 to May 2021; the subjects were divided into 2 groups:

Patients Group:

Thirty Patients with type 2 diabetes mellitus with microangiopathic complications . Of which 18 Postmenopausal females (60%) and 12 age matched males (40%). They were recruited from Diabetes, endocrine and internal medicine clinic, Beni-Suef university hospital.

Control Group:

Healthy volunteers (61) were age and sex matched with the patients group.

They were 24 females (39%) and 37 males (61%).

2.1 Inclusion criteria:

Postmenopausal women and age matched male patients with type 2 DM were diagnosed according to criteria for the diagnosis of diabetes [13].

Fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h*.

OR

2hours post prandial plasma glucose (2h PG) ≥200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

OR

A1C ≥6.5% (48 mmol/mol).

OR

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

2.2Exclusion criteria:

Patients with the following criteria will be excluded from this study:

1. kidney disease like nephritis and nephropathy (other than diabetic nephropathy), infections, fever or other conditions that could influence urine protein content.
2. Retinal diseases like retinal tear and age-related macular degeneration (other than diabetic retinopathy).
3. Causes of peripheral neuropathy (other than diabetic neuropathy) like disc prolapse.

Methods:

Participants were subjected to the followings:

1-History taking, stressing on diabetic duration, manifestations, complications, treatment and family history.

2-Clinical Examination (blood pressure, Body Mass Index (BMI), Waist circumference

(WC), microfilament test detection of nerve affection).

3-Laboratory investigations include Complete blood count (CBC), lipid profile, liver enzymes, Creatinine, Urea, eGFR, Hemoglobin A1c, fasting blood glucose ,2hr P.P. blood glucose, urine albumin/creatinine ratio .

4-pelvi-abdominal ultrasound.

5-fundus examination.

Genetic analysis:

Genomic DNA extraction and analysis for polymorphism rs6815464 of the MAEA gene using the polymerase chain reaction-real time (PCR-real time) method of blood sample.

3.Statistical methodology

Data were analyzed using SPSS software version 18 (USA).

- Description of quantitative variables was presented in the form of mean± standard deviation (SD).
- Description of qualitative variables was presented in the form of numbers (No.) and percent's (%).
- One way ANOVA (for parametric data) was used to detect the difference between the three groups regarding scale.
  variables (Kruskal Wallis for non-parametric data).
- Chi-Square test (or fisher exact) and odd ratio was used to detect the difference between groups regarding the categorical variables.
- The significance of the results was assessed in the form of P-value that was differentiated into:
  - Non-significant when P-value > 0.05
  - Significant when P-value ≤ 0.05

4. Results:

The current study was conducted at Diabetes, endocrine and internal medicine clinic in Beni-Suef university hospital. Approval No: FMBSUREC/09022020/Abd-El Azeim.

Table 1: Demographic and clinical data among the studied groups:

Variables	DM with complications (N= 30)	Control subjects (N=61)	P value
Age (Years)	61.4 ± 5.5	57.5 ± 5.4	0.07
Sex n, % **Male** **Female**	 12 (40%) 18 (60%)	 37 (61%) 24 (39%)	 X^2= 3.4 P= 0.18
BMI (kg/m^2)	26.1 ± 4.6	24.08 ± 3.7	0.06
Waist circumference (Cm)	87.1 ± 5.7	88 ± 6.2	0.53
A/C Ratio	561.2 ± 112	11 ± 6.1 a	<0.0001*

Data are represented as mean ± SD or N (%). Data are analyzed using One-way ANOAV followed by tukey test or chi square test. No significant difference between the studied groups regarding their age, sex distribution, waist circumference and BMI.

Table 2: Genotypes and alleles distribution of MAEA rs6815464 among the studied groups:

Variables	DM with complications (N= 30)		Control subjects (N=61)	P value
Genotypes				
CC	28		53	X^2= 0.85 P= 0.36
CG	2		8	
GG	0		0	
Alleles				
C	58		114	X^2= 0.8 P= 0.37
G	2		8	

Data are represented as N (%). Data are analyzed using chi square test/ Fischer exact. There was no significant difference between the two studied groups regarding MAEA rs6815464 Genotypes and alleles distribution.

Table 3: Genotypes and alleles distribution of MAEA rs6815464 among diabetic patients with nephropathy:

Variables	DM with nephropathy		Odd Ratio	95% CI	P value
	Yes (N= 27)	No (N=3)			
Genotypes					
CC	25 (93%)	3 (100%)	Ref		
CG	2 (7%)	0 (0%)	0.68	0.02-17.4	0.81
GG	0 (0%)	0 (0%)	-	-	-
Alleles					
C	52 (96%)	6 (100%)	Ref		
G	2 (4%)	0 (0%)	0.61	0.02-14.3	0.76

There was no significant relation between different genotypes and alleles of MAEA gene and diabetic nephropathy.

Table 4: Genotypes and alleles distribution of MAEA rs6815464 among diabetic patients with retinopathy:

Variables	DM with retinopathy		Odd Ratio	95% CI	P value
	Yes (N= 29)	No (N= 1)			
Genotypes					
CC	27 (93%)	1 (100%)	Ref		
CG	2 (7%)	0 (0%)	0.27	0.008-8.6	0.46
GG	0 (0%)	0 (0%)	-	-	-
Alleles					
C	56 (97%)	2 (100%)	Ref		
G	2 (3%)	0 (0%)	0.22	0.008-5.9	0.36

There was no significant relation between retinopathy and genotype and allele frequency of MAEA rs6815464 among diabetic patients with complications.

Table 5: Genotypes and alleles distribution of MAEA rs6815464 among diabetic patients with neuropathy:

Variables	DM with neuropathy		Odd Ratio	95% CI	P value
	Yes (N=20)	No (N= 10)			
Genotypes					
CC	18 (90%)	10 (100%)	Ref		
CG	2 (10%)	0 (0%)	2.8	0.12-64.8	0.51
GG	0 (0%)	0 (0%)	-	-	-
Alleles					
C	38 (95%)	20 (100%)	Ref		
G	2 (5%)	0 (0%)	2.6	0.12-58.1	0.53

There was no significant relation between neuropathy and genotype of MAEA rs6815464 among diabetic patients with complications.

Table 6: Prevalence of NAFLD among the studied groups:

Variables	DM with complications (N= 30)	Control subjects (N=67)	P value
NAFLD Yes	12 (40%)	0 (0%)	<0.0001*
NAFLD No	18 (60%)	61 (100%)	

Data are represented as N (%). Data are analyzed using Fischer exact. NFLD was significantly higher in diabetic patients with complications than controls.

Table 7: Prevalence of complications among diabetic patients:

Variables	DM with complications (N= 30)	P value
Nephropathy	27 (90%)	
Retinopathy	29 (97%)	
Neuropathy	21 (70%)	
Fundus examination		
NPDR	26 (87%)	
PDR	3 (10%)	
Microaluminuria	11 (37%)	
Macroalbuminuria	16 (53%)	
Treatment of DM		
Insulin	23 (77%)	0.5
Oral	7 (23%)	

Data are represented as N (%). Data are analyzed using Chi square (X^2). No significant difference between diabetic patients with complications regarding insulin and oral hypoglycemic drugs use.

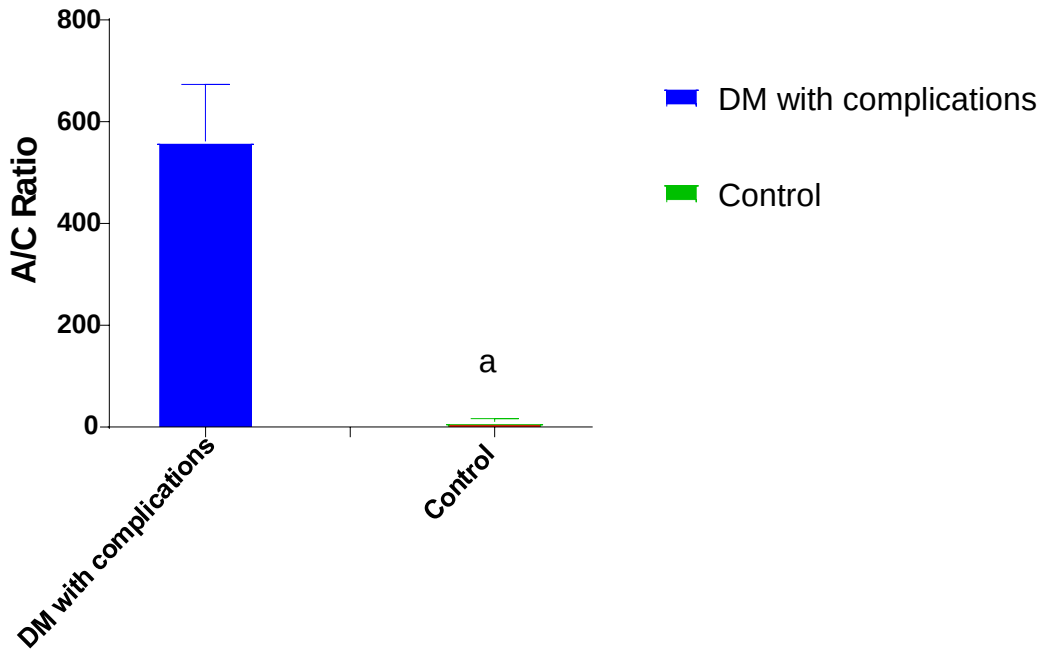

Figure 1: A/c ratio among the studied groups. There was a significant difference between the studied groups regarding A/C ratio. Control group has significantly lower concentration as regard A/C ratio.

5. Discussion:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, which is caused by a deficiency in insulin secretion or by biological action or both. long standing DM causes chronic damage and dysfunction of various organs, in particular to eye, kidney, heart, blood vessels, and nerve [1].

In type2 diabetes chronic hyperglycemia causes an increase in products of glycosylation which induce inflammation and injury to arterial walls, causing changes in vascular tissue and atherosclerosis leading to increase the risk of coronary artery diseases, stroke and peripheral vascular disease, which called macrovascular complications, smaller blood vessels also may be affected causing microvascular complications such as diabetic peripheral neuropathy, retinopathy and nephropathy [14].

MAEA gene is located in chromosome 4p16.3, and some genome wide association studies (GWAS) have demonstrated that an SNP rs6815464 in an intron of MAEA gene is associated with type 2 diabetes mellitus. Among eight new loci in various genes reported from the GWAS, MAEA rs6815464 showed the strongest signal [15].

This study was designed to assess MAEA gene rs6815464 polymorphism in a group of T2DM patients with complications and controls to emphasize its possible role as a risk factor to microangiopathic complications in diabetics. Our patients were selected according to their urinary Albumin /creatinine ratio, eGFR, fundus examination, microfilament test.

Our study showed that 29 (97%) patients had diabetic retinopathy and 26 (87%) patients of those had non-proliferative diabetic retinopathy (NPDR) while the remaining 3 (10%) patients had proliferative diabetic retinopathy (PDR). This is in agreement with Ramanathan RS. et al., [16], which showed that diabetic Retinopathy was the most common complication.

Our study showed that MAEA rs6815464 genotype frequency didn't differ significantly between diabetic patients with complications and free healthy controls.

To my knowledge, this is the first study to evaluate the association between MAEA gene polymorphism and microangiopathic complications of T2DM.

In contrast with our study, Cho, Y.S., et al., [17] a study was conducted to

identify susceptibility loci for type 2 diabetes (T2D) in East Asian populations. It showed that MAEA-rs6815464 was reported as a T2DM risk variant in Asians.

Our study also showed that NAFLD was significantly higher in diabetic patients with complications than in controls.

This is in agreement with Gastaldelli A. et al. [18] study which showed that NAFLD is associated with a 2- to 3-fold increased risk of developing type 2 diabetes (T2DM).

Also, Hazlehurst JM, et al., [19] a study demonstrated that presence of both NAFLD and T2DM increases the likelihood of the development of complications of diabetes (including both macro- and micro- vascular complications).

In Our study, there was no significant difference between the studied groups regarding their age, sex distribution, waist circumference and BMI.

This is in contrast to Zeid, et al., study [20] in which diabetic patients have mean BMI 26.60 ± 2.081kg/m2, in controls mean BMI was 27.92 ± 3.378 kg/m2 with p = 0.042*.

In conclusion, our study suggests that there is no significant association of the MAEA rs6815464 polymorphism with the incidence microangiopathic complications in type 2 diabetic patients. Also, T2DM increases risk of NAFLD.

Recommendations

Further studies on larger populations with MAEA gene rs6815464 polymorphism are required.

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