Original Research Article

Endothelial Nitric Oxide Synthase (eNOS) Gene Polymorphism (G894T) as a Risk Factor for obesity in the Egyptian Population

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Abstract

Background and Objective: Endothelial nitric oxide synthase gene polymorphism (eNOS) is one of three isoforms that synthesize nitric oxide (NO), that participates in several biological processes have been associated with obesity. This study was undertaken to determine if eNOS gene (G894T) was associated with susceptibility of obesity. **Materials and Methods:** the study was carried out on 200 cases divided into 100 obese patient and 100 healthy as control. The mean age cases was (27.02 ± 10.90) they include 79 female and 21 males. All participants were subjected to an estimation of their body mass index(BMI), weight hip ratio (WHR),in addition to random blood sugar (RBS) ,total cholesterol, triglyceride(TG),and lactate dehydrogenase enzyme (LDH). DNA was amplified using PCR-SSP for detection of relation between polymorphism and endothelial nitric oxide synthase gene in G894T. **Results:** All cases showed that there were no significant difference between cases and controls regarding to their chemical lab's analysis (TG, Cholesterol, LDL and HDL).All cases showed significant frequency of G894T GG, TT and GT (*P* <0.001) vs. Controls **Conclusion:** The polymorphism G894T of eNOS considered as a potential risk factor for obesity.

Key Word: Endothelia Nitric oxide, gene, polymorphism, obesity

Abbreviations: *Endothelial nitric oxide (eNOS), polymerase chain reaction with sequence specific primers PCR-SSP., Nitric oxide NO.*

Introduction

Obesity is a medical condition in which surplus body fat accumulated to the range that it might had a negative effect on health. **(1),** People are generally considered obese when their body mass index (BMI),A measurement obtained by dividing a person's weight by the square of the person's height, is over 30 kg/m2, with the range 25–30 kg/m2 defined as overweight ***(1)*** ,Some East Asian countries use lower values***.(2)*** Obesity increases the incidence of various diseases and conditions, specially cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, definite types of cancer, osteoarthritis and depression***. (3), (4*****).**

Obesity is most commonly caused by a mixing of excessive food intake, lack of physical activity, and genetic susceptibility***.(1),(5)*** A few cases are caused firstly by genes, endocrine disorders, medications, or mental disorder**. (6)** on the other hand obese people eat little next to gain weight because of a slow metabolism is not medically supported***.(7)*** .On average, obese people have a greater energy usage than their normal people because of the energy required to maintain an increased body mass***.(7) , (8).***

Obesity might be a cause of death which can be preventable worldwide, with increasing rates in adults and children ***(1).*** In 2015, 600 million adults (12%) and 100 million children were obese in 195 countries. **(9)** Obesity is more common in women than men ***(1)***. Several studies viewed that obesity is one of the most dangerous public health problems of the 21st century. **(10)** In 2013, obesity is classified as a disease by the American Medical Association***. (11), (12).***

Impaired NO. Production is involved in the pathogenesis of several diseases such as hypertension, diabetes mellitus, obesity, erectile dysfunction, and migraine. In this regards, a large number of studies showed that polymorphisms in NOS3 gene affect the susceptibility to these diseases. Although NOS3 is a highly polymorphic gene, three genetic polymorphisms in this gene have been widely studied: the single nucleotide polymorphisms (SNPs) g.-786T>C located in NOS3 promoter and in exon 7, respectively, and the variable number of tandem repeats 4b/4a (VNTR) characterized by 27 bp repeat in intron 4***. (13).*** The C allele for the T786C polymorphism, which results in reduced eNOS expression and NO.Production was associated with increased risk for hypertension***, (14).*** The VNTR in intron 4 affects eNOS expression***, (15).*** And the susceptibility to hypertension, ***(14).*** Obesity, ***(16).***

Materials and Methods

Study group: This study includes 200 cases 100 obese patients they were recruited from the department of diabetes and endocrine unit in specialized medical hospital Mansoura University, as well as Ministry of Health Hospitals of Dakahlia, Egypt during the period September 2016 to May 2018, as 100 patients obese. The mean age of cases were 27.02 ± 10.90 years they were in the form of 21 male and 79 female. According to the definition of metabolic syndrome given by WHO, ATP and IDF (75%) of patient were classified as having metabolic syndrome while the rest, (25%) were not complicated and were characterized as just having simple obesity

Control group: for comparison 100 healthy controls were selected.

Biochemical analysis: After 12 h of fasting, a blood was collected from each case and control in an empty tube blood sample for biochemical analysis. If the sample were not analyzed immediately, they will frozen and stored at -70 C. Total cholesterol, triglyceride (TG), LDL and HDL were measured by enzymatic methods on automatic biochemistry analyzer.

Capture column kit extraction and purification:

The generation DNA purification capture column kit (Gentra System, USA) is based on a proprietary system that uses two reagents, a DNA purification solution and a DNA elution solution, along with a specially formulated purification matrix. In this kit, a sample is applied directly to the purification matrix contained a spin column .the cells contained in sample lyse upon contact with the matrix. Once the cells were lysed, DNA was captured by the matrix material which make it possible to efficiently wash away contaminants, leaving the DNA bound to the matrix. Contaminants, including protein, heme and RNA are removed from the matrix by washing with DNA purification solution. Following removal contaminants, the DNA released from the matrix using DNA elution solution and heat .samples of purified DNA were ready for analysis and not require precipitation.

PCR amplifications of each eNOS studied: Single nucleotide polymorphism (SNPs) for nitric oxide synthase gene (eNOS) were genotyped in this case-control study G894T, T786Cand 27bp polymorphism using polymerase chain reaction PCR. Amplification were performed in sequence-specific primer polymerase chain reaction (SSP-PCR) employing a forward and reverse primer for each part. The region containing one (Restriction Fragment Length Polymorphisms) RFLPs within the eNOS gene was amplified with tag DNA polymerase, PCR buffer, Mgcl2 and dNTPs. The entire reaction volume plus 5 micro L of bromophenol blue track dye were loaded into 2% agarose gel (Bohringer Mannheim) containing ethidium bromide. And for 30 minutes at 100V Gels were electrophoresed, then photographed under UV light (320 nm) and then detect the presence or absence of an allele specific bands.

Statistical analysis

Statistical analysis of data was done using the software statistical package (SPSS program version 17). The student t-test was used to compare the numerical values related to cholesterol, other chemical parameter and body mass index whereas CHI square test used to compare frequencies of different genotypes and alleles between cases and controls.

Results

Cases and controls showed a non-significant difference regarding to their age (p = 0.74). However, cases showed a significant levels of BMI, cholesterol, TG, HDL-C and LDL-C (p =0.001). (Table 1)

Regarding to descriptive data of studied cases of obesity, cases showed a significant difference vs. control (normal, no disease) with p = 0.001 (Table 2)

Comparing all cases with obesity and healthy controls regarding their genotype distribution of eNOS gene polymorphism (in G894T) (Table 3): all genotypes (GG), (GT), and (TT) were highly significant (p=0.001) vs. controls. While on alleles analysis both (G) and (T) were significantly. (P =0.02)

Table 1 : Descriptive data of studied cases of obesity and healthy controls.

	Patients (N=100)	**Control (N=100)**	**t**	***P***
Hip	122.69 ± 12.96	89.26 ± 17.18	15.536	<0.001*
Weight	106.03 ± 16.95	68.66 ± 17.77	15.216	<0.001*
Height	162.47 ± 8.26	166.38 ± 7.55	3.495	0.001*
BMI	40.13 ± 6.40	25.02 ± 7.67	15.132	<0.001*
WHR	0.95 ± 0.14	0.82 ± 0.12	7.268	<0.001*
waist	116.16 ± 15.47	74.57 ± 24.76	14.245	<0.001*
Age	27.02 ± 10.90	27.51 ± 10.26	0.327	0.744
Cholesterol	246.32 ± 60.23	181.16 ± 44.48	8.703	<0.001*
TG	140.76 ± 95.91	101.74 ± 47.85	3.640	<0.001*
HDL-C	49.94 ± 15.60	37.54 ± 13.48	6.014	<0.001*
LDL-C	168.85 ± 64.86	124.10 ± 40.89	5.835	<0.001*

N: number of cases, t: Student t-test, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, *p =0.001 (significant).

Table 2: descriptive data of studied cases of obesity.

	Patients		Control		χ^2	*p*
	N	**%**	**N**	**%**		
disease						
obesity	53	53.0%	0	0.0%	200.000	<0.001*
obesity+D.M	21	21.0%	0	0.0%		
obesity+HTN	12	12.0%	0	0.0%		
obesity+D.M+ HTN	14	14.0%	0	0.0%		
normal, no disease	0	0.0%	100	100.0%		

N: Number of cases, %: percentage of cases, χ^2: Chi-square test

D.M.: Diabetes Mellitus, HTN. : Hypertension

Tablet 3: comparison between all cases with obesity and healthy controls regarding their genotype distribution of eNOS gene polymorphism in (G894T).

G894T		Patients		Control		χ^2	*p*
		N	**%**	**N**	**%**		
Genotype	GG	70	70%	40	40%	36.257	<0.001**
	GT	14	14%	54	54%		
	TT	16	16%	6	6%		
Alleles	(G)	154	77%	134	67%	4.960	0.026*
	(T)	46	23%	66	33%		

N= number of cases, % = percentage of cases, TT = thymine thymine, GT = guanine thymine, GG= guanine guanine, T =thymine, G=guanine. Significance using χ^2: Chi-square test:

**p*=0.026 (significant)

***p*=0.001 (extremely significant)

Electrophoresis result of PCR showing enzymatic digestion of G894T polymorphism of eNOS gene:

Wild type GG is found which appears at 206 b only lanes 4 and 6. Digestion of PCR product of G894T polymorphism of eNOS gene using Mbo1 enzyme. Which digests the 206-bp fragment into 119 and 87 bp fragments (heterozygous mutated genotype GT which has 206, 119, 87 bp fragments lanes 2 and 7) (homozygous mutated genotype TT is found which has 119, 87 bp fragments lanes 1, 3, 5) by using DNA size marker 50bp.

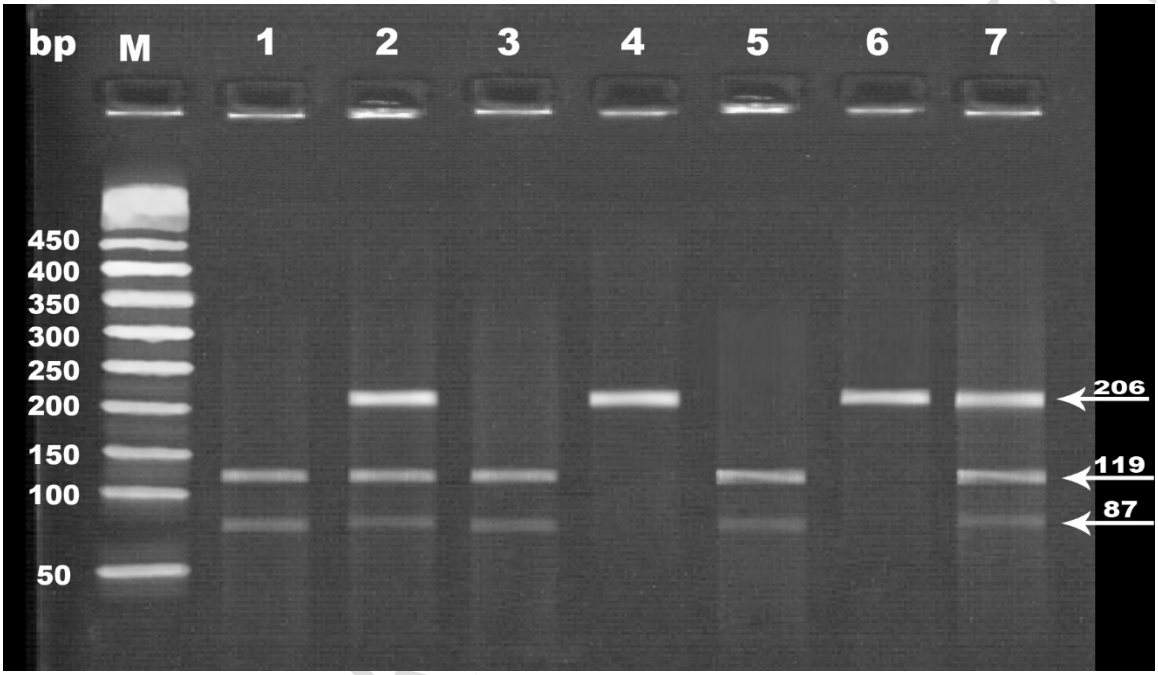

Fig 1: Enzymatic digestion of G894T polymorphism of eNOS gene.

Discussion

Overweight and obesity are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries ***(1).***

Obesity is one of the leading preventable causes of death worldwide ***(17), (18).***Growing evidence supports the association of diseases with NOS3 haplotypes (combination of alleles in close proximity, within a DNA block). This approach may be more informative than the analysis of genetic polymorphisms one by one. ***(19).*** Haplotypes including the SNPs g.-786T>C and Glu298Asp, g- G894T and the VNTR in intron 4 affected the susceptibility to hypertension, **(20).** And there is association between NOS3 and the susceptibility to obesity ***(16).*** And diabetes mellitus ***(21).***

The present study aims mainly to investigate the association of the eNOS gene polymorphism (G894T) with the possibility of occurrence obesity, the study results showed that homozygous mutated GG and homozygous mutated TT genotypes ,mutant G and T allele of G894T polymorphism had significant frequency between cases of obesity compared with controls.

In agreement with the prestent study, **Hala Ben Nasr et al. (22)** Have reported that among tunisian patients, eNOS gene polymorphism G894T was significantly associated with obesity.

The present research exhibited a significant association of G894T with occurrence of obesity and these results in harmony with results of **M.wrzosek et al. (23)**

Data obtained from study in Chinese population suggested that the G894T mutation in the endothelial nitric oxide synthase gene may serve as a major risk factor of essential hypertension in obese patients. **(24)**

Bressler J. et al. (25) in the United States in a study carried in four communities suggested that interaction between incidence of obesity and NOS3.

The G894T polymorphism of eNOS gene is a risk factor of obesity these result in accordance with the results of **Rihab Sendesni et al. (26)**

On the contrary to the present research **Roberta Fernanda da Silva et al. (27)** did not demonstrate a significant difference in plasma NO2 concentration blood pressure and obesity taking into account the haplotype results (-786T/C, 4b/4a, and 894G/T).

Our study reported that endothelial nitric oxide gene polymorphism (G894T) is a risk factor for development of obesity.

CONCLUSIONS

This study found that The eNOS gene polymorphism (G894T) is a risk factor for development of obesity. And mutant G, T alleles, (GG and TT genotypes of G894T), significantly considered a risk factor for development of obesity.

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ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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