Journal of King Saud University – Science 32 (2020) 920–923

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

C677T genotypes in *methyltetrahydrofolate reductase* gene in student obesity

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ARTICLE INFO

Article history:
Received 1 May 2019
Revised 15 May 2019
Accepted 27 May 2019
Available online 1 June 2019

Keywords:
Obesity
C677T
MTHFR gene
GCUF students
Pakistan population

ABSTRACT

Obesity is known as weight gaining programme which leads to increase in body mass index (BMI), is known for significant risk factors for chronic diseases. The prevalence of obesity has been rising in South Asian countries, specifically in Pakistan. Numerous studies have shown the association with metabolic diseases (ex; obesity) with single nucleotide polymorphisms. The present study has been designed to investigate the association between C677T polymorphism in methylenetetrahydrofolate reductase (*MTHFR*) gene with obesity in Pakistan population. In this case-control study, we have enrolled 200 obese students and 200 non-obese or control students based on the BMI. Genomic DNA was isolated with enrolled 400 blood samples and PCR was performed with C677T polymorphism in the *MTHFR* gene to analyse the frequency. Later on restriction enzyme length polymorphism analysis was carried out for the undigested PCR products. Genotyping frequencies for C677T polymorphism were compared between obesity and non-obesity students. Genotype, allele and various genetic model of inheritances were calculated and documented the significant association ($p < 0.05$). However, Anova analysis couldn't document the positive association when we measured with C677T polymorphism in obesity cases versus anthropometric measurement such as age, weight and BMI ($p > 0.05$). The present study concludes C677T polymorphism in *MTHFR* gene was genetically associated between the obesity risks in Pakistan population. However, relation with Anova analysis showed the negative association with C677T polymorphism.

1. Introduction

The meaning of obesity is defined as excess of fat absorbance present in the human body. Obesity is explained as the complex disorder amongst which excess fat has been deposited in the human body, which significantly increases the morbidity and mortality (Mozafarizadeh et al., 2019). Based on WHO, obesity is confirmed as BMI (Body Mass Index) is exceeding more or equal to 30 kg/m^2 (Ayoub et al., 2019). Presently, obesity is known as major global health problem in both males and females' and later on associated with additional metabolic diseases such as hypertension, atherosclerosis, type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS), cancer and cardiovascular diseases (CVD) like stroke, myocardial infarction and coronary artery diseases (Zamani et al., 2019). Obesity is also known as socio-economic issues with predisposing factors which is considered as behavioral disorder (Shahid and Hasnain, 2018)and also associated with the activation of inflammation and its specific pathways as well as with production of abnormal cytokines (Barati et al., 2019). Since 1975, the global prevalence of obesity has been tripled. The consequence between non-communicable diseases and adiposity are defined with anthropometric modifiable factors like BMI, WC (waist circumference) and skin-fold thickness (Shen et al., 2019). The obesity disease is related with growth in plasma concentrations of free fatty acids, obstructing insulin stimulation and uptake of glucose. Incidence of overweight and obesity is expanding extremely all over the world. Globally, 1.1 billion people are overweight and obesity subjects has been crossed more than 310 million (Bego et al., 2019). The prevalence of obesity is varying from rural and

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2019.05.009
1018-3647/

urban areas; in urban areas men were effected with 56% and in 67% in women, whereas in the overall Pakistan, women were proned with 38% and men with 28% of obesity (Siddiqui et al., 2018) and Pakistan is facing an important substitution from communicable to non-communicable disease, is expanding from children to adolescents.

GWAS (genome-wide association studies) has documented with minimum of 15 candidate genes responsible for the growth in visceral depots; commonly associated with metabolic disorders (ex; T2DM, CVD and MetS). However, it is important to document genetic loci are associated with obesity or not (McCarthy, 2010). BMI and WC are the basic catalogues used to measure the overweight and obesity. For abdominal obesity, WC is a good marker and BMI is an ever green marker for the measurement of currently visible adiposity in the humans. However, in the neck circumference is an extra marker to measure the obesity (Ma et al., 2017). Confirmatory documents with twin and family-based studies suggests strong heritability of obesity ranging from 40 to 70%. Genes and molecules regulates the intake of energy and output have been studied widely (Ren et al., 2019). Earlier studies have been documented the relationship between obesity and hypertension; as both these diseases have similar genetic architecture and loci. GWAS have identified large number of similar SNPs (single nucleotide polymorphisms) such as *ATP2B1*, *CSK*, *MTHFR* (Methylenetetrahydrofolate reductase) and *CYP17A1* and more that is commonly associated with obesity and hypertension (Fu et al., 2018). The *MTHFR* gene is regulating for the remethylation pathway (Matam et al., 2015). Pirozzi et al. (2018) has documented a study implementing C677T polymorphism in Brazilian obese subjects. Limited studies have been performed with C677T polymorphism in Pakistan population but not with the obesity. So, the current study aimed to investigate the C677T genetic variant in *MTHFR* gene with Pakistani obesity.

2. Materials and methods

2.1. Students selection

Approval of ethical grant was the first step in designing this study. This work has been started by collecting 200 obesity and 200 non-obesity subjects from Government College University Faisalabad (GCUF) in Pakistan. The GCUF is one of the major universities in Pakistan with the total strength of 28,000 students in the campus. The obesity cases were selected based on the BMI above 30 kg/m^2 with in the GCUF premises. The inclusion criteria of the controls were whose BMI is <25 kg/m^2. We have excluded whose BMI is >25 kg/m^2 and GCUF students who were suffering from fever, typhoid or diarrhea and any other medical problem. This study was carried out between December 2014-June 2015 with in the GCUF premises. All the involved students have filled the questionnaire and signed the consent form.

2.2. Questionnaire details

The participated student has documented the details such as, age, gender, marital status, height, weight, BMI, and family history of obesity. Additionally, we have taken the physical activity and nutritional details from participants. Weight and height of the participants was measured with Quetelet's equation for BMI (Khan et al., 2015).

2.3. Blood analysis

From the 400 students, we have enrolled 1.0 mL of the EDTA blood from GCUF premises. Using genomic DNA isolation kit, genomic DNA has been extracted (Promega, USA). NanoDrop was used to quantify the genomic DNA. C677T genotyping was performed with Polymerase chain reaction (PCR) and then the PCR product was digested with *HinfI* restriction enzyme. PCR was performed with 50 μL reaction and primers were selected from Khan et al (Khan et al., 2015) studies. Digested (T allele 175/23 bp) and undigested (C allele 198 bp) PCR products were run on 2% agarose gel electrophoresis.

2.4. Statistical analysis

SPSS software (Version 19.0, USA) were implemented. Using the t-tests between Obesity cases and non-obese (controls), the continuous variables were calculated. Clinical characteristics were expressed as mean and standard deviation. Hardy Weinberg equilibrium (HWE) deviates the genotype distribution. Allele and genotype frequencies were calculated. Anova (one-way) analysis was measured between the non-modifiable risk factors and C677T genotypes in obesity cases. A P value < 0.05 is considered as significant.

3. Results

3.1. Non-modifiable and modifiable risk factors

This is a case-control study carried out from GCUF in Pakistan and 400 students have been enrolled and defined as 200 obesity cases and 200 non-obesity control subjects. The clinical characteristics have been shown in Table 1. We have planned to recruit 45% of male and 55% of female students in both the cases and control subjects ($p = 0.99$). The mean age was apparent to be similar in obesity (25.9 ± 4.1) and non-obesity (25.7 ± 3.9) students ($p = 0.48$). Weight and BMI was significantly associated ($p < 0.05$). There was a negative association between height, family history of obesity/T2DM ($p > 0.05$). The modifiable risk factors such as physical activity, nutritional diet and smoking has been recorded in Table 2. The physical inactivity and smoking were found to be high between the cases versus controls ($p < 0.05$). Physical activity, non-smoking and non-vegetarian were negatively associated ($p > 0.05$).

Table 1
Non-modifiable risk factors between obesity and non-obesity subjects.

Non-modifiable parameters	Obesity (n = 200)	Non-obesity (n = 200)	P-value
Age (Years)	25.9 ± 4.1	25.7 ± 3.9	0.48
Gender (Male: Female)	90 (45%):110 (55%)	90 (45%):110 (55%)	0.99
Weight (kg)	88.2 ± 14.32	65.7 ± 10.21	0.0002
Height (Cms)	164.1 ± 13.1	165.8 ± 12.9	0.82
BMI (Kg/m^2)	32.8 ± 6.1	23.9 ± 5.8	<0.0001
Family History of Obesity	64 (32%)	58 (29%)	0.17
Family History of T2DM	140 (70%)	122 (61%)	0.01

Table 2
Modifiable risk factors between obesity and control subjects.

Life-style	Obesity (n = 200)	Controls (n = 200)	t-tests
Physical activity	32 (16%)	125 (62.5%)	0.17
Physical inactivity	168 (84%)	75 (37.5%)	0.0001
Smoker	35 (17.5%)	12 (6%)	0.001
Non-smoker	165 (82.5%)	188 (94%)	0.12
Vegetarian	0 (0%)	0 (0%)	0.00
Non-vegetarian	200 (100%)	200 (100%)	0.99

Table 3
Genotypic calculation for C677T polymorphism for obesity and non-obesity.

C667T	Obesity cases (n = 200)	Non-obesity (n = 200)	P values	Odds ratios with 95% confidence intervals
CC genotypes	140 (70%)	161 (80.5%)	Reference	Reference
CT genotypes	49 (24.5%)	38 (19%)	0.10	1.48 (0.91–2.39)
TT genotypes	11 (5.5%)	1 (0.5%)	0.002	12.65 (1.61–87.1)
CT + TT vs CC	60 (30%)	39 (19.5%)	0.01	1.76 (1.11–2.80)
TT vs CC + CT	189 (94.5%)	199 (99.5%)	0.003	11.58 (1.48–90.5)
CT vs CC + TT	151 (75.5%)	162 (81%)	0.18	1.38 (0.85–2.23)
C alleles	329 (0.82)	360 (0.90)	Reference	Reference
T alleles	71 (0.18)	40 (0.10)	0.001	1.94 (1.28–2.94)

Table 4
Correlation between non-modifiable risk factors and C667T genotypes using Anova analysis.

ANOVA	CC genotype (n = 140)	CT genotype (n = 49)	TT genotype (n = 11)	P value
Age (Years)	26.8 ± 4.1	24.9 ± 4.4	26.2 ± 4.0	0.82
Weight (kgs)	88.1 ± 13.9	82.4 ± 14.62	94.2 ± 14.45	0.90
BMI (kg/m^2)	32.8 ± 6.4	33.6 ± 6.2	32.1 ± 5.9	0.92

3.2. Genotyping analysis

C677T polymorphism was found to be in HWE. The CC (70%), CT (24.5%) and TT (5.5%) genotypes were present in obesity cases, whereas in non-obese CC, CT and TT genotypes were 80.5%, 19% and 0.5% appeared. T allele was recorded higher in cases (18%) when compared with the controls (10%). The obesity cases had 82% of c-allele and 90% of them were perceived in non-obese (Table 3). Genotype (CT vs CC; p = 0.002, TT vs CC; p = 0.01), allele (T vs C; p = 0.001) and genetic mode of inheritances such as dominant (CT + TT vs CC; p = 0.01) and recessive (TT vs CC + CT; p = 0.003) modes of inheritance showed the significant association.

3.3. Anova analysis

There was no association when we compared the Anova analysis in age, weight and BMI with C677T genotypes in obesity cases (p > 0.05; Table 4).

4. Discussion

The current study discusses the issue with C677T polymorphism in the *MTHFR* gene in obesity students in Pakistan population. The present study was carried out with 200 obese and 200 controls recruited from GCUF campus in Faisalabad city in Pakistan. Till now, no genetic studies have been documented, and the current study is the initial study to be carried out with significant association in CT and TT genotypes, T allele, dominant and recessive models.

Genetic polymorphisms in human individuals are affected due to the variation, which may be genetic or non-genetic is recognized as SNPs. C677T polymorphism is the most clinically investigated in human diseases apart from numerous SNPs located in the *MTHFR* gene. The chromosome position is located at 1p36.3 (Matam et al., 2015). The catalyze enzyme transforms the 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. C677T polymorphism in *MTHFR* gene (rs1801133) lower enzyme activity gives rise for the dietary requirement in folic acid to maintain the normal homocysteine to remethylation to methionine (Yi et al., 2019). Hyperhomocysteinemia has been associated with a variety of metabolic disorders and diseases and obesity is one of them. T allele in C677T polymorphism is associated with an enormous number of disease risks such as obesity, hypertension, insulin resistance, T2DM, Mets and lipid disorders. Furthermore, this polymorphism may affect the DNA methylation and hypomethylation (Wang et al., 2018). Obesity is connected with hypertension, T2DM and heart diseases such as stroke, MI and CVD. The combination of obesity with homocysteine levels in *MTHFR* gene is documented as a risk factor (Papandreou et al., 2007). Obesity is known as complex disorder, proved as relatively difficult to elucidate and is accompanied by life threatening complications. In general, it is arising from genetic and environment risk profile as excess intake of calorie, physical inactivity, insufficient sleep, gastrointestinal microbiome and BMI heritability was found to be 40–70% (Fairbrother et al., 2018, Goodarzi, 2018). In the literature, GWAS has been documented with BMI, waist-hip ratio and adiposity related SNPs available (Goodarzi, 2018). C677T polymorphism in Pakistani population is carried out in male infertility, MI, rheumatoid arthritis, breast cancer, hyperhomocysteinemia, genetic birth defects and in general population (Akram et al., 2012, Iqbal et al., 2015, Iqbal et al., 2016, Mansoor et al., 2009, Michael et al., 2008, Shi et al., 2003, Ullah et al., 2019, Yakub et al., 2012). Limited polymorphism studies have been documented in Pakistani population (Qureshi et al., 2017, Rana et al., 2018, Shabana and Hasnain, 2015a,b, Shabana et al., 2018, Shahid et al., 2013). Globally, case-control and *meta*-analysis studies showed the significant association with obesity (Fan et al., 2015, Kao and Muller, 2013, Lewis et al., 2008, Tsang et al., 2015) and our study is also in accordance with the prior global studies. However, exome-sequencing studies have been carried out in global population with obesity (da Fonseca et al., 2017, Gerhard et al., 2013, Jiao et al., 2015, Parente et al., 2017) and none of them could detect the genetic marker.

Screening the obesity students with C677T polymorphism in the *MTHFR* gene is one of the limitations of this study. Skipping of homocysteine levels were another limitation of this study. Choosing young obesity students is the current strengths of this study. In conclusion, the current study confirms the C677T polymorphism in *MTHFR* gene is associated with the obesity risk in Pakistani population. Future studies recommend to carry out similar study with many SNPs in large sample size to rule out the genetic role with obesity.

Declaration of Competing Interest

None.

Acknowledgements

"The authors (SM and KAAG) would like to express their sincere appreciation to the Deanship of Scientific Research at the King

Saud University for its funding of this research through Research Group Project No. RG-1435-012".

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