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Non synonymous A803G Polymorphism of N-acetyltransferase 2 Gene and Impaired lipid profile in Egyptian Obese Children and Adolescents

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Abstract

Background: Childhood overweight and obesity remains an important public health concern. The prevlance of obesity and overweight among children and adolescents aged 5-19 years has risen dramatically from 4% to over 20%. The aim of the present study was to assess A803G polymorphism in the NAT2 gene in Egyptian obese children and adolescents and to detect the relation between this gene mutation and impaired lipid profile in them. Patients and methods: this cross sectional study included 100 obese children and adolescents divided into two groups according to their HbA1c results : group 1(pre-diabetic obese children and adolescents) and group 2 (Non- diabetic obese children and adolescents) , both groups are compatible as regard age and sex and had equal numbers (50). Whole blood samples were collected and underwent genotyping todetect NAT2 A803G gene polymorphism using Taqman allelic discrimination assay. Results: There was highly statistically significant higher total cholesterol, triglycerides and LDL among Group 1 than Group 2. But regarding HDL, it was highly statistically significant higher among Group 2 than Group 1. Heterozygous A allele of Group 1 group had the worst lipid profile characteristics (higher cholesterol, triglycerides and LDL and lower HDL) but this difference was statistically significant only regarding LDL while regarding otherpararmeters, they were not statistically significant. While, heterozygous G allele of Group 2 had the worst lipid profile characteristics (higher cholesterol, triglycerides and LDL and lower HDL) but this difference was statistically significant only regarding LDL while regarding other parameters, they were not statistically significant. Conclusion: Our study concluded that Egyptian obese children and adolescents who carrying the NAT2 A803 allele might be at a high risk of impaired lipid profile and consequent increased future risk to develop secondary metabolic diseases.

Keywords: N-acetyltransferase 2, lipid profile, Obese Children

INTRODUCTION

The issue has grown to epidemic proportions, with over 4 million people dying each year as a result of being overweight or obese in 2017 according to the global

burden of disease. Rates of overweight and obesity continue to grow in adults and children. From 1975 to 2016, the prevalence of overweight or obese children and adolescents aged 5–19 years increased more than four-fold from 4% to 18% globally **(1)**. The most important complication of obesity is the early onset of metabolic syndrome, defined by the presence of dyslipidaemia, hypertension and impaired glucose homeostasis **(2)**. In response to IR, pancreas increases its insulin production to maintain glucose homeostasis, but the progressive fat accumulation in pancreatic β cells leads to β cells failure with impaired and defective insulin secretion. This condition clinically manifests with IGT (also defined as prediabetes) or T2D. However, an individual genetic susceptibility to develop IGT or T2D exists and in last decades the role of genetic polymorphisms has been extensively investigated **(3)**.

N-acetyltransferase 2 (NAT2) is a liver enzyme necessary for the detoxification and metabolism of foreign chemicals and various drugs, such as caffeine, isoniazid, sulfamidine, hydrazine, dapsone, procaine amide, sulfapyrimidine, nitrazepam amide, sulfapyridine, and nitrazepam **(4)** .

Numerous single nucleotide polymorphisms (SNPs) in the coding exon of the NAT2 gene, inherited as NAT2 haplotypes and genotypes, confer rapid, intermediate, and slow acetylator phenotypes dependent on the degree of alteration of gene and protein products **(5)**. NAT2*4 is one of most prevalent NAT2 haplotypes which classified as the wild type and is associated with fast (normal) acetylator status, as are the NAT2*12A (c.803A>G) and NAT2*13A (c.282C>T) haplotypes **(6)** .

Therefore, this study aimed to asses A803G polymorphism in the NAT2 gene in Egyptian obese children and adolescents

PATIENTS AND METHODS

This comparative cross sectional study was carried out in the pediatric endocrinology unit outpatient clinic, the Pediatric Department at Zagazig University Children's Hospital and the Microbiology and Immunology Department in collaboration with Zagazig Scientific and Medical Research Center Faculty of Medicine, Zagazig University, Egypt, in the period spanning from December 2019 to December 2020.

Inclusion criteria:

1-patients includes children and adolescents with age ranging from 5to 18 y. BMI of casesis greater than 2SD above the WHO Growth Reference Median. Patients do not have T1DM. Patients do not have secondary forms of obesity.

Full history and clinical examination with stress on anthropometric measures, hemoglobin A1c levels, HOMA IR and lipid profile were performed for each participant.

Lipid profile assessment: 5 ml morning blood samples (without anticoagulant) were collected from all children after a minimum of 12-h fasting, and with the routine procedure (determination of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), and TG was performed by colorimetric enzyme method on a RA-50 Chemistry Analyser spectrophotometer (Bayer), using LABTEST reagents), and using an automated instrument, these investigations for lipid profile assessment were done **(7)**.

Whole blood samples were collected and underwent genotyping to detect NAT2 A803G gene polymorphism using Taqman allelic discrimination assay. DNA was isolated using Gene JET Whole Blood Genomic DNA Purification Mini Kit. The identification of the gene polymorphism was carried out using Real Time polymerase chain reaction (PCR). Genotypes were assigned and confirmed byindependent laboratory investigators who were unaware of the patients'phenotypes. Patients were divided into two groups: pre-diabetic obese (group 1)and non-diabetic obese (group2) according their HbA1c results and the both groups wereincluded the assigned polymorphism (A803G) gene (homozygous mutant,homozygous wild and heterozygous A&G allelegenotypes).

Statistical analysis:

Data were analyzed using SPSS version 23 for data processing. The following statistical methods were used for analysis of results of the present study.Data were expressed as number and percentage for qualitative variables and mean + standard deviation (SD) for quantitative one. The arithmetic mean ($\overline{X}$) as an average describing the central tendency of observations. Chi- square test (X2) used to find the association between row and column variables. The student "t" test for comparison of means of two independent groups. Mann Whitney testwas used to calculate difference between quantitative variables in not normally distributed data in two groups.

ANOVA (F-test) test was used to calculate difference between quanitative variables in more than two groups. The threshold of significance was fixed at 5% level (P-value), P value of > 0.05 indicates non-significant result and P value of < 0.05 indicates significant results.

RESULTS

This comparative cross section study included 100participants, divided into two groups; group 1and group 2, both had equal numbers (50). There was no statistically significant difference between the groups 1 and 2 in age and sex **(Figure 1)**. There was statistically significant higherwaist circumference and Waist/Hip ratio among group 1 than group 2. But regarding weight, height, BMI, BMI-SDS and Waist/Height ratio, there was no statistically significant difference between group 1 and group 2 **(Tables 1)**.

The present study showed a systolic and diastolic blood, HBA1c and HOMAIR were highly statistically significant higher among Group 1 than Group 2 **(Figure 2)**. There was highly statistically significant higher total cholesterol, triglycerides and LDL among Group 1 than Group 2. But regarding HDL, it was highly statistically significant higher among Group 2 than Group 1**(Tables 2)**.

There was statistically significant difference in N-acetyltransferase 2 genotyping between Group 1 and Group 2 where homozygous wild was present only among Group 2 (4.0%) while heterozygous A allele was found only among Group 1 (12.0%) **(Tables 3)**.

The present study showed that Heterozygous A allele of Group 1 group had the worst lipid profile characteristics (higher cholesterol, triglycerides and LDL and lower HDL) but this difference was statistically significant only regarding LDL while regarding otherpararmeters, they were not statistically significant **(Tables 4)**. While, heterozygous G allele of Group 2 had the worst lipid profile characteristics (higher cholesterol, triglycerides and LDL and lower HDL) but this difference was statistically significant only regarding LDL while regarding other parameters, they were not statistically significant **(Tables 5)**.

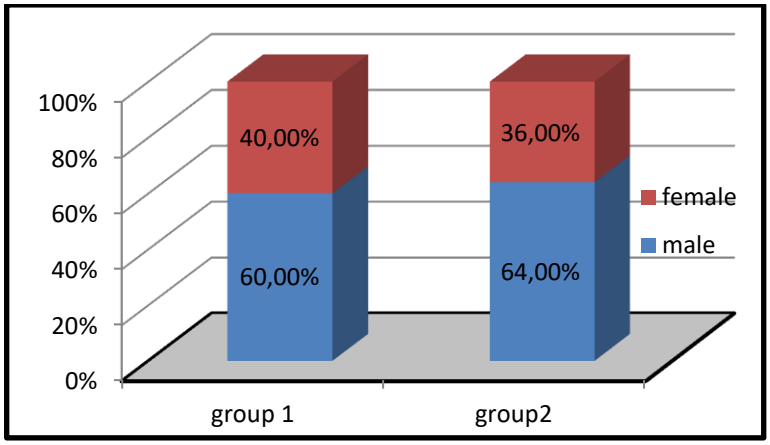

Figure (1): Bar chart for matched sex in the Group 1 and Group 2

Table (1): Comparing anthropometric measures between Group 1 and Group 2:-

Variables	**Group 1 (50) mean ± SD (Range) median**	**Group 2 (50) mean ± SD (Range) median**	**t-test**	**p-value**
Weight (Kg)	59.9±16.7 (30-94) 66	52.3±19.3 (28-95) 45	1.8	0.06
Height(Cm)	133.9±23.7 (95-170) 138	125.3±22.6 (95-170) 117	1.8	0.07
BMI	32.3±1.2 (31-36.2) 32.3	32.57±1.75 (30.8-39.5) 32.2	0.7	0.4
BMI-SDS	3.08±1.2 (2-6.22) 2.5	3.47±1.4 (2.15-6.31) 3.04	1.4	0.1
Waist circumference (Cm)	80.9±13.8 (57-98) 89	74.6±13.8 (55-96) 69	2.2	**0.02***
Waist/Hip ratio	0.61±0.04 (0.55-0.71) 0.6	0.59±0.025 (0.55-0.65) 0.59	1.9	**0.04***
Waist/Height ratio	0.64±0.05 (0.59-0.71) 0.61	0.57±0.03 (0.55-0.68) 0.56	1.5	0.2

* Statistically significant difference ($P \leq 0.05$)

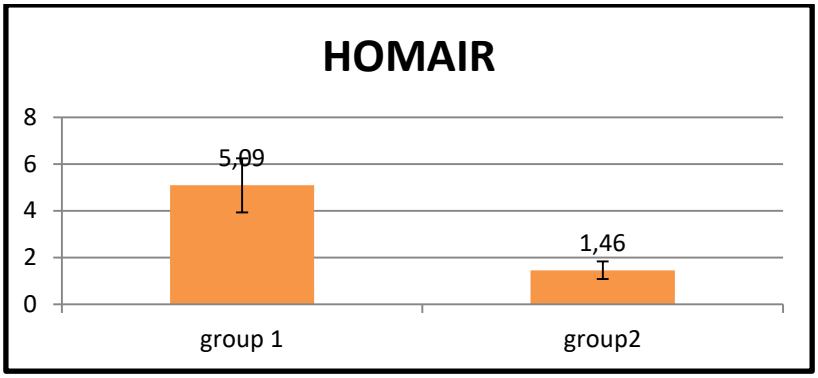

Figure (1): Bar chart for HOMAIR in the Group 1 and Group 2

Table (2): Comparing lipid profile between Group 1 and Group 2:-

Variables	**Group 1 (50) mean ± SD (Range) median**	**Group 2 (50) mean ± SD (Range) median**	**t-test**	**p-value**
Total Cholesterol	171.4±22.6 (127-211) 180	142.5±8.6 (133-160) 139.1	8.4	**0.001****
Triglycerides	131.9±39.1 (52-189) 130	89.2±16.2 (72.5-140) 86.7	7.1	**0.001****
HDL	43.3±8.1 (34-57.9) 40.7	49.2±5.8 (38.9-60.7) 50.2	4.2	**0.001****
LDL	118.9±20.2 (63-151.4) 117	69.4±7.4 (60-85) 68.5	16.2	**0.001****

** Statistically highly significant difference ($P \leq 0.001$)

Table (3): Comparing N-acetyltransferase 2 genotyping polymorphism (NAT2) between Group 1 and Group 2:-

NAT2	**Group 1**		**Group 2**		**χ^2**	**p-value**
	No(50)	**%**	**No(50)**	**%**		
Homozygous wild	0.0	0.0%	2	4.0%	10.6	**0.01***
Homozygous mutant	40	80.0%	38	76.0%		
HeterozygousA allele	6	12.0%	0.0	0.0%		
HeterozygousG allele	4	8.0%	10	20.0%		

Table (4): Relation between N-acetyltransferase 2 genotypingand lipid profile among the Group 1:-

Variable	*Homozygous mutant* (40) mean ± SD range	*HeterozygousA allele* (6) mean ± SD range	*HeterozygousG allele* (4) mean ± SD range	F- test	p-value	LSD
Total Cholesterol	170.5±22.5 (127-211)	185±3.9 (180-188)	160.5±35.2 (130-191)	1.6	0.2	0.1(1) 0.4(2) 0.09(3)
Triglycerides	130.7±54.1 (66-186)	132.5±39.3 (52-189)	127.2±3.2 (124.4-130)	0.03	0.9	0.9(1) 0.8(2) 0.9(3)
HDL	44.0±9.34 (37-56)	42.7±7.8 (34-57.9)	48.3±8.83 (40.7-56)	0.9	0.4	**0.7(1)** 0.2(2) 0.4(3)
LDL	115.6±19.1 (63-151.3)	136.6±11.4 (128.5-151.4)	124.6±30.89 (97.9-151.4)	3.2	**0.04***	**0.01*(1)** 0.4(2) 0.3(3)

* Statistically significant difference (P ≤ 0.05)

Table (5): Relation between N-acetyltransferase 2 genotyping and lipid profile among the Group 2:-

Variable	*Homozygous wild(2)* mean ± SD range	*Homozygous mutant(38)* mean ± SD range	*HeterozygousG allele(10)* mean ± SD range	F-test	p-value	LSD
Total Cholesterol	137.0±0.1 (137-137)	141.1±7.1 (133-160)	148.8±11.5 (133.1-159)	4.1	**0.02***	0.4(1) 0.06(2) **0.01*(3)**
Triglycerides	72.5±0.1 (72.5-72.5)	86.7±9.4 (72.5-100.9)	90.8±17.4 (72.5-140)	1.3	0.2	0.1(1) 0.2(2) 0.4(3)
HDL	53.7±6.1 (43.7-60.7)	48.4±5.3 (38.9-60.7)	43.7±0.1 (43.7-43.7)	4.8	**0.01***	0.2(1) **0.02*(2)** **0.009*(3)**
LDL	63±0.1 (63-63)	68.4±6.9 (60-85)	74.8±7.5 (63-80.3)	4.1	**0.02***	0.2(1) **0.03*(2)** **0.01*(3)**

* Statistically significant difference (P ≤ 0.05)

DISCUSSION:

Childhood overweight and obesity remains an important public health concern. The prevlance of obesity and overweight among children and adolescents aged 5-19 years has risen dramatically from 4% tin 1975 to over 18 % in 2016 (**1**).

It is well known that obesity represents the main modifiable risk factor for insulin resistance in children and adolescents; obesity-induced insulin resistance in children is the most important risk factor for developing cardiovascular diseases and type 2 diabetes in adulthood. The mechanisms through which obesity causes insulin resistance are complex and not completely known to date **(Tagi et al., 2020).**

Our results revealed no significant gender difference between our two group. Our results revealed a significant higher waist circumference and waist/Hip ratio among group 1 than group 2. But regarding weight, height, BMI and BMI-SDS and waist/height ratio, there was no statistically significant difference between both groups in agreement with **Marzuillo et al., (9)** who observed that there was no significant difference among the different NAT2 A803G genotypes for Waist/Height ratio.

Our results revealed statistically significant higher Waist/Hip ratio among Heterozygous A803 allele of group 1 than other genotypes , but no significant difference among the NAT2 A803G genotypes regarding waist/Hip ratio in group 2. Our results revealed no statistically significant difference in age, sex, weight, height, BMI and BMI-SDS among group1 and group2 with different N-acetyltransferase 2 (A803G) genotypes in agreement with **Marzuillo et al., (9)** who observed that there was no significant difference among the NAT2 A803G genotypes for age, sex, weight, height, BMI, BMI-SDS and waist circumference.

Our study revealed significantly higher systolic and diastolic blood pressure, HBA1c and HOMAIR among group1 than group 2. Our study revealed a statistically significant higher systolic blood pressure and HOMAIR among Heterozygous A803 allele of case group than other genotypes. Regarding diastolic blood pressure and HBA1c, they were higher among Heterozygous A803 allele of group 1. This difference was not statistically significant. These results were in agreement with **Knoweles et al., (10)** who observed association of A803 allele at rs1208 with HbA1c. In contrary, **Marzuillo et al., (9)** revealed no significant difference among the studied groups of NAT2 A803G genotypes regarding systolic or diastolic blood pressure, HBA1c and HOMAIR among NAT2 A803G genotypes.

Our study showed highly statistically significant higher total cholesterol, triglycerides and LDL among group 1 than group 2. But regarding HDL, it was highly statistically significant higher among group2 than group 1. Our results revealed that Heterozygous A allele of group 1 has the worst lipid profile characteristics (higher cholesterol, triglycerides and LDL and lower HDL) but this difference was statistically significant only regarding LDL while regarding other parameters, they were not statistically significant in agreement with **Knoweles et al., (10**) in their GWAS observed association for the A803 allele at rs1208 regarding TG levels, total and LDL cholesterol but no Significance association with HDL cholesterol levels .

In contrary **Marzuillo et al., (9)** revealed no significant difference regarding cholesterol, triglycerides, LDL and HDL among NAT2 A803G genotypes. **Nielsen et al., (11)** demonstrated that the risk of developing lipid disorders is 2.8 times higher in obese children (BMI > 90th percentile) than in children with normal body weight.

Thus, Obese children present with increased LDL cholesterol and triglycerides levels and decreased HDL cholesterol levels. Elevated LDL cholesterol may lead to atherosclerosis and trigger chronic inflammation. Abnormal lipid levels are present in 12 to 17% of children and adolescents who are overweight **(12)**.

CONCLUSION

Our study concluded that Egyptian obese children and adolescents who carrying the NAT2 A803 allele might be at a high risk of impaired lipid profile and consequent increased future risk to develop secondary metabolic diseases.

No Conflict of interest.

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