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Correlation between Nonsynonymous A803G Polymorphism of N-acetyltransferase 2 Gene and Impaired Glucose Homeostasis in Egyptian Obese Children and Adolescents

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ABSTRACT

Background: Two thirds of the world's populations live in countries where obesity-related illness is a significant cause of death. There is a considerable increase in adult obesity and there is good evidence that more children are also becoming obese especially over the last 30 years period.

Objective: To assess A803G polymorphism in the NAT2 gene in Egyptian obese children and adolescents and to detect the relation between this gene mutation and development of impaired glucose homeostasis in them.

Patients and methods: 100 children and Adolescents were investigated in the study. They were divided into 2 groups according to their HbA1c: 50diagnosed as pre-diabetic obese were enrolled as group (1) and 50 diagnosed as non-diabetic obese were enrolled as group (2). **Results:** correlation between HbA1c and HOMAIR showed that HbA1c was statistically significantly positively correlated with weight, waist circumference, systolic and diastolic blood pressures, HOMAIR, total cholesterol and LDL. While, HbA1c was statistically significantly negatively correlated with HDL and BMI-SDS, with no statistically significant correlations with other variables among Group 1. HOMA-IR was statistically significantly positively correlated with weight, waist circumference and systolic and diastolic blood pressures. While, HOMAIR was statistically significantly negatively correlated with BMI-SDS among Group 1. HbA1c was statistically significantly positively correlated with weight and waist with no statistically significant correlation with other variables among Group 2.

Conclusion: Egyptian obese children and adolescents who are carrying NAT2 A803 allele might be at a high risk of developing impaired glucose homeostasis and consequent increased future risk to develop diabetes mellitus type 2.

Keywords: Glucose homeostasis, NAT2 A803G, BMI-SDS.

INTRODUCTION

Overweight and obesity are serious public health problems that are caused by energy imbalance between calories consumed and energy gained. Obesity are caused by many interrelated factors as dietary behaviour, physical inactivity, family socio-demographic characteristics, environmental factors, and genetic factors [(1)]. Childhood obesity and its related complications such as impaired glucose tolerance (IGT), type 2 diabetes (T2D), and nonalcoholic fatty liver disease have been constantly increasing during the last decades with a strong impact on children's well-being [(2)]. Genetic analysis is a powerful tool to identify metabolic risk loci, and alleles that contribute to IR and T2D risk [(3)].

The strongest association for a nonsynonymous SNP in NAT2 [rs1208 (803A>G, K268R)] with the ancestral "A" allele (frequency 0.57) associated with a greater degree of IR in analyses are adjusted for age, gender, and BMI in a large adult population. The same ancestral "A" allele at rs1208 was associated with IR-related traits, including increased fasting glucose, hemoglobin A1c, total and LDL cholesterol, triglycerides, and coronary artery disease [(4)]. The NAT2 A803 allele seems to play a role in worsening the destiny of obese children carrying it, predisposing them to impaired glucose homeostasis and then to a possible future T2D [(5)]. This study aimed to asses A803G polymorphism in the NAT2 gene in Egyptian obese children and adolescents and to detect the relation between this gene mutation and development of impaired glucose homeostasis in them.

PATIENTS AND METHODS

A comparative cross-sectional study was carried out in the Pediatric Endocrinology Unit Outpatient Clinic, Pediatric Department, 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. The study was conducted in the period from December 2019 to December 2020.

100 children and adolescents were investigated in the study then divided into 2 groups according to their HbA1c: 50 diagnosed as pre-diabetic obese were enrolled as group 1 and 50 diagnosed as non-diabetic obese were enrolled as group 2. The mean age was 9.4 ± 4.2 years. 62 males and 38 females were included in both cases and controls.

Inclusion criteria: Children and adolescents with age ranging from 5 to 18 years. BMI of cases was greater than 2 SD above the WHO Growth Reference Median. Patients do not have T1DM. Patients do not have secondary forms of obesity.

Exclusion criteria: Patients outside age group, patients that their parents refuse to share in the study, patients

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lack the diagnostic criteria of simple obesity and patients have T1DM or secondary forms of obesity.

All children and adolescents included in the study were subjected to full medical history, clinical examination and anthropometric measurements including: Body weight, height, waist to height ratio (WTHR) and Body mass index (BMI). Obesity is defined for children aged between 5-19 years as BMI is greater than 2 SD above WHO Growth Reference Median [(6)].

Laboratory investigation:

- **HbA1c**: 2ml peripheral venous blood samples were collected in an EDTA tubes. For HbA1c test to classify as normal, or in the non-diabetic range the value must be below 5.7 %. Anyone with anHbA1c value of 5.7 % to 6.4 % is considered to be prediabetic, while diabetes can be diagnosed with HbA1c of 6.5% or higher [(7)].
- **HOMA-IR:** 5 ml morning blood samples were collected from all children and adolescents after a minimum of 8-h fasting for glucose and insulin measurements by immune fluorometric method. HOMA-IR was calculated by multiplying the value of fasting glucose and fasting insulin divided by 22.5. The score more than 4.0 is classified as insulin resistance and the score less than 4.0 is considered as insulin sensitive according to the formula: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5 [(8)].

Detection of NAT2 A803G genepolymorphism using Taqman allelic discrimination assay:

All the reagents were highly purified analytical PCR-materials. All the tubes, pipettes tips used for DNA extraction were DNAse, RNAse free tubes to avoid contamination purchased from thermo scientific (Waltham. USA). The following steps were performed for all patients: 1) Genomic DNA extraction was performed from whole blood sample. 2) Real time PCR amplification of the NAT2 A803G gene using a specific primers. 3) Taqman allelic discrimination assay allow to genotype 2 possible variants at the site of SNP measuring the change in the fluorescence of the dyes associated with probes in Real Time PCR.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Data were analyzed using SPSS version 23 and expressed as number and percentage for qualitative variables and standard deviation (SD) for quantitative one. Chi- square test (X^2) used to find the association between row and column variables. The student "t" test for comparison of means of two independent groups. Mann Whitney test was 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 quantitative 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 study included 100 participants, divided into two equal groups; group 1and group 2. There was statistically significant higher Waist/Hip ratio among Heterozygous A allele of group 1 than other genotypes but regarding age, sex, BMI, BMI-SDS and waist circumference, there was no statistically significant difference among group 1 with different N-acetyl transferase 2 genotypes (Table 1).

There was statistically significant higher systolic blood pressure and HOMA-IR among Heterozygous A allele of group 1 than other genotypes. Regarding diastolic blood pressure and HbA1c, they were higher among Heterozygous A allele of group 1 but this difference was not statistically significant (Table 2).

Regarding group (2), there was no statistically significant difference regarding age, sex, BMI, BMI-SDS, Waist/Hip ratio and waist circumference among group 2 with different N-acetyltransferase 2 genotypes (Table 3).

There was statistically significant higher systolic blood pressure among Heterozygous G allele of group 1 than other genotypes. Regarding diastolic blood pressure, HbA1cand HOMA-IR, they were higher among Heterozygous B allele of group 2 s group but this difference was not statistically significant (Table 4).

Concerning correlation between HbA1cand HOMA-IR, HbA1c was statistically significantly positively correlated with weight, waist circumference, systolic and diastolic blood pressure, HOMA-IR, total cholesterol and LDL and statistically significantly negatively correlated with HDL and BMI-SDS, with no statistically significant correlation with other variables among group 1. HOMA-IR was statistically significantly positively correlated with weight, waist circumference, systolic and diastolic blood pressures, total cholesterol and LDL and statistically significantly negatively correlated with BMI-SDS, with no statistically significant correlation with other variables among Group 1 (Table 5). While, HbA1c was statistically significantly positively correlated with weight and waist with no statistically significant correlation with other variables among group 2. HOMA-IR was statistically significantly positively correlated with weight, waist circumference, systolic and diastolic blood pressures, total cholesterol and LDL and statistically significantly negatively correlated with BMI-SDS, with no statistically significant correlation with other variables among group 2 (Table 6).

Table (1): Relation between N-acetyltransferase 2 genotyping, socio-demographic and anthropometric measures among group 1

Variable	Homozygous mutant (40) mean ± SD	Heterozygous A allele (6) mean ± SD	Heterozygous G allele (4) mean ± SD	F- test	p-value	LSD
Age (years)	9.2 ± 4.4	14 ± 3.1	11 ± 5.8	2.4	0.06	0.1(1) 0.4(2) 0.3(3)
BMI (kg/m^2)	32.4 ± 1.3	32.4 ±1.2	31.7 ± 0.69	0.6	0.5	0.9(1) 0.2(2) 0.3(3)
BMI-SDS	2.17 ± 0.23	3.27 ± 1.3	2.55 ± 0.53	2.5	0.06	**0.04*(1)** 0.2(2) 0.6(3)
Waist circumference (Cm)	79.1 ± 13.8	93.3 ± 1.36	79.5 ± 16.7	2.9	0.06	**0.01*(1)** 0.9(2) 0.1(3)
Waist/Hip ratio	0.59 ± 0.03	0.61 ± 0.03	0.56 ± 0.01	3.2	0.04*	**0.01*(1)** 0.4(2) 0.1(3)

K.W=Kruskal-Wallis test, * Statistically significant difference (P ≤ 0.05)

Table (2): Relation between N-acetyltransferase 2 genotyping, blood pressure and glucose level among group 1

Variables	Homozygous mutant (40) mean ± SD	Heterozygous A allele (6) mean ± SD	Heterozygous G allele (4) mean ± SD	F- test	p-value	LSD
Systolic blood pressure	115.8±7.5	125±4.5	117.5±8.6	4.1	**0.02***	**0.007*(1)** 0.6(2) 0.1(3)
Diastolic blood pressure	75.3±8.1	81.67±6.8	72.5±8.7	2.1	0.1	0.07(1) 0.5(2) 0.08(3)
HBA1c	5.88±0.2	6.07±0.05	5.9±0.11	2.3	0.1	**0.03*(1)** 0.9(2) 0.2(3)
HOMAIR	4.85±0.93	6.93±1.34	4.75±0.51	12.5	**0.001****	**0.001**(1)** 0.8(2) **0.001****(3)

** Statistically highly significant difference (P ≤ 0.001) * Statistically significant difference (P ≤ 0.05)

Table (3): Relation between N-acetyl-transferase 2 genotyping, socio-demographic and anthropometric measures among group 2

Variables	Homozygous wild (2) mean ± SD	Homozygous mutant (38) mean ± SD	Heterozygous G allele(10) mean ± SD	F- test	p-value	LSD
Age	4±0.1	8.5±4.3	9.2±3.8	1.2	0.3	0.1(1) 0.1(2) 0.6(3)
BMI	33.4±0.1	32.4±1.9	32.6±0.7	2.1	0.1	0.06(1) 0.7(2) 0.07(3)
BMI-SDS	4.1±0.1	3.37±1.3	3.3±1.4	K.W 2.2	0.2	0.06(1) 0.07(2) 0.8(3)
Waist circumference (Cm)	60.0±0.1	74.3±14.1	78.8±12.4	1.6	0.2	0.1(1) 0.8(2) 0.08(3)
Waist/Hip ratio	0.6±0.01	0.59±0.02	0.59±0.02	0.7	0.5	0.5(1) 0.9(2) 0.3(3)

Table (4): Relation between N-acetyltransferase 2 genotyping, blood pressure and glucose level among the group 2

Variables	Homozygous wild(2) mean ± SD	Homozygous mutant(38) mean ± SD	Heterozygous G allele(10) mean ± SD	F- test	p-value	LSD
Systolic blood pressure	100±0.1	108.5±5.	111.8±6.7	3.4	**0.03***	0.06(1) **0.01*(2)** 0.1(3)
Diastolic blood pressure	60.0±0.1	68.4±5.7	71±6.1	3.1	0.06	**0.04*(1)** **0.01*(2)** 0.2(3)
HBA1c	5.0±0.1	5.04±0.23	5.08±0.2	0.2	0.7	0.6(1) 0.2(2) 0.5(3)
HOMAIR	1.00±0.001	1.44±0.37	1.62±0.30	2.4	0.09	0.1(1) **0.03*(2)** 0.2(3)

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

Table (5): Correlation between HBA1cand HOMAIR with patients' characteristics and laboratory data among group 1

Variables	Group 1					
	HBA1c			HOMAIR		
	r	p	Sig	r	p	Sig
Age (years)	0.04	>0.5	NS	0.01	>0.5	NS
Weight (Kg)	**0.6**	**0.001****	**HS**	**0.7**	**0.001****	**HS**
Height(Cm)	0.07	>0.5	NS	0.09	>0.5	NS
BMI	0.06	>0.5	NS	0.02	>0.5	NS
BMI-SDS	**-0.6**	**0.001****	**HS**	**-0.5**	**0.001****	**HS**
Waist circumference (Cm)	**0.7**	**0.001****	**HS**	**0.6**	**0.001****	**HS**
Waist/Hip ratio	-0.4	>0.5	NS	-0.1	>0.5	NS
Waist/Height ratio	0.06	>0.5	NS	0.03	>0.5	NS
Systolic blood pressure	**0.4**	**0.001****	**HS**	**0.6**	**0.001****	**HS**
Diastolic blood pressure	**0.3**	**0.01***	**S**	**0.5**	**0.001****	**HS**
HBA1c	----------			**0.7**	**0.001****	**HS**
HOMAIR	**0.7**	**0.001****	**HS**	----------		
Total Cholesterol	**0.3**	**0.006***	**S**	**0.4**	**0.001****	**HS**
Triglycerides	0.2	>0.5	NS	0.2	>0.5	NS
HDL	**-0.3**	**0.03***	**S**	-0.2	>0.5	NS
LDL	**0.3**	**0.04***	**S**	**0.4**	**0.003***	**S**

*Statistically significant difference ($P \leq 0.05$) **Statistically highly significant difference ($P \leq 0.001$)
S=Significant, HS=highly significant.

Table (6): Correlation between HBA1c and HOMAIR with patients' characteristics and laboratory data among group 2

Variables	Group 2 group					
	HBA1c			HOMAIR		
	r	p	Sig	r	p	Sig
Age (years)	0.03	>0.5	NS	0.06	>0.5	NS
Weight (Kg)	**0.3**	**0.01***	**S**	**0.7**	**0.001****	**HS**
Height(Cm)	0.09	>0.5	NS	0.01	>0.5	NS
BMI	0.03	>0.5	NS	0.1	>0.5	NS
BMI-SDS	0.2	>0.5	NS	**-0.6**	**0.001****	**HS**
Waist circumference (Cm)	**0.3**	**0.01***	**S**	**0.4**	**0.003***	**S**
Waist/Hip ratio	-0.2	>0.5	NS	-0.01	>0.5	NS
Waist/height ratio	0.08	>0.5	NS	0.04	>0.5	NS
Systolic blood pressure	0.3	>0.5	NS	**0.5**	**0.001****	**HS**
Diastolic blood pressure	0.04	>0.5	NS	**0.49**	**0.001****	**HS**
HBA1c	----------			0.1	>0.5	NS
HOMAIR	0.1	>0.5	NS	----------		
Total Cholesterol	0.09	>0.5	NS	**0.5**	**0.001****	**HS**
Triglycerides	0.2	>0.5	NS	0.1	>0.5	NS
HDL	-0.2	>0.5	NS	**-0.7**	**0.001****	**HS**
LDL	0.1	>0.5	NS	**0.4**	**0.001****	**HS**

*Statistically significant difference ($P \leq 0.05$) **statistically highly significant difference ($P \leq 0.001$)
S=Significant, HS=highly significant.

DISCUSSION

The current study aimed to assess the correlation between the Non-synonymous A803G acetyltransferase 2 gene and impaired glucose homeostasis in Egyptian obese children and adolescents at Zagazig University Children's Hospital.

Genetic susceptibility to T2D can be mediated by genetic variability at sites related to several processes, including β-cell function, proinsulin production, obesity, IR, and lipodystrophy/ peripheral adipose storage capacity [(9)]. Recognition of these "clusters" driving T2D risk not only provides biological insight into genetic susceptibility but also has clinical implications as certain clusters, especially the IR and lipodystrophy clusters, are more strongly associated with adverse cardiovascular outcomes [(10)].

Other studies have implicated a connection between IR and reduced mitochondrial function. Among these is GWAS (genom wide association studies), which analysed data from non-diabetic participants of 5000 Europeans who provided DNA for genome wide genotyping and underwent direct measurement of insulin sensitivity. The study identified that human N-acetyltransferase 2 (NAT2) is a novel insulin sensitivity gene [(11)].

The present study was planned and performed to assess Non-synonymous (A803G) N- acetyltransferase 2 gene polymorphism in Egyptian obese children and adolescents and to detect the relation between this gene mutation and impaired glucose homeostasis in them .

Our results revealed no statistically significant difference in age, sex, weight, height, BMI and BMI-SDS among group 1 and group 2 with different N-acetyltransferase 2 (A803G) genotypes. There were higher systolic and diastolic blood pressures, HbA1c and HOMA-IR among group 1 than group 2. A higher systolic blood pressure and HOMA-IR 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 agree with **Cho and Kim** [(11)] who showed that children and adolescents with obesity had a higher prevalence of elevated blood pressure and hypertension. Also, **Leggio *et al.*** [(12)]**, Mameli *et al.*** [(13)] reported that with obesity, there is excess of adipose tissue, which is dysfunctional, leading to a cascade of events resulting in elevation of blood pressure (BP). Insulin resistance inhibits adipose tissue lipolysis, which accelerates the release of free fatty acids (FFA) into circulation. Elevated levels of FFA increase alpha-adrenergic vascular effects leading to increased arterial tone.

Our results revealed that HbA1c was statistically significantly positively correlated with weight and waist circumference in group 1 and group 2. This is in agreement with **Seo *et al.*** [(14)] who reported that children and adolescents with obesity had elevated levels of glycated hemoglobin. Our results revealed that HbA1c was statistically positively correlated with systolic and diastolic blood pressures, HOMA-IR, total cholesterol and LDL and statistically significantly negatively correlated with HDL and BMI-SDS in group 1. But in group 2, HbA1c was not statistically significantly correlated with any variables. **The American Diabetes Association** [(7)] recommends screening of children and adolescents after the onset of puberty or after 10 years of age, whichever occurs earlier, in overweight (body mass index [BMI] ≥ 85th percentile) or obese (BMI ≥ 95th percentile) children and youth who have one or more risk factors for diabetes, every 3 years using HbA1c and recommends using HbA1c as a screening measure in children for the diagnosis of type 2 diabetes except in cases of rare disorders such as cystic fibrosis, haemoglobinopathies, etc. The move toward use of HbA1c in recent years, has gained traction due to several advantages as a diagnostic test. It also provides a better measure of overall levels of glycaemia by reflecting longer-term exposure intervals (~3 months, the average half-life of a red blood cell), has the practical advantage of not requiring fasting at collection, higher repeatability and serves as a better guide to clinical management [(15)].

Our results revealed that HOMA-IR in both groups was statistically significantly positively correlated with weight, waist circumference, systolic and diastolic blood pressures, total cholesterol and LDL and statistically significantly negatively correlated with BMI-SDS, with no statistically significant correlation with other variables. Insulin sensitivity index cannot be used for routine clinical practice, because it requires an OGTT, and therefore cannot be proposed on a large-scale basis. Thus, insulin resistance can be more easily accessed by calculating HOMA-IR , which in a recent study, was negatively associated with Metabolic Healthy Obese (MHO) children and adolescents regardless of the criteria used [(16)].

Our results revealed that HOMA-IR was statistically significantly higher among Heterozygous A803 allele of group 1 (pre-diabetic) than other genotypes. This disagrees with **Marzuillo *et al.*** [(5)] who found no association between the NAT2 A803 allele and HOMA-IR. They reported that obese children and adolescents who are carrying NAT2 A803 allele presented with higher risk to show impaired glucose tolerance not by increasing insulin resistance but by its effect on both the early pancreatic β-cellular response to glucose load and the capacity of pancreatic β-cells to neutralize the insulin resistance. Supporting our results, the study done by **Knowels *et al.*** [(4)] who observed the strongest association for a nonsynonymous SNP in NAT2 [rs1208 (803A > G, K268R)], with the A803 allele (frequency 0.57) associated with a greater degree of IR in analyses adjusted for age, gender, and BMI ($P = 2.8 \times 10–6$). Also, it was associated with various cardiometabolic traits, including higher fasting glucose,

HbA1c, TG levels, total and LDL cholesterol, and coronary artery disease in the expected direction.

CONCLUSION

Our study concluded that Egyptian obese children and adolescents who are 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.

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REFERENCES

1. **Achari A, Jain S (2018):** Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int J Mol Sci., 18 (6): 1321-23.
2. **Güngör N (2014):** Overweight and obesity in children and adolescents. J Clin Res Pediatr Endocrinol., 6 (3): 129-43.
3. **Melvin A, O'Rahilly S, Savage D (2018):** Genetic syndromes of severe insulin resistance. Curr Opin Genet Dev., 50: 60–67.
4. **Knowles J, Xie W, Zhang Z *et al.* (2015)**: Identification and validation of N-acetyltransferase 2 as an insulin sensitivity gene. J Clin Invest., 125 (4): 1739–1751.
5. **Marzuillo, P, Di Sessa A, Umano G *et al.* (2017)**: Novel association between the nonsynonymous A803G polymorphism of the N-acetyltransferase 2 gene and impaired glucose homeostasis in obese children and adolescents. Pediatr Diabetes, 18: 478–484.
6. **WHO and ARE-Ministry of Health & Population (2018)**: Egypt National stepwise Survey of Non Communicable Diseases Risk Factors 2017 Fact Sheet. https://www.who.int/ncds/surveillance/steps/Egypt_STEPS_Survey_2017_Fact_Sheet.pdf
7. **American Diabetic Association (2018**): Diagnosing diabetes and learning about prediabetes. Available at http://www. diabetes. org/ diabetes-basics/diagnosis/?loc=db slabnav.
8. **Oliveira C, Cotrim H, Stefano J *et al.* (2010):** Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arq Gastroenterol., 47 (2): 165-9.
9. **Udler M, Kim J, von Grotthuss M *et al.* (2018):** Christopher D. Anderson on behalf of METASTROKE and the ISGC. Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: A soft clustering analysis. Plos Med., 15 (9): 654-59.
10. **Sangwung P, Petersen K, Shulman G *et al.* (2020)**: Mitochondrial Dysfunction, Insulin Resistance, and Potential Genetic Implications: Potential Role of Alterations in Mitochondrial Function in the Pathogenesis of Insulin Resistance and Type 2 Diabetes. Endocrinology, 161: 17-23.
11. **Cho H, Kim J (2020)**: Secular trends in hypertension and elevated blood pressure among Korean children and adolescents in the Korea National Health and Nutrition Examination Survey 2007-2015. J Clin Hypertens., 22: 590-597.
12. **Leggio M, Lombardi M, Caldarone E *et al.* (2017)**: The relationship between obesity and hypertension: an updated comprehensive overview on vicious twins. Hypertens Res., 40: 947–63.
13. **Mameli C, Zuccotti G, Carnovale C *et al.* (2017):** An update on the assessment and management of metabolic syndrome, a growing medical emergency in paediatric populations. Pharmacol Res., 119: 99–117.
14. **Seo J, Hwang S, Kim J *et al.* (2018)**: Distribution of glycated haemoglobin and its determinants in Korean youth and young adults: a nationwide population-based study. Sci Rep., 8: 1962-68.
15. **Centers for Disease Control and Prevention (2017):** National Diabetes Statistics Report, 2017. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Department of Health and Human Services. https://dev.diabetes.org/sites/default/files/2019-06/cdc-statistics-report-2017.pdf
16. **Vinciguerra F, Tumminia A, Baratta R *et al.* (2020)**: Prevalence and Clinical Characteristics of Children and Adolescents with Metabolically Healthy Obesity: Role of Insulin Sensitivity. Life, 10 (8): 127-133.