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Detection of Single Nucleotide Polymorphism (SNP) (rs34819629) and its Association with Pediatric Type 1 Diabetes Mellitus

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Abstract:

Background: Diabetes mellitus (DM) is a chronic metabolic disorder caused by an absolute or relative deficiency of insulin, an anabolic hormone. Insulin is produced by the beta cells of the islets of Langerhans located in the pancreas, and the absence, destruction, or other loss of these cells results in type 1 diabetes, so we aim to investigate the presence of a genetic association between single nucleotide polymorphism (SNP) and pediatric T1DM in a group of pediatric Egyptian patients. **Patient and method:** The study was a case control study conducted on 80 diabetic subjects aged 5-15 years recruited from the endocrine clinic Pediatrics, Beni-Suef University, and 76 apparently healthy controls with matched age and sex.

All subjects were subjected to history taking, full clinical examination, laboratory tests (hemoglobin A1C, TSH, free T4, serum high density lipoprotein, serum low density lipoprotein, serum triglycerides) and SNP (rs34819629) was done by allelic discrimination technique using real time PCR. **Results:** In our study, the diabetic patients were 28 males (35.0%) and 52 females (65.0%), with a mean age of 10.0 ± 3.2 SD.AS for the control group they were 52 females (68.4%) and 24 males (31.6%) with a mean age 8.8 ± 2.8 SD and we found that Female cases affected more than males and No association was found in SNP (rs34819629) with Type 1 diabetes mellitus.**Conclusion*:*** Microalbuminuria was the most complication in our results and no association was found between SNP (rs34819629) and Type 1diabetes mellitus.

Keywords : Diabetes mellitus - genetic polymorphisms –pediatric.

1. Introduction:

The increased prevalence of diabetes mellitus is considered one of the greatest public health challenges nowadays. Type 1 diabetes mellitus (T1DM), a polygenic autoimmune disease, might be incited by both genetic and environmental factors. Although T1DM has a lower prevalence compared to type 2 diabetes mellitus (T2DM), it is the most common form of diabetes in childhood caused by T cell-mediated autoimmune damage to pancreatic B-cell evidenced by raised titers of GAD [glutamate decarboxylase] antibodies and has a greater impact on the quality of life (1).There are strong genetic associations between T1DM and MHC class II genes: DQA, DQB and DRB. T1DM presents in genetically susceptible individuals with a more rapid onset which is more evident in pre-school children than adolescents. Symptoms appear when approximately 90% of B-cells have been destroyed. Individuals with T1D are at increased risk of other autoimmune-mediated diseases such as coeliac and thyroid disease (2). Most pediatric patients with diabetes have type 1 diabetes mellitus (T1DM) and a lifetime dependence on exogenous insulin. Diabetes mellitus (DM) is a chronic metabolic disorder caused by an absolute or relative deficiency of insulin, an anabolic hormone. Insulin is produced by the beta cells of the islets of Langerhans located in the pancreas, and the absence, destruction, or other loss of these cells results in type 1 diabetes (insulin-dependent diabetes mellitus [IDDM]). A possible mechanism for the development of type 1 diabetes is shown in the. (3). Type 2 diabetes mellitus: non–insulin-dependent diabetes mellitus [NIDDM]) is a heterogeneous disorder. Most patients with type 2 diabetes mellitus have insulin resistance, and their beta cells lack the ability to overcome this resistance. Although this form of diabetes was previously uncommon in children, in some countries, 20% or more of new patients with diabetes in childhood and adolescence have type 2 diabetes mellitus, a change associated with increased rates of obesity. Other patients may have inherited disorders of insulin release, leading to maturity onset diabetes of the young (MODY) or congenital diabetes. This topic addresses only type 1 diabetes mellitus (4).Clear evidence suggests a genetic component in type 1 diabetes mellitus. Monozygotic twins have a 60% lifetime concordance for developing type 1 diabetes mellitus, although only 30% do so within 10 years after the first twin is diagnosed. In contrast, dizygotic twins have only an 8% risk of concordance, which is similar to the risk among other siblings (5).The frequency of diabetes development in children with a mother who has diabetes is 2-3%; this figure increases to 5-6% for children with a father who has type 1 diabetes mellitus. The risk to children rises to almost 30% if both

parents are diabetic (6).Human leukocyte antigen (HLA) class II molecules DR3 and DR4 are associated strongly with type 1 diabetes mellitus. More than 90% of whites with type 1 diabetes mellitus express 1 or both of these molecules, compared with 50-60% of the general population. (7)

2. Patients and Methods:

The current case control study included eighty type I diabetic pediatric patients, enrolled from the pediatric Outpatient clinic of Beni-Suef Faculty of Medicine University Hospital. Seventy-six age and sex matched healthy control subjects were included. The patients and the healthy controls were informed about the study and their consent was taken. The study was conducted from December 2019 till May 2020.

The diagnosis of type 1 diabetes was done based on the plasma glucose criteria, either the fasting plasma glucose (no caloric intake for at least 8 h) (FPG) ≥ 126mg/dL (0.7mmol/L) or the 2-h plasma glucose (2-h PG) ≥ 200mg/dL (11.1mmol/L) after 1.75g/kg up to maximum of 75-g oral glucose tolerance test (OGTT) or the hemoglobin A1C ≥ 6.5%(48 mmol/mol). (8).

2.1 Inclusion criteria:

1. Age: 5-15 years old children
2. Patients with type 1 diabetes mellitus.

2.2 Exclusion criteria:

- Patients with diabetes associated with syndromes (e.g. Down syndrome).
- Patient with diabetes associated with genetic disorders.

2.3 All patients were subjected to:

1. History taking with: special emphasis on onset and duration of diabetes, family history of diabetes and hypertension or coronary heart disease.

2. Complete clinical examination: General examination including cardiac, chest, abdominal examination and arterial blood pressure measurement

3. Anthropometric measurements:

A- Weight & weight standard deviation (SD),

B- Height and height SD,

C- Body mass index (BMI) and BMI SD

D- Pubertal assessment.

A- Weight:

- From 1 – 2years, patients were weighed wearing light clothing on a digital scale.
- Older patients were weighed while standing on scale with weight equally placed on both feet.
- Weight was measured and recorded to the nearest 0.1 kg and then plotted on the curves and standard deviation scores.

B-Height

Standing height: From 2-3 years onwards, a wall mounted stadiometer was used for height measurement. Patients were standing as erect as possible with his/her head, thorax, spine, buttocks and heels, all placed together touching the vertical plane of the stadiometer. Head is centered and positioned with **the Frankfurt plane** parallel to the floor. Length/Height is recorded in centimeters, to the nearest 0.1 cm and then plotted on the curves and standard deviation scores.

C- Body mass index (BMI):

BMI was calculated by applying the following formula:

$$\text{BMI} = \frac{\text{weight in kg}}{(\text{length or height in m})2}$$

Then by using Egyptian Growth Percentile, Charts patients whose BMI equal or above the 95th percentile were considered obese. Patients with BMI equal or above the 85th percentile but less than the 95th percentile are considered overweight. But patients whose BMI are below the 3rd percentile, are considered significantly underweight.

D- Pubertal assessment:

Puberty was assessed by Tanner staging, the onset was defined by the beginning of breast development (B_2) in girls and the enlargement of testicular volume to 4 ml (G_2) in boys.

4- Investigations

- New HbA1c at the time of study (when needed).
- Lipid profile (triglycerides, LDL &HDL).
- Microalbuminuria in urine.

- TSH &free T4.

. Special Laboratory investigations:

Detection of SNP (rs34819629) By Allelic discrimination technique using RT- PCR.

Statistical methodology:

Statistical analysis was done using IBM SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Pearson's Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. Quantitative data were tested for normality using Kolmogorov-Smirnov test and Shapiro-Wilk test. For normally distributed data, comparison of quantitative data between two groups was done using Student t-test. Allele frequencies were determined by allele counting and departure from hardy-Weinberg equilibrium were checked using Chi square tests. Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation; using logistic regression and adjusted for age, gender and BMI. All tests

were two-tailed. A p-value < 0.05 was considered significant.

3- Results

The present work is a case control study that included 80 type1diabetic children and adolescents and 76 unrelated healthy controls.

The patients were recruited from pediatric Endocrinology Clinic, Beni-Suef University.

The diabetic patients were 28 males (35.0%) and 52 females (65.0%), with a mean age of 10.0 ± 3.2 SD.

AS for the control group they were 52 females (68.4%) and 24 males (31.6%) with a mean age 8.8 ± 2.8 SD.

Table (1) : Demographic data of T1DM cases and controls:

variables	Cases N=80	Controls N=76
Age (mean± SD)/year	10.0 ± 3.2	8.8 ± 2.8
Gender :		
Female	52 65.0%	52 68.4 %
Male	28 35.0%	24 31.6 %
Weight (mean ± SD)/Kg	33.8 ± 12.4	30.3 ± 9.8
Height (mean± SD)/cm	132.4 ± 19.3	131.1 ± 15.4
BMI (mean± SD)	18.9 ± 5.0	17.1 ± 1.9

BMI: Body mass index, SD: standard deviation

Table (1) shows demographic and comparative Data of T1DM cases and controls as following :

- **As regards age**, T1DM patients' ages ranged between 5 and 16 years with a mean value of 10.0 ± 3.2 SD, while controls' ages ranged between 5 and 15 years with a mean value of 8.8 ± 2.8 SD.
- **As regards sex distribution**, 52 patients were females (65.0%) and 28 were males (35.0%), while in controls 52 were females (68.4%) and 24 were males (31. 6%).
- **As regards weight,** T1DM patients' weights ranged from 17 to 60 kg with a mean value of 33.8 ± 12.4 SD, while controls' weights ranged from 17 to 50 kg with a mean value of 30.3 ± 9.8 SD.
- **As regards height**, T1DM patients' heights ranged from 100 to 186 cm with a mean value of 132.4 ± 19.3 SD, while controls 'heights ranged from 100 to 160 cm with a mean value of 131.1

± 15.4 SD.

- **As regards BMI**, T1DM patients' BMI ranged from 14.5 to 44.6 with a mean value of 18.9 ± 5.0 SD, while controls' BMI ranged from 13.6 to 20.5 with a mean value of 17.1 ± 1.9 SD.

Table (2): Descriptive data of the T1DM cases as regards the presence of micro vascular complications:

Variables	No (%)
Microalbuminuria	16 20.0%
Diabetic neuropathy	0 %
Diabetic retinopathy	0 %

Table (2): shows that 16 (20.0%) of diabetic cases had microalbuminuria while 64 (80.0%) of diabetic cases did not have.

Table (3): Lab and clinical data of type 1 DM cases at the onset of diagnosis:

	N	Mean	Standard deviation	Median	Minimum	Maximum
FBG	80	189.1	38.6	180.0	135.0	290.0
2.HPPBG	80	296.8	47.5	300.0	210.0	390.0
HA1C	80	9.7	2.0	9.9	5.8	14.3
Systolic BP	80	98.2	12.5	100.0	50.0	120.0
Diastolic BP	80	68,1	9.0	70.0	50/0	80.0

FBG: fasting blood glucose, **2HPPBG:** 2 Hours post prandial blood glucose, **BP**: Blood pressure

Table (3) shows Lab and demographic data of type 1 DM cases as following:

- As regards Fasting blood glucose level, it ranged from 135 to 290 with a mean value of 189.1 ± 38.6 SD**.**

- As regards 2 hour post prandial, it ranged from 210 to 390 with a mean value of 296.8 ± 47.5 SD.
- As regards HbA1c, it ranged from 5.8 % to 14.3 % with a mean value of 9.7 ± 2 SD.
- As regards systolic BP, it ranged from 50 to 120 with a mean value of 98.2 ± 12.5 SD.
- As regards diastolic BP, it ranged from 50 to 80 with a mean value of 68.1 ± 9.0 SD.

Table (4) Frequency distribution of rs34819629 (C\T) gene polymorphism in T1DM Cases and controls

		Group		Total
		DM Type I	Control	
rs34819629 C/C	Count	80	76	156
	% within Group	100.0%	100.0%	100.0%
Total	Count	80	76	156
	% within Group	100.0%	100.0%	100.0%

Table (4) shows that rs34819629 (C\T) gene polymorphism wasn't associated with type 1 diabetes mellitus.

4- Discussion:

The increased prevalence of diabetes mellitus is considered one of the greatest public health challenges nowadays. Type 1 diabetes mellitus (T1DM), a polygenic autoimmune disease, is resulted from both genetic and environmental factors. Although T1DM has a lower prevalence compared with type 2 diabetes mellitus (T2DM), it is the most common form of diabetes in childhood and has a greater impact on the quality of life**.** (9). T1DM is an autoimmune disease whereby antigen-specific T cells selectively destroy insulin-producing pancreatic β cells. The activated T cells first invade the islets, leading to "insulitis" phenomena. This is followed by destruction of the islets, mediated by a complex interaction between the activated lymphocytes, cytokines and macrophages (10). The affected individuals will develop the disease, in accordance with genetic susceptibility mainly influenced by certain haplotypes linked to the major histocompatibility complex (HLA) Class II molecules), plus another group of genes classified as non-HLA. Its etiology is extremely complex, since environmental factors interact with genetic predisposition, leading to an irreversible autoimmune attack against insulin-producing cells located in the

pancreas. Environmental factors include viral infections, toxins and diet. Furthermore, due to this heterogeneity, some loci might present stronger effects on particular populations or a subset of families (11) The aim of this work was to detect the presence of Genetic association between single nucleotide polymorphism (rs34819629) and Pediatrics T1DM in a group of pediatric Egyptian patients. Our study was a case control study that included 80 T1DM cases and 76 unrelated healthy controls The cases were recruited from Pediatric Endocrinology Clinic, Beni-Suef University. They were 52 females (65.0%) and 28 males (35.0%) diabetic patients, and 52 females (68.4%) and 24 males (31.6%) control group, with an average age of 10.0 ± 3.2 SD for diabetic group and 8.8± 2.8 SD for control group. Our study showed that the female patients affected more than the males.

Our result was on accordance with **El-Ziny et al** (12) study that showed a significant female predominance among the total patient population in both rural and urban areas. Also this was to be in line with literature data where female predominance was significant among Libyan **(Kadiki and Roaeid) (13)** Saudi (**Abduljabbar et al**) **(14)** and Turkish **(Demirbilek, et al) (15)** T1DM patients. Only a slight and not significant higher incidence of T1DM in Sudanese females **(Elamin, et al) (16)** and Kuwaiti females **(Shaltout, et al) (17)** was reported, while no gender difference was observed among Tunisians **(Ben Khalifa, et al) (18)**.

Our results showed that 16 (20.0%) of diabetic cases had microalbuminuria while 64 (80.0%) of diabetic cases did not have.

In the Oxford Regional Prospective Study of young people with Type 1 DM by **Schultz et al (19)** found that the prevalence of elevated microalbuminuria was 13–26% and the prevalence of persistent microalbuminuria was 5%.

Gallego et al (20) found that the prevalence rates of 6–18% for elevated microalbuminuria in cases with Type 1 DM.

In a Swedish cohort of 426 young patients with Type 1 DM, **Svensson et al (21)** found a prevalence of microalbuminuria of 5.6%.

In this thesis, we showed that no association was found between SNP (rs34819629) and T1DM in Egyptian populations. Our results are in accordance with study conducted by **Qian et al (22) and Hameed et al (23)** who showed that no significant association between SNP (rs34819629) and T1DM In the contrary to our results **Ni et al (24)** detected that there was significant association between SNP (rs34819629) and T1DM in Japanese children (OR: 3.16. CI: 1.09-9.15. **P value = 0.0290*).**

5. Conclusion and Recommendations:

Our results indicated no association was found between SNP (rs34819629) and T1DM

This results recommend that more researches are needed to demonstrate the gene map of T1DM cases and participate to benefit from the novel strategies that use these gen maps in prevention and treatment of T1DM.and more studies are needed using larger samples to detect the association of other single nucleotide polymorphisms with type 1 diabetes mellitus.

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