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Evaluation the Role of Cytotoxic T-lymphocyte Antigen-4 (rs231775) Gene Polymorphism in Patients with Type 1 Diabetes Mellitus

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ABSTRACT

Background: Type I Diabetes Mellitus (T1DM) is categorized by autoimmune destruction of the pancreatic islets, largely driven by T-cell activity. Numerous genetic factors contribute to this autoimmune process. The CTLA-4 gene acts as a key suppressor of T-cell proliferation, thereby influencing the body's propensity for autoimmune conditions.

Objectives: This research aimed to estimate the role of cytotoxic t-lymphocyte antigen-4 CTLA-4 (rs231775) gene polymorphism in cases having type I diabetes mellitus (T1DM).

Subjects and Methods: A total of 112 children participated, split into two equally matched groups: 56 with T1DM and 56 healthy controls matched for age and gender. Genotyping for CTLA-4 rs231775 was performed using allelic discrimination PCR with TaqMan probes.

Results: The incidence of both the GG genotype and the G allele at the rs231775 locus was notably greater in kids with T1DM than in the control group. Statistical analysis revealed strong associations between this CTLA-4 polymorphism and several variables, including patients' age, duration of illness, body mass index, as well as a range of laboratory measures such as fasting glucose, random glucose, two-hour postprandial glucose, glycated hemoglobin (HbA1c), blood urea, creatinine, lipid profile and the albumin-to-creatinine ratio.

Conclusion: Carrying the G allele or being homozygous (GG) for rs231775 in the CTLA-4 gene is related with higher odds for developing T1DM.

Keywords: CTLA-4 gene, Gene polymorphism, rs231775, Type 1 diabetes mellitus.

INTRODUCTION

T1DM is a chronic autoimmune disorder in which the body's immune defenses mistakenly target and eliminate the pancreatic beta cells responsible for producing insulin. This destruction results in a marked lack of insulin and necessitates that affected individuals, particularly children, rely on insulin therapy for glucose regulation throughout their lives [(1)].

Clinical features of T1DM in pediatric patients often arise abruptly and include symptoms such as polyphagia, unintentional weight loss, polydipsia, polyuria, nocturnal enuresis, fatigue, and overall weakness. If timely diagnosis and management are not achieved, affected individuals can develop diabetic ketoacidosis (DKA), a severe and potentially fatal condition. DKA often necessitates emergency care involving fluid resuscitation, administration of insulin, correction of electrolyte imbalances, and continuous medical supervision. It is noteworthy that about one in three children present with DKA at the initial onset of T1DM [(2)].

The onset of T1DM results from a combination of underlying genetic susceptibility and environmental influences. While disease-associated gene polymorphisms are well characterized, the environmental triggers involved are not yet fully clarified despite considerable investigative efforts [(3)].

Several studies highlight the substantial contribution of genetic factors to T1DM risk. The major genetic determinant lies within the HLA region on chromosome 6, notably alleles such as DR3, DR4, DQA1*0501, DQB1*0201, DQA1*0301, and DQB1*0302, which collectively account for approximately 40–50% of the risk of developing T1DM [(2)]. Additionally, around sixty non-HLA genetic loci were implicated in illness susceptibility [(3)].

Cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) functions as a critical regulator in maintaining T-cell mediated immune homeostasis and tolerance [(4)].

Also recognized as CD152, the CTLA-4 protein is a negative regulator of T-cell activation; it delivers inhibitory signals that suppress cytokine production, T-lymphocyte activation, and proliferation. Variants in the CTLA-4 gene can disturb immune equilibrium by permitting abnormal T-cell activity, leading to tissue infiltration and subsequent dysfunction, a mechanism implicated not only in T1DM, but also in the pathogenesis of systemic lupus erythematosus, autoimmune thyroid illness, multiple sclerosis, myasthenia gravis, and rheumatoid arthritis [(5)].

With respect to the Egyptian population, data concerning the epidemiology and genetic contributions to DM remain inadequate. However, the International Diabetes Federation (IDF) indicates that Egypt ranks 9th globally in diabetes occurrence, with about 8.85 million adult cases in early 2020 and an occurrence rate of 15.2%. Egypt is included within the Middle East and North Africa (MENA) region, where diabetes cases are projected to double by 2045, reaching an estimated 108 million individuals [(6)].

There remains a scarcity of research regarding the impact of the CTLA-4 rs231775 gene polymorphism on

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T1DM risk specifically in Egypt. Addressing this gap, the current research aims to investigate the potential influence of the CTLA-4 rs231775 gene variant among Egyptian cases diagnosed with T1DM.

SUBJECTS AND METHODS

Subjects:

The present study took place at the Clinical Pathology and Pediatric Departments of the Faculty of Medicine, Menoufia University, during the period from October 2023 to October 2024.

Sample size estimation:

This is analytical cross-sectional research proposing to estimate the role of CTLA-4 (rs231775) gene polymorphism in cases with T1DM. A previous study revealed that the incidence of the G allele has been significantly raised in cases than in controls (Twenty-eight percent vs. seven percent) (**Kheiralla, 2021**) [(7)]. So, at least 112 participants should be recruited to the study, with at least 56 in each group, which is the size of sample to examine the findings of the current research with a significant P below 0.05.

112 subjects were included in this study; their ages ranged between 8-15 years. The subjects were divided into:

Group I (Case group): this group included 56 subjects selected from out-patient in Pediatric Department of Menoufia University Hospital and diagnosed as T1DM regarding criteria of American Diabetes association (ADA).

Group II (Control group): involved 56 apparently healthy subjects, age and gender matched with cases. Patients with malignancies, with other autoimmune diseases and with liver or heart impairment were excluded from the study.

Every subject underwent a comprehensive interview to collect medical history, in addition to a thorough physical examination and complete laboratory investigations.

Anthropometric measurements included height and weight, which have been applied to compute the body mass index (BMI).

Sample collection: was conducted under strictly aseptic conditions. Each participant provided a total of 3 ml of venous blood. This was split between two main tubes: one plain tube (1 ml) for immediate analysis of random blood sugar (RBS), serum creatinine and urea. A second tube containing EDTA (2 ml), which was further divided for hemoglobin A1C % (HbA1c%) assay and genetic analysis of the rs231775 polymorphism. For fasting blood tests, such as fasting blood sugar (FBS) and lipid profile (High density lipoprotein (HDL-C), low density lipoprotein (LDL-C), triglycerides (TG), total cholesterol (TC)), an additional 2 ml sample was drawn after a minimum 12-hour fast. 2 hour post prandial glucose (2hppG) was measured two hours after a meal. For albumin / creatinine ratio (ACR), urine specimen has been collected to measure albumin and creatinine in urine.

Laboratory assessments, covering FBG, RBS, 2h ppG, serum creatinine, urea, lipid profile and ACR, were performed using the AU680 auto-analyzer from (Beckman Coulter Indianapolis, USA), HbA1c% was run on the Capillaries 3 Octa **(**Sebia, France).

To identify the CTLA-4 rs231775 A/G genotype; analysis was done by allelic discrimination PCR. DNA was isolated from samples in EDTA tubes via a DNA extraction Kit (Thermo Fisher Scientific, United States of America) following manufacturer directions, then maintained at –80°C up to the genotyping step.

The identification relied on a TaqMan allelic discrimination assay from (Applied Biosystems, USA). making use of probe sequences specific to each allele [VIC for A allele/ FAM for G allele] GCACAAGGCTCAGCTGAACCTGGCT[**A/G**]CCA GGACCTGGCCCTGCACTCTCCT. The PCR reaction total volume was twenty microliter including10 µl TaqMan Genotyping Master Mix, one microliters Custom TaqMan assay (primer/probe) 40×, and five microliters genomic DNA, and four microliters nuclease free water. PCR cycling consisted of an initial heat step at ninety-four degrees Celsius for fifteen minutes, followed by 50 cycles of denaturation (94°C, 15 seconds) and annealing (62°C, 30 seconds). Real-time PCR results were analyzed with Quant Studio 5 software. (Applied Biosystems, USA) **(Figure 1).**

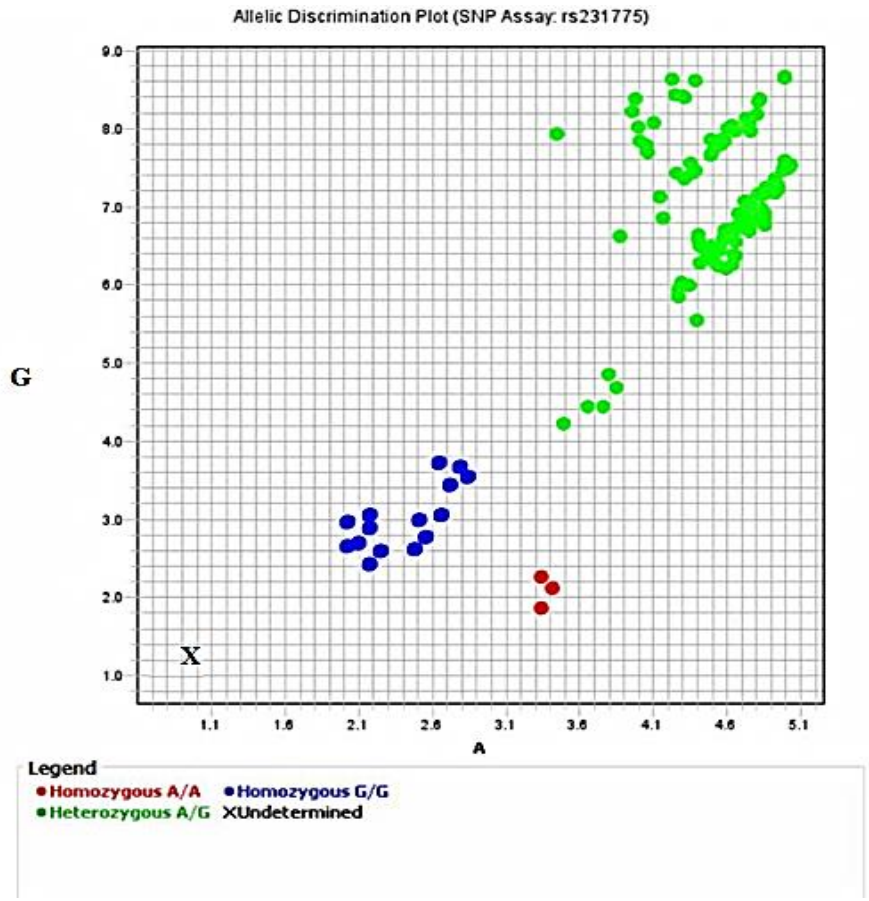

Fig. 1: Allelic discrimination plot for CTLA4 (rs231775).

Ethical Consideration: The research was validated by the Ethics Committee of Clinical Pathology Department, Faculty of Medicine, Menoufia University (IRB: 7/2023 CPATH), which approved the protocol of the research. Before enrollment, written informed consent has been collected from the caregivers of the participants. This investigation aimed to conduct research on humans in line with the Declaration of Helsinki, the code of ethics of the World Medical Association.

Statistical Analysis

All data have been statistically analyzed applying IBM SPSS Statistics Software, Version 20. Qualitative variables have been presented as counts and percentages, and variances between groups have been assessed with the Chi-square test. Quantitative variables have been defined using the range, standard deviation, mean, as well as interquartile range (IQR), and median. For comparisons between two normally distributed groups, independent t-test was used, and between two groups involving abnormally distributed data, the Mann-Whitney U test has been applied. When comparing more than 2 groups, if the information has been normally distributed, one-way analysis of variance (ANOVA; F value) has been utilized, and for abnormally distributed data, the Kruskal-Wallis test (K value) has been used. A Monte Carlo test isA statistical test that uses computer simulation to estimate the p-value of a hypothesis test.

Univariate regression analysis has been performed to detect the majority of independent factors for influencing T1DM. P-values below or equal to 0.05 have been deemed as statistically significant.

- P1: p value between g1 and g2
- P2: p value between g1 and g3
- P3: p value between g2 and g3

RESULTS

A total of 112 participants have been enrolled in this study, involving 61 males and 51 females. Both the case control and T1DM groups were comparable in terms of gender. A statistically significant difference in age was observed between the groups ($p = 0.049$), the mean ages were relatively close.

Significantly higher weight and BMI values were observed among T1DM patients compared to controls, while insignificant variance has been found in height among the groups **(Table 1).**

Table (1): Demographic and anthropometric data of the studied groups

	Parameters	Case (n = 56)		Control (n = 56)		Test of Sig.	p
		No.	%	No.	%		
Gender	Male Female	30 26	53.6 46.4	31 25	55.4 44.6	X^2= 0.036	0.850
Age (years)	Min. – Max. Mean ± SD.	8.0 – 15.0 12.25 ± 2.03		8.0 – 15.0 11.55 ± 1.68		t= 1.977	0.049*
Weight (kg)	Min. – Max. Mean ± SD.	30.0 – 66.0 52.14 ±12.13		25.0 – 54.0 40.36 ±6.56		6.396	* <0.001
Height (cm)	Min. – Max. Mean ± SD.	125.0 – 165.0 147.82 ±10.02		117.0 – 162.0 144.39 ±10.12		1.801	0.074
BMI (kg/m^2)	Min. – Max. Mean ± SD.	17.80 – 27.40 23.50 ±3.34		16.50 – 21.40 19.21 ± 0.84		9.313*	* <0.001

SD: Standard deviation, t: Student t-test, X^2: Chi square test, *: Statistically significant, BMI: body mass index.

Biochemical assessment revealed that FBS, RBS, 2hppG, HbA1c%, serum creatinine, urea and ACR have been all significantly elevated in the T1DM group than controls. Regarding lipid parameters; (TC, TG and LDL-C) were significantly higher among cases, while HDL-C has been found to be higher in healthy controls **(Table 2).**

Table (2): Comparison of laboratory data of the studied groups

Parameters	Case (n = 56)	Control (n = 56)	Test of Sig.	p
FBs (mg/dl) Mean ± SD.	143.5 ±13.84	88.14 ± 5.52	t= 27.827*	<0.001*
RBs (mg/dl) Mean ± SD.	269.8 ± 38.48	119.02 ±7.08	t= 28.841*	<0.001*
2hr pp (mg/dl) Mean ± SD.	288.3 ±39.67	132.1 ± 4.98	t= 29.237*	<0.001*
HbA1c (%) Mean ± SD.	8.65 ±1.30	5.0 ± 0.22	t= 20.690*	<0.001*
Urea (mg/dl) Median (IQR)	78.50 (34.00 – 92.50)	16.0 (13.0 – 17.0)	U= 0.001*	<0.001*
Creatinine (mg/dl) Median (IQR)	1.80 (0.67 – 2.55)	0.54 (0.50 – 0.58)	U= 310.50*	<0.001*
Albumin/creatinine ratio (mg/g) Median (IQR)	340.0(140.0 – 380.0)	10.0 (8.0 – 11.0)	U= 0.001*	<0.001*
TG (mg/dl) Mean ± SD.	100.0 ±20.41	79.23 ± 4.54	7.439*	<0.001*
Cholesterol (mg/dl) Mean ± SD.	174.1 ± 24.76	130.0 ± 6.59	t= 12.864*	<0.001*
HDL-C (mg/dl) Mean ± SD.	42.54 ±5.57	49.68 ±1.76	t= 9.147*	<0.001*
LDL-C (mg/dl) Mean ± SD.	111.52 ±26.43	64.45 ±6.84	t= 12.904*	<0.001*

IQR: Inter quartile range; SD: Standard deviation; t: Student t-test; U: Mann Whitney test; *: Statistically significant; FBs: Fasting blood sugar; RBs: Random blood sugar; 2hr PP: 2 hour post prandial blood glucose; HbA1c: Hemoglobin A1c; TG: Triglyceride; HDL: High density lipoprotein LDL: Low density lipoprotein.

Analysis of CTLA-4 (rs231775) genotypes and allele frequencies **(Table 3)** demonstrated a significant overrepresentation of the A/G and G/G genotypes and the G allele in T1DM patients. Conversely, the A/A genotype and A allele were more represented among the control group.

The G/G and A/G genotypes displayed substantially increased risk for T1DM; G/G: OR (9.75), A/G: OR (3.75). Moreover, the presence of the G allele, dominant model (AG+GG), and recessive model (GG alone) all conferred significantly elevated risk for T1DM; G allele: OR (2.525), (AG+GG) genotypes: OR (4.500), GG genotype: OR (3.930) **(Table 3).**

Table (3): The risk of development of diabetes mellitus type 1 with different genotypes and alleles.

Parameters	Case (n = 56)		Control (n = 56)		p	OR (LL – UL 95% C.I)
	No.	%	No.	%		
Genotype (rs231775)						
AA	8	8.9	24	42.9		Ref
AG	35	67.9	28	50.0	0.006*	3.750(1.462 – 9.618)
GG	13	23.2	4	7.1	0.001*	9.750(2.460 – 38.639)
Allele						
A	51	45.5	76	67.9		Ref
G	61	54.5	36	32.1	0.001*	2.525(1.467 – 4.349)
Dominant						
AA	8	8.9	24	42.9		
AG + GG	48	85.7	32	57.1	0.001*	4.500(1.799 – 11.254)
Recessive						
AA + AG	43	86.8	52	92.9		
GG	13	23.2	4	7.1	0.024*	3.930(1.194 – 12.935)
Over dominant						
AA + GG	21	37.5	28	50.0		
AG	35	67.9	28	50.0	0.184	1.667(0.785 – 3.539)

OR: Odds ratio; CI: Confidence interval; LL: Lower limit; UL: Upper limit; *: Statistically significant at $p \leq 0.05$

The study also found a significant relation between the CTLA-4 rs231775 genotype and several clinical and laboratory variables, including age, illness duration, weight, BMI, FBS, RBS, 2hppG, HbA1c, serum creatinine, urea, creatinine, triglycerides, cholesterol HDL, and LDL **(Table 4).**

Table (4): Relation between Genotype (rs231775) with different parameters in case group (n= 56)

Parameters	Genotype (rs231775)						Test of sig.	p
	AA (n = 8)		AG (n = 35)		GG (n = 13)			
	No.	%	No.	%	No.	%		
Gender								
Male	3	37.5	21	60.0	6	46.2	X^2=1.70	MCp= 0.474
Female	5	62.5	14	40.0	7	53.8		
Age (years) Mean ± SD.	10.38 ± 1.92		12.54 ± 1.88		12.62 ± 1.98		F=4.497	0.016*
Consanguinity								
No	4	50.0	26	74.3	12	92.3	X^2=4.753	MCp= 0.072
Yes	4	50.0	9	25.7	1	7.7		
Family history								
No	5	62.5	14	40.0	6	46.2	X^2=1.350	MCp= 0.546
Yes	3	37.5	21	60.0	7	53.8		
Age of onset (years) Median (Min. – Max.)	10.0 (8.0 – 14.0)		9.0 (1.0 – 13.0)		8.50 (2.0 – 15.0)		H=3.007	0.222
Duration of illness (years) Median (Min. – Max.)	0.10 (0.02 – 1.0)		4.0 (0.04 – 13.0)		5.0 (0.05 – 12.0)		H=11.232	0.004* P1=0.004 P2=0.009 P3=1
Weight (kg) Mean ± SD.	38.38 ± 7.13		54.60 ± 10.62		54.0 ± 13.31		F=7.435	0.001* P1=0.0011 P2=0.006 P3=0.984
Height (cm) Mean ± SD.	141.4 ± 11.33		148.5 ± 8.87		150.1 ± 11.31		F=2.141	0.128
BMI (kg/m^2) Mean ± SD.	19.06 ± 1.18		24.50 ± 2.87		23.54 ± 3.31		F=12.091	<0.001* P1=0.000 P2=0.002

Parameters	Genotype (rs231775)						Test of sig.	p
	AA (n = 8)		AG (n = 35)		GG (n = 13)			
	No.	%	No.	%	No.	%		
								P3=0.550
FBs (mg/dl) Mean ± SD.	129.1 ± 7.77		146.3 ± 13.29		145.0 ± 13.39		F=6.036	0.004* P1=0.003 P2=0.0201 P3=0.947
RBs (mg/dl) Mean ± SD.	224.4 ± 15.91		279.1 ± 34.84		272.7 ± 39.51		F=8.438	0.001* P1=0.0004 P2=0.0075 P3=0.832
2hr pp (mg/dl) Mean ± SD.	242.5 ± 16.69		297.6 ± 36.33		291.5 ± 40.64		F=7.924	0.001* P1=0.0006 P2=0.0092 P3=0.857
HbA1c (%) Mean ± SD.	7.01 ± 0.29		9.0 ± 1.14		8.72 ± 1.39		F=10.153	<0.001* P1=0.0001 P2=0.0041 P3=0.7282
Urea (mg/dl) Median (Min. – Max.)	34.0 (30.0 – 35.0)		82.0 (25.0 – 130.0)		76.0 (30.0 – 128.0)		H=8.554	0.014* P1=0.06 P2=0.01 P3=1
Creatinine (mg/dl) Median (Min. – Max.)	0.64 (0.56 – 0.85)		2.0 (0.44 – 3.50)		1.60 (0.51 – 3.50)		H=8.518	0.014* P1=0.06 P2=0.01 P3=1
Albumin/creatinine ratio (mg/g) Median (Min. – Max.)	122.5 (95.0 – 150.0)		350.0 (90.0 – 400.0)		350.0 (120.0 – 400.0)		*H* = 12.445	<0.001* P1=0.01 P2=0.002 P3=1
TG (mg/dl) Mean ± SD.	80.0 ± 3.78		104.3 ± 19.39		100.8 ± 22.68		F=5.367	0.008* P1=0.0053 P2=0.0466 P3=0.837
Cholesterol (mg/dl) Mean ± SD.	148.3 ± 8.81		177.4 ± 22.94		181.0 ± 27.29		F=6.138	0.004* P1=0.0053 P2=0.0064 P3=0.877
HDL-C (mg/dl) Mean ± SD.	50.25 ± 1.91		40.97 ± 4.62		42.0 ± 5.72		F=13.116	<0.001* P1=0.000 P2=0.0007 P3=0.774
LDL-C (mg/dl) Mean ± SD.	82.0 ± 7.78		115.5 ± 24.13		118.8 ± 28.79		F=7.232	0.002* P1=0.0021 P2=0.0033 P3=0.905

X^2: Chi square test; MC: Monte Carlo test; H: H for Kruskal Wallis test; F: F for One way ANOVA test; *: Statistically significant at $p \leq 0.05$; FBs: Fasting blood sugar; RBs: Random blood sugar; 2hr PP: 2 hour post prandial blood glucose; HbA1c: Hemoglobin A1C; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein.

Univariate logistic regression analysis demonstrated that the weight, BMI, serum creatinine, lipid profile and CTLA-4 (rs231775) AG+GG VS AA were considered dependent factors affecting T1DM.

DISCUSSION

T1DM manifests as an autoimmune condition uniquely affecting the pancreas, leading to the infiltration of lymphocytes into pancreatic islets and the existence of pancreas-specific autoantibodies in the blood. Various genetic susceptibility factors are acknowledged as contributors to the autoimmune mechanisms underlying T1DM [8].

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) acts as a vital modulator of T cell activity, playing a fundamental role in maintaining immune homeostasis and promoting tolerance. Polymorphisms in the CTLA-4 gene, particularly the rs231775 variant, have been found to impact T cell regulation by altering gene function. Specifically, the A-to-G substitution at position 49 of the CTLA-4 gene (known as A49G) findings in an amino a` substitution from threonine to alanine at position 17, consequently modifying protein function and potentially heightening the risk of T1DM development [9].

In this research, patients with T1DM exhibited higher body weight and BMI than the healthy control group, a finding consistent with researches by **De Keukelaere *et al.*** [10] and **Fellinger *et al.*** [11] who also reported increased weight and BMI among children with T1DM.

These studies point to significant metabolic changes in pediatric diabetic populations. The underlying reasons for excessive weight gain in these patients may include elevated insulin concentrations—due to both exogenously administered and residual endogenous insulin during partial remission—excess caloric consumption prompted by a fear of hypoglycemia, increased appetite, and inaccuracies in assessing food intake, as suggested by **Kaminsky *et al.*** [12].

However, **Çam *et al.*** [13] found no statistically significant variance among children with T1DM and their healthy peers in terms of weight and BMI, indicating variability across populations.

This study also revealed that FBS and HbA1c% concentrations were significantly greater in the diabetic group, supporting observations made by **Çam *et al.*** [13] and **Tihić-Kapidžić *et al.*** [14].

Further, lipid profile assessments showed that children with T1DM had elevated levels of TG, TC, and LDL-C, whereas HDL-C levels were greater in controls; these results align with prior reports by **Homma *et al.*** [15], **Mostofizadeh *et al.*** [16], and **Melo *et al.*** [17].

The underlying reasons for these dyslipidemic shifts are still being explored. Some papers suggest that a mix of chronic inflammation, lipodystrophies, increased atherogenesis, insulin resistance, mitochondrial disorders that influence carbohydrate and lipid metabolism There are also reports tying these changes to particular issues with how HDL cholesterol is manufactured and managed, and with defects in the 'reverse cholesterol transport' system [18].

In this research, there was statistically significant variance among the cases and the controls regarding ACR, which was higher in the patient group, consistent with **Metwalley *et al.*** [19] findings among Egyptian patients.

The pathophysiology here appears to be multifactorial. Persistently high blood sugar in T1DM affects how the kidneys function, causing both metabolic and hemodynamic shifts, which can injure the fragile glomerular filtration barrier. This can be further exacerbated by hypertension, another common issue in people with diabetes, leading to increased pressure within the glomeruli and more damage to the filtering surfaces. Over time, these effects combine to make the kidneys leak more albumin and, eventually, impair glomerular filtration rate (GFR) [18]. Ongoing monitoring of estimated GFR as well as the urinary albumin-to-creatinine ratio is widely recommended in diabetes management guidelines, as these are important markers for diagnosing and following diabetic nephropathy [20].

Genotypic analysis in this study showed that the A/G and G/G genotypes of CTLA-4 rs231775 were substantially more common in the T1DM group, while the A/A genotype was predominant among healthy controls. The G allele was also more commonly detected in diabetic cases, contrasting with the A allele's higher frequency in control subjects. These findings are supported by **Kheiralla *et al.*** [7], whose research demonstrated both the GG and AG genotypes, as well as the G allele of CTLA-4 Thr16, to be more prevalent in patients, highlighting the correlation of CTLA-4 +49 A/G polymorphism with greater T1DM possibility in Sudanese children. **Gunavathy *et al.*** [21] further corroborated this, indicating that T1DM patients were more likely to carry G allele-containing genotypes (GG+AG), which convey heightened disease susceptibility, whereas the AA genotype appeared to confer some protection and was associated with later disease onset.

Evidence from two meta-analyses by **Si *et al.*** [22] and **Chen *et al.*** [23] further reinforced the significant link among the CTLA-4 +49A/G polymorphism and T1DM risk among Caucasian and Asian populations, implicating the G allele as a risk factor. Contrarily, **Rodríguez *et al.*** [24] didn't find a significant association among CTLA4 polymorphisms and T1DM in the Colombian population, and **Sousa *et al.*** [25] similarly reported no relationship between CTLA-4 +49A/G (rs231775) or -318 C/T (rs5742909) and T1DM in a southern Brazilian cohort. **Al-Isawi *et al.*** [26] echoed these negative findings, concluding there was insignificant link among CTLA-4 +49A>G genotype and T1DM susceptibility in their research population.

Moreover, a significant relationship has been detected among the CTLA-4 (rs231775) genotype and multiple clinical and laboratory features, such as age at diagnosis, illness duration, weight, BMI, FBS, RBS,

2hppG, HbA1c%, serum creatinine, urea ACR, and lipid measures.

This observation is in agreement with **Mosaad *et al*.** [27] who found that the GG genotype of CTLA-4 is correlated with a younger age of onset. That said, **Tawfik *et al*.** [28] did not observe any differences in age or HbA1c% among various CTLA-4 (+49A/G) genotype groups.

Conclusion: These findings support the potential of CTLA-4 rs231775 genotyping as a predictive marker for T1DM risk among Egyptian children, particularly due to its influences on lipid and glucose metabolism. Discrepancies among different studies may arise from the multifactorial and genetically complex etiology of T1DM, varied environmental factors, or limited sample sizes. Therefore, future research with larger, diverse samples and investigations into additional CTLA-4 gene variants is warranted to clarify the gene's role in childhood T1DM susceptibility within Egyptian and broader populations.

DECLARATIONS

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