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Association of expression C/C .G/G, C/G Genotypes with Glycated hemoglobin (HbA1c) levels in Sudanese children with type 1 Diabetes

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ABSTRACT

Background: Type 1 diabetes (T1D) is a common complex metabolic disease characterized by the autoimmune destruction of insulin-producing B cells of the pancreatic islets. Due to a drastic loss of B cells, resulting in no to lowinsulin production,. Genetic factors play a significant role in the aetiology of T1D, However, the main causes of morbidity and mortality today are the complications that arise from even mild hyperglycaemia in T1D cases. Glycemic control is often measured using an assay called haemoglobin A1C (HbA1c), that provides an estimate of average blood glucose in individuals over a 3month period. Aim of the study to correlate between the frequencies of C/C,G/G and C/G genotypes and HBA1C levels in T1D patients.

Objective: To evaluate the significant correlation between frequencies of genotypes and HBA1C levels.

Methods: A total of 100 Sudanese subjects with T1D were enrolled in this study, on the average age between 5 to 18 years. The study was conducted in diabetes central hospitals in Khartoum state. In order to determine the HLA gene polymorphism, the allele-specific-refractory mutation system-polymerase chain reaction (ARMS-PCR) method was utilized.

Results: There were no significant correlation (r= 0.074, P-value = 0.497) between frequencies of genotypes and HBA1C levels.

Conclusion: This study identified that there's no significant correlation between frequencies of genotypes and HBA1C levels.

Keywords: Genotypes, Glycated hemoglobin (HbA1c), Sudanese children, type 1 Diabetes

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Introduction

Type 1 diabetes (T1D) is an autoimmune disease in which insulin is functionally absent because of the destruction of the B cells in the pancreas by the immune system [1].The triggers for the autoimmune attack are not fully understood, but it is now widely accepted that both environmental and genetic factors contribute to it [2]. T1D was originally described as "juvenile diabetes" and, later, "insulin-dependent diabetes mellitus" (IDDM). T1D occurs most commonly in juveniles but can occur in adults, especially those in their

late 30s and early 40s [1]. The incidence of T1D has risen over the past 20 years [3].T1D is one of the most frequently studied complex genetic disorders [4]. The strong genetic contribution to T1D is illustrated by the fact that by age 60, 65% of identical twins of T1D probands will develop T1D themselves [5]. Furthermore, children born to a family with an affected family member have a 5% risk of T1D by age 20, compared with a 0.3% risk for children without affected family members [6], yet the great majority of T1D cases do not have a first-degree relative with diabetes [1] . The major histocompatibility complex (MHC) is reported to account for approximately 40% to 50% of the familial aggregation of T1D [7, 8]. The gene products from this region were originally discovered on the surface of white blood cells, so they became known as leukocyte antigens; thus, the human MHC is also referred to as the HLA complex. HLA molecules were originally studied for their ability to confer tolerance (histocompatibility) following tissue grafts [9]. The HLA genotype, which is the combination of HLA alleles inherited from both parents, is key for the development of T1D [10]. Glycated hemoglobin (HbA1c) reflects the level of blood glycemia and is commonly used to monito glycemic control in diabetes and to diagnose type 2 diabetes. Genome-wide association studies (GWAS) have revealed .60 genetic variants to be associated with HbA1c in individuals without diabetes (11–12). Arecent GWAS of multiple ethnic groups confirmed 18 variants previously associated with HbA1c and added 42 new loci (12). Some of the variants affect biological pathways maintaining glucose homeostasis. For example, single nucleotide polymorphisms (SNPs) in or near glucokinase (GCK) and melatonin receptor 1B (MTNR1B) are known to be associated with HbA1c, fasting plasma glucose, and b-cell function (13). HbA1c is further influenced by genes and pathways that affect erythrocytes. In particular, SNPs in hemochromatosis (HFE) and hexokinase 1 (HK1) (13) have an effect on HbA1c through nonglycemic pathways that affect erythrocyte physiology and survival HbA1c reflects the glycemic load over ;3 months (14), and traits such as hemoglobinopathies, alterations in intracellular glucose metabolism, or defects of glucose transport into erythrocytes affect HbA1c In addition, diet,drugs, and insulin administration affect HbA1c in individuals with type 1 diabetes (T1D) who have little to no endogenous insulin production and, thus, rely on an external source of insulin to ensure glucose uptake in cells. Despite a strong environmental stimulus, HbA1c levels are correlated between both dizygotic twins and monozygotic twins concordant or discordant for T1D (15).

Materials and Methods: This study was performed on 100 Sudanese subjects on the average age between 5 to 18 years. 100 cases with type1diabetes mellitus according to the diagnostic criteria established by National Diabetes Data Group (NDDG). And 100 (non – diabetic) with no clinical evidence or T1D history in their family, as a control groupThe DNA was extracted from

peripheral blood samples of the patients and controls by utilizing a commercially available kit (G-DEXTM11b Genomic DNA Extraction Kit (Blood)\200T catalog numbers 17241).

Genotyping: We designed an amplification refractory mutation system polymerase chain reaction (ARMS-PCR) for detection of rs3104413 (C/G) (Table1) [10]. Polymerase chain reaction (PCR) was performed by using commercially available PCR premix (AccuPower PCR Premix; BIONEER, Daejeon, Korea) according to the manufacturer's instructions. Briefly, 1 µl template DNA (~100 ng/µL), 1 µl of each primer (10 pmol/µl), and 15 µL DNase-free water were added to AccuPower PCR Premix. It was done in 20 µl reaction volume containing 100 ng of genomic DNA. The following thermal profiles were run; 3 min. at 95ºC for initial denaturation, followed by 30 cycles of 95ºC for 20s, 60ºC for 30s and 72ºC for 40s and final extension at 72°C for 5 min. for position rs3104413 (C/G). The amplified PCR products were analyzed by 2% agarose gel electrophoresis and ultraviolet visualization. The length of the expected PCR products were 372 bp for rs3104413 (C/G) polymorphisms. (Table1), Glycated protein is formed post-translationally through the slow, non enzymatic reaction between glucose and amino groups on protein..HbA1 is considered as a more reliable parameter in monitoring glycemia over the glycemic reading with the conventional glucometer (15). Test Procedure: 100 µl of hemolysis buffer was Drew and transfer it into detection buffer tube, 5µl of fingertip blood or tube blood was Drew using 5µl capillary tube, the capillary tube was putted into the detection buffer tube, the lid of the detection buffer tube was closed and the sample was mixed thoroughly by shaking it about 15 times, the cartridge half was take out from I-Chamber slot, 75µl of the sample was pipetted out, mixtured and loaded it into a sample well in the teats cartridge, waited till the sample mixture flow appears in the windows, the cartridge was -inserted into I-Chamber slot (30C◦), the cartridge was leaved in I-Chamber for 12 minutes before removing, to scan the sample – loaded

(Table1) The sequences of primers used in the study for rs3104413 single-nucleotide Polymorphism

Gene polymorphism HLA rs1304413	Primers Sequence (5′ to 3′)	Tm (˚C)	Product size
Reverse (C allele)	GGAGAAGCACGACAATAGGAC	59	C and G allele: 327 b p
Reverse (G allele)	GGAGAAGCAAGCCAATAGGAG	59	
Forward (common)	CTGCTTTTCACACCAACCTCT	60	

HbA1c: cartridge was inserted it in to the cartridge holder of the instrument for I- chroma tests, the select button was Pressed on the instrument for I-chroma testes to start the scanning process, instrument for i-chroma tests was start scanning the sample loaded cartridge immediately and test result was read on the display screen of the instrument for I-chroma teast. Statistical

Analysis:Data was examined using the statistical package of social science (IBMSPSS version 26.0) for windows software package. A P. value of ≤ 0.05 was interpreted as statistically significant. Categorical variables, alleles and genotypes frequency were analyzed using a Pearson's Chi square test. Comparison of groups was done by Kruskal Wallis test. The strength of significant was done by calculating the contribution to chi square of each cell using adjusted residuals P values (adjusted P value =0.05/number of new adjusted residuals or cells).

Results

A total of 200 Sudanese subjects were enrolled in this study, on the average age between 5 to 18 years .100 cases with type1diabetes mellitus and 100 (non – diabetic) as a Control group. The study was conducted in diabetes central hospitals in Khartoum state. The patients' mean age were (12.00±3.735) and controls were (12.25±3.686) as shown in (Table1), in the control group, male frequency was (49%) and female was (51%) while the patients group, male (48.3%) and female was (51.7%) as shown in (Table2).

Table 1: The Correlation between the frequencies of Genotypes and HBA1C levels in patients:

GROUPS			**FREQUENCY**
CONTROL	Gender	Females	51.0(%)
		Males	49.0(%)
	Age	(mean ±Std.)	12.25±3.686
PATIENTS	Gender	Females	51.7(%)
		Males	48.3(%)
	Age	(mean ±Std.)	12.00±3.735

There were no significant correlation (Spearman's rho, r= 0.074, P value = 0.497) between frequencies of genotypes and HBA1C levels as shown Fig 1.

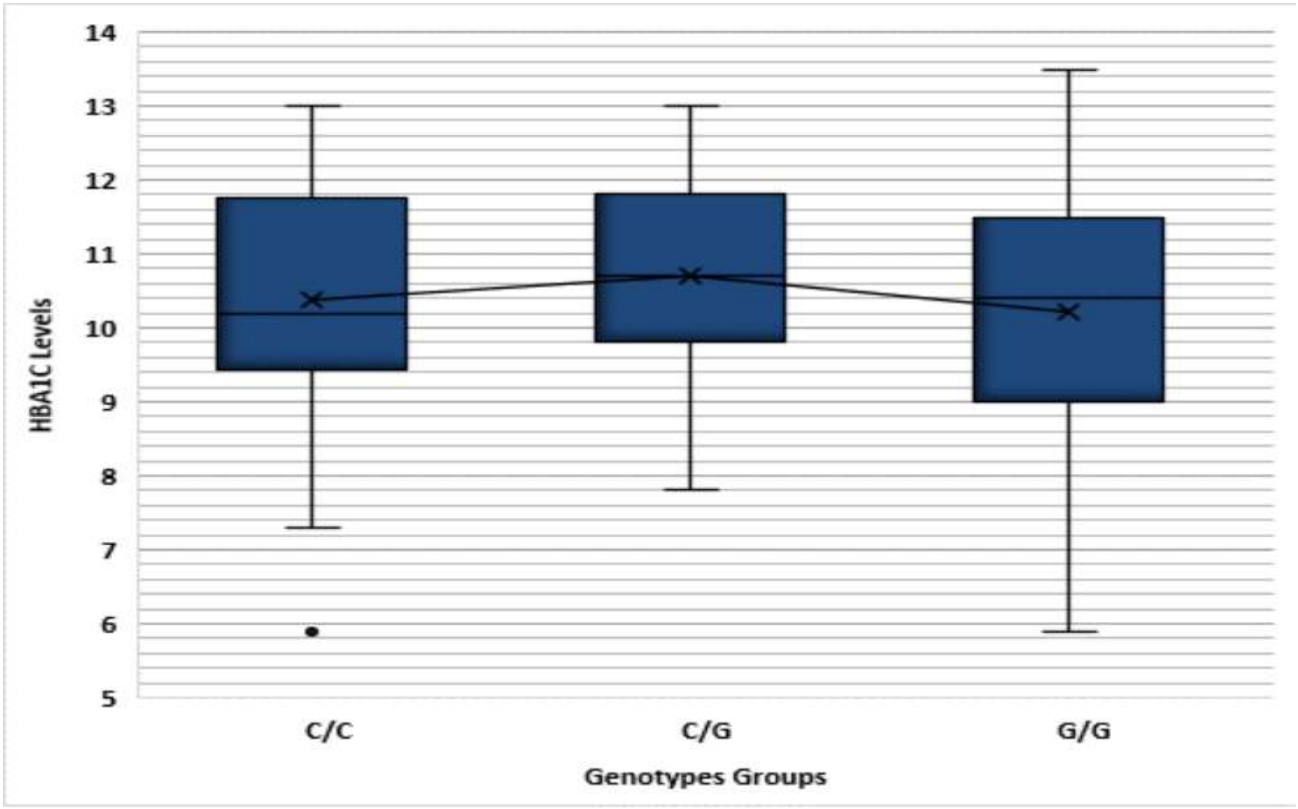

Fig1: The Correlation between the frequencies of Genotypes and HBA1C levels in patient

Discussion

Once T1D is diagnosed, the average time to develop micro and macro vascular complications of the disease is 15-20 years (19). The epidemiological risk factors associated with the development of complications include: duration of diabetes, glycemic control (typically as measured from hemoglobin A1c (HbA1c)), age, weight and waist-hip ratio, smoking status, cholesterol, triglycerides, and blood pressure. Of these. (16,18 ,17) Glycemic control of T1D can be measured by frequent monitoring of glucose in the blood or urine or by the percent of glycated haemoglobin in the blood, so called- haemoglobin A1C (HbA1c). Normal levels of HbA1c are lower than 6%, while a HbA1c value of 6.5% or more can be diagnostic of hyperglycemia (19). HbA1c levels have been shown to be heritable, with heritability estimates ranging from 47% to 59% (20). A genome wide association study identified 11 single nucleotide polymorphisms (SNPs) in 10 different genetic loci associated with HbA1c levels (21). Collectively, they explained approximately 2.4% of the variance in HbA1c levels and 5% of heritability of HbA1c. However, environmental factors also influence variation in HbA1c; age and levels of C-reactive protein (a measure of stress) were found to be the strongest determinants of variation in HbA1c levels among Finnish men without diabetes (22). Nevertheless, HbA1c is a marker of long-term glycemia. Prolonged hyperglycemia causes a number of micro and macro-vascular complications tissues, and plays a causative role in the micro-vascular complications in T1D,

including retinopathy, nephropathy and neuropathy (23). High HbA1c can also cause an increase in the number of reactive oxygen species inside RBCs, which can alter the cell membrane properties and lead to blood cell aggregation and impaired blood flow, or lead to inflammation that can result in atheroscelerotic plaques, both of which become risk factors for CVD (24). This study to Correlation between the frequencies of Genotypes and HBA1C levels in a total of 100 Sudanese subjects with type1 diabetes mellitus , on the average age etween 5 to 18 years, our study found that there were no significant correlation (Spearman's rho, r= 0.074, P value = 0.497) between frequencies of genotypes and HBA1C levels. Another study Anna Syreeni, Niina Sandholm etal identified that: a locus on chromosome 13 near RXFP2 that is associated with HbA1c in individuals with T1D (25) Sara Victoria Good found that weakly suggestive associations between imputed gene expression and mean HbA1c for nine genes expression with Glycated haemoglobin (HbA1c) levels in people with type 1diabetes. (26) Conclusion This study identified that there's no significant correlation between frequencies of genotypes and HBA1C levels. Data Availability When all data is publicly available, this manual is a review of pub-lished research. Conflicts of Interest The authors declare no conflicts of interest. Authors' Contributions Hiba Omer- designed the project. Data collection. xperimental design. Lab working . .Data analysis. Manuscript writing. Sababil Salih- Co manuscript writing. . Data collection Sakeena NourEldine - Data collection Abdulaze Abdulsalam – Manuscript reviewing data analysis. Mohamed Abdelgadir – Assisting in study design, lab working, data analysis

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