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Vitamin D Receptor Gene (Fok-I) Polymorphisms in Type 1 Diabetic Children; Case Study in Zagazig University Hospitals

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ABSTRACT

Background: Many meta-analyses studied the association between vitamin D receptor (VDR) gene polymorphism and type 1 diabetes (T1DM) susceptibility. **Objective:** This study was designed to assess the role of VDR gene (FOK-I) polymorphisms in type 1 diabetic children from Zagazig University Hospitals in Egypt. **Patients and Method:** In this case-control study, the genotypes of VDR gene (FOK-I) polymorphisms were assessed in 180 type 1 diabetic children and 120 healthy matched age controls by PCR-RFLP analysis. **Results:** A high statistical difference between patient and control regarding VDR gene (FOK-I) polymorphisms, where 44% of the patient group had heterozygous genotype (AG) compared to 8.3% in the control group. AG genotype has almost a higher risk nine times odds ratio (OR) = 8.8 than AA genotype in diabetic patients. There was a significant increase in the G allele in the patient group. Moreover, a significant association between (FOK-I) polymorphisms and T1DM complications was also observed.

Conclusion: (AG) genotype of VDR gene (FOK-I) polymorphisms could be a risk factor for T1DM complications. So, VDR gene (FOK-I) polymorphisms should be performed with other genetic studies for early prediction, detection and prevention of microvascular complications of T1DM that adversely affect health-related quality of life of Egyptian children and burden the primary care units.

Keywords: FOK-I, Genotype, Polymorphisms, T1DM, VDR gene.

INTRODUCTION

Type 1 diabetes (T1DM) is the most frequent form of diabetes in children and young adults and is one of the most common chronic diseases in children[(1)]. The worldwide incidence of T1DM is increasing by 2-5% annually[(2)]. Egypt represents the highest incidence of childhood T1DM in the Middle East[(3)]. Approximately 96,000 children are anticipated to develop T1DM annually worldwide[(4)].

HLA haplotypes are related to 50% of disease heritability[(5)]. Vitamin D plays a possible role in immune regulation[(6)]. VDR gene provides instructions for making a protein that allows the body to respond to vitamin D[(7)]. Four common single nucleotide polymorphisms for vitamin D receptor gene have been investigated[(8)]. VDR polymorphisms are associated with T-cell mediated autoimmune diseases [(9)].

This study was designed to assess the role of VDR gene (FOK-I) polymorphisms in type 1 diabetic children from Zagazig University Hospitals in Egypt.

PATIENTS AND METHODS

The study included two groups; 180 patients of type 1 diabetes mellitus and 120 non-diabetic completely healthy. Patient inclusion criteria were: age → 1-16 years, gender → male and female and blood glucose level→fasting >126 mg/dl, 2 hours postprandial >200 mg/dl. While patient exclusion criteria: age > 16 years, patient with a history of chronic renal disease, patient with a history of chronic liver disease, patient with a history of chronic infection or inflammation. Patients were subjected to the following: history taking regarding age, together with the onset and duration of the disease, complete clinical examination. Routine investigations as fasting and 2 hours postprandial blood glucose, hemoglobin A1C levels, liver, and kidney function tests, and albumin/creatinine ratio (ACR) and lipid profile.

All laboratory investigations were done using Cobas Hitachi, Roche diagnostics (Japan). Specific investigations: genotyping of vitamin D receptor gene (FOK 1) using DNA sequencing Genetic analyzer 3500 was done for patients and control groups. Polymerase chain reaction amplicons were generated using the following primer pairs: for FOK 1:

Forward 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3' and reverse 5'-ATGGAAACACCTTGCTTCTTCTTCTCCCTC-3'

Ethical approval:

This study was carried out in the Clinical and Chemical Pathology Department and Pediatric Department in Zagazig University Hospitals in Egypt. The study was approved by the Ethical Committee of Zagazig Faculty of Medicine (hospital IRB approval number 4813). An informed consent was obtained from all patients and their parents in this research. Every patient and parent received an explanation for the purpose of the study. All given

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data were used for the current medical research only. 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

All data were collected, tabulated, and statistically analyzed using SPSS version 19. Continuous quantitative variables were expressed as the mean ± SD and median (interquartile range), and categorical qualitative variables were expressed as absolute frequencies (number) and relative frequencies (percentage). Mann-Whitney test was used to compare two groups of abnormally distributed data. Independent student's t-test was used to compare two groups of normally distributed data. Categorical data were compared using the chi-square test or Fisher's exact test. The odds ratio was used for risk quantification. The tests were two-sided with a p-value < 0.05 was considered statistically significant and p-value < 0.001 was considered highly statistically significant.

RESULTS

This is a case-control study that included 180 T1DM children from 1 to 16 years and 120 matched healthy children from 2 to 15 years. Biodemographic data are described in table 1.

Table (1): Bio-demographic data among the studied groups

Variable	DM patients (n=180)		Control group (n=120)	
Age: (years) Median Range	12.7 1-16		13 2-15	
	No:	%	No:	%
Sex: Male Female	 100 80	 55.6 44.4	 60 60	 50 50

120 patients have a family history of DM. Family history was significantly higher in DM patients when compared to the control group as shown in table 2.

Table (2): Family history among the studied groups

Variable	DM patients (n=180)		Control group (n=120)		P value
	No:	%	No:	%	
Family history Negative Positive	 60 120	 33.3 66.7	 120 0	 100 0	 <0.001**

**: Highly significant

HbA1c, fasting blood glucose, two hours post-prandial blood glucose, albumin/creatinine ratio (ACR), total cholesterol, LDL and triglycerides were significantly higher in the DM group when compared to the control group.

WBC and HDL were found to be significantly higher among the control group compared to the DM group. The laboratory data are described in table 3.

Table (3): Comparison of laboratory data among the studied groups

Variable	DM patients (n=180)	Control group (n=120)	P value
Glycosylated hemoglobin (HbA1C %) Mean ± SD	10.8 ± 1.90	5.13 ± 0.27	<0.001 **
Fasting glucose mg/dl Mean ± SD	214.9 ± 29.5	89.6 ± 10.2	<0.001 **
Post-prandial glucose mg/dl Mean ± SD	293.8 ± 6.1	110.6 ± 14.7	<0.001 **
Albumin/ Creatinine Ratio (ACR) mg/g Mean ± SD	206.5 ± 41.3	17.08 ± 3.85	<0.001 **
White Blood Cells count (WBCs) × 10^9/L Mean ± SD	8.19 ± 1.00	10.2 ± 1.90	<0.001 **
Total cholesterol mg/dl Mean ± SD	153.3 ± 36.6	120.5 ± 26.9	<0.001 **
Low Density Lipoprotein (LDL mg/dl Mean ± SD	74.3 ± 4.68	54.4 ± 3.36	<0.001 **
High Density Lipoprotein (HDL) mg/dl Mean ± SD	45.5 ± 8.44	60.9 ± 7.69	<0.001 **
Triglycerides mg/dl Mean ± SD	87 ± 15.9	62 ± 10.1	<0.001 **

**: Highly significant

There was a significant difference between the studied groups regarding genotypes, as AG genotype was found to be higher among the patients when compared to the control group as shown in table 4.

Table (4): Frequency distribution of genotype polymorphisms among the studied groups

Variable	DM group (n=180)		Control group (n=120)		OR (95% CI)	P-value
	No	%	No	%		
Genotype AG AA	 80 100	 44.4 55.6	 10 110	 8.3 91.7	 8.8 (4.322-17.917)	 <0.001**
Allele frequency A allele G allele	 280 80	 77.8 22.2	 230 10	 95.8 4.2	 6.57 (3.329-12.973)	 <0.001**

**: Highly significant

There was also a significant difference between the AG and AA genotypes and laboratory data among the patients as shown in table 5.

Table (5): Relationship between genotype polymorphisms and laboratory data among the patients

Variable	AG (n=80)	AA (n=100)	P value
HbA1C % Mean ± SD	11.9 ± 1.82	9.89 ± 1.46	<0.001**
Albumin g/dl Mean ± SD	3.38 ± 0.71	4.08 ± 0.39	<0.001**
AST U/L Median	14.8 ± 3.32	8.58 ± 2.53	0.001*
Creatinine mg/dl Mean ± SD	0.55 ± 0.08	0.44 ± 0.06	<0.001**
ACR mg/g Median	196.1 ± 33.6	122 ± 16.3	0.02*
Hemoglobin g/dl Mean ± SD	10.6 ± 0.56	11.7 ± **1**.22	<0.001**
Total cholesterol mg/dl Mean ± SD	186.3 ± 40.3	127 ± 21.3	<0.001**
Triglycerides mg/dl Mean ± SD	104.5 ± 19.6	73 ± 9.64	<0.001**

*: Significant, **: Highly significant

DISCUSSION

Vitamin D and VDR genes are engaged in the development of T1DM and its complications by multiple mechanisms that may be significant in the etiology and treatment of the disease and its problems[10]**.** In this study, positive family history was found in 66.6% of our patients. These results were more or less similar to what was reported by **Khalid**[8] who found that among diabetic patients, 59.2% were having a positive family history of diabetes mellitus**.**

As expected, HbA1C showed a highly statistically significant difference between patients and control groups. These results agreed with that reported by **Ali *et al.*** [9]**.**

Albumin/creatinine ratio (ACR) levels were significantly higher in the DM group compared to the control non-diabetic group. This is consistent with **Swamy *et al.*** [10]**.** Moreover, persisted increase in glycated hemoglobin may be an indicator of ACR and diabetic nephropathy [17]. However, **Gogas Yavuz *et al.*** [11] showed a non-significant association between microalbuminuria and T1DM which may be due to errors associated with the collection of 24 hours urine. That is why we preferred to use ACR rather than micro albuminuria**.**

The current study showed that total cholesterol, LDL, and triglycerides were significantly higher among the DM group when compared to the control group. HDL was found to be significantly higher among the control group compared to the DM group. Similar results were reported by **Begum *et al.*** [12]**.**

As regards VDR gene (FOK-I) polymorphisms, there was a statistically significant difference between the patient and control groups. The frequencies of the A and G alleles in the patient group were statistically significantly different between the patient and control group. The majority of the patient group had (AG) heterozygous type of polymorphism, while in the control group about one-tenth had (AG) heterozygous polymorphism and the majority showed (AA) wild type with no polymorphism. This finding suggests a positive link between AG genotype of (FOK-I) with a higher T1DM risk and that the G allele may be a risk allele for the disease, which goes in agreement with **Mukhtar *et al.*** who found that VDR polymorphisms were identified as susceptible regions for T1DM development in the Pakistani population[13]**.** Moreover, in Alexandria University, **El-Kafoury *et al.*** studied (FOK-I), and (Bsm-1) polymorphism in T1DM using the RFLP technique and admitted an association between VDRG polymorphisms and T1DM[14]**.**

A meta-analysis of 13 case-control studies involving 2,538 cases and 2,679 healthy people was also conducted on the Asian population. Five of the studies were from West Asia, while the rest were from East Asia. The (FOK-I) polymorphisms confer vulnerability to T1DM in the West Asian population, according to this meta-analysis [15].

However, contrary to our results, **Thorsen *et al.*** reported that VDRG polymorphisms link with T1DM was not confirmed among the Danish population, which may be due to the interconnection with other genetic or environmental factors implicated in the mechanisms of development T1DM and its complications as it is supposed that it has several etiological pathways[16]**.**

Our study also highlighted a significant association between the ACR among (AG) genotypes. These results agreed with **Razi *et al.*** [17] in which ACR showed a highly statistically significant difference between different genotypes, with the G allele encoded as a risk factor for diabetes and its complications**.** Also, **Ezhilarasi *et al.*** [18] called attention to a significant

linkage between VDRG polymorphisms and higher creatinine levels**.**

There was also a significant association between (FOK-I) polymorphisms and total cholesterol and triglycerides. These findings were in harmony with those of **Xia *et al.*** [(19)] who also reported a significant association between (FOK-I) polymorphisms and dyslipidemia although they reported very similar fasting blood glucose and 2-hour postprandial glucose between the two groups of genotypes. This suggests that the development dyslipidemia is not directly related to hyperglycemia**.** Similarly, **Sun *et al.*** [(20)] stated that VDRG polymorphisms were associated with risks of cardiovascular complications pathogenesis including dyslipidemia as triglyceride levels were significantly higher in (AG) group compared to (AA) group**.**

In general, discrepancies between our results and others may be attributed to ethnicity and the substructure of children even among heterogeneous populations. In the Pakistani community, **Mukhtar *et al.***[(13)] revealed a positive association while **Nasreen *et al.*** [(21)] showed no association**.**

CONCLUSION

VDR gene (FOK-I) polymorphisms, especially heterozygous (AG) genotype, may be a risk factor for T1DM and the (G) allele is a risk allele influencing the disease. There was a statistically significant association between VDRG polymorphisms and T1DM complications.

Quality of life of the studied diabetic children may be negatively affected by the development of microvascular complications of T1DM.

So, performing other studies using large-scale collaborative genome-wide association studies (GWAS) in Egypt to search for genetic factors controlling T1DM should be done to construct susceptibility profiles that will help in the prediction, prevention, and early detection of the disease.

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