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ASSOCIATION OFCYTOMEGALO VIRUS WITH TYPE I DIABETES MELLITUS AMONG CHILDREN IN MINIA GOVERNORATE

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ABSTRACT

Background: Type I diabetes (T1D) is the most common form of diabetes in most parts of the world.Aim: The association between cytomegalovirus (CMV)and T1D mellitus was studied, with comparison to healthy subjects and to correlate its level with different clinical and laboratory parameters.

Materials & Methods: This study included 68 children and adolescents who were classified into two groups. GroupI comprised 53 patients diagnosed with T1D and having regular follow up in the pediatric endocrinology out-patient clinic, Minia University children's hospital. Group II comprised 15 apparently healthy subjects, age and sex matched to the diseased group. According to the onset of diabetes, we divided the diabetic group into two sub-groups. Group Ia (newly diagnosed) comprised 20 patients, with ages ranging between 7 and 18 years; 10 were males (50 %), and 10 were females (50 %). Whilst group Ib (duration of disease >1 year) comprised 33 patients, with ages ranging between 6 and 17 years; 17 were males (49%) and 18 were females (51%). The studied groups were subjected to the following: thorough history taking, clinical examination and laboratory investigations (random blood glucose levels) and HbA1c%. DNA was extracted using QIAamp Min elute kit protocol for detection of cytomegalo virus by RT-RCR.

Results:The frequency of cytomegalovirus was significantly higher in T1D children than the control and in group Ia than group Ib.

Conclusion: T1D children had significantly higher serum cytomegalovirus than the control group, as did those newly diagnosed compared to those with longer duration of illness.

Keywords:T1D, Cytomegalo-virus, Haemoglobin A1C, Polymerase chain reaction

Key Messages: Dose of insulin, significant +ve correlations, RT-PCR

ASSOCIATION DU CYTOMEGALOVIRUS AVEC DIABETE SUCRE DE TYPE 1 CHEZ LES ENFANTS DANS LE GOUVERNORAT DE MINIA.

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RESUME

Contexte : Diabète de type 1(DT1) est la forme la plus courante du diabète dans la plupart des régions du monde.

But : L'association entre le cytomégalovirus et DT1 sucre a été étudiée avec une comparaison aux sujets sains et pour corréler son niveau avec les divers paramètres cliniques et laboratoires.

Matériaux et Méthodes : Cette étude a inclus 68 enfants et adolescents qui ont été classés en deux groupes. Le Groupe I a compris 53 patients diagnostiqués avec DT1 et ayant suivi régulier dans la clinique endocrinologie pédiatrique ambulatoire, l'Université hôpital d'enfants de Minia. Le Groupe II a compris 15 sujets apparemment sains, l'age et le sexe adapté au groupe malade. Selon l'attaque du diabète, nous avons divisé le groupe diabétique en deux sous – groupes. Le Groupe Ia (nouvellement diagnostiqué), a compris 20 patients, dont l'âge est compris entre 7 et 18 ans ; 10 étaient males (50%), et 10 étaient femelles (50%). Alors que le Groupe Ib (la durée de malade >1 ans) comprenait 33 patients ; 17 étaient males (49%), et 18 étaient femelles (51%). Les groupes étudiés ont été soumis a la suivante : grâce a la prise de l'histoire, examen clinique et examen de laboratoire (nouveau de glycémie aléatoire) et HbA1c%. L'ADN a été extrait en utilisant QIAamp Min elute protocole du kit pour le dépistage du cytomégalovirus par RT – RCR.

Résultats : La fréquence du cytomégalovirus était considérablement plus élevée dans les enfants DT1 que le contrôlé et dans le Groupe Ia que le Groupe Ib.

Conclusion : Les enfants DT1 ont eu sérum cytomégalovirus plus élevé que le groupe contrôlé, comme ceux nouvellement diagnostiqués par rapport a ceux qui ont une plus longue durée de la malade.

Mots Clés : DT1, Cytomégalovirus, Hémoglobine A1C, la réaction en chaine de la Polymérase.

Messages Clés : Dose d'insuline, Significative +ve corrélations, RT – PCR.

INTRODUCTION

Type I diabetes (T1D) is the most common form of diabetes in most parts of the world; variations exist between the incidence rates of different populations. In type 1 DM, a viral infection in genetically susceptible individuals was hypothesized as triggering the disease. Human cytomegalovirus (HCMV) can infect and alter functions of poly morphonuclear leukocytes, lymphocytes and macrophages.Type 1 diabetes (T1D) is one of the most common chronic diseases in developed countries and represents about 10% of all cases of diabetes. It is caused by a selective destruction of insulin-producing beta cells (β-cells) in the pancreas (1). An increasing incidence of T1D has been observed in the last few decades, especially in young individuals (less than five years old) (2). The cause of T1 Dis still unknown. Several factors interact and lead to the development of the disease. An inflammatory is let infiltrate (insulitis) can be observed at the symptomatic onset of T1D, and reflects the immune response to β-cells (3). An autoimmune destructive process, which plays a central role in the development of T1D, is facilitated by the subject's own genetic susceptibility and by non-genetic factors. Non-genetic factors include viral infections, toxic chemicals, and others (4). Specific viruses can infect humans and may cause diabetes mellitus through different mechanisms, such as pancreatitis or hepatitis and their subsequent complications (5). Cytomegalo-virus (CMV) is an important factor thought to be associated with type I diabetes mellitus, owing to its ability to induce immunological damage to β-cells (6) CMV is an ubiquitous virus in the herpes group, causing chronic life-long infection in affected participants. It is a widely distributed virus, belonging to herpes virinae, subfamily of herpes viridae. Molecular mimicry is one of the principal immunological mechanisms that lead to destruction of pancreatic β-cells. This mimicry could be in volvedincy to megalo virus-induced diabetes by inducing is reactive antibodies. The loss of T-cell tolerance to self (GAD65) may be due to processing and presentation of a molecular mimic of cytomegalo virus protein pUL57 by dendritic cells (7). A Real-time PCR assay was described as an accurate and rapid test for CMV quantization by Leruez-Ville et al (2004) (8) The purpose of this study is to find out the association between cytomegalovirus and type 1 diabetes mellitus with comparison to healthy subjects, and to correlate its level with different clinical and laboratory parameters.

MATERIALS & METHODS

This study included 68 children and adolescents who were classified into two groups. Group I comprised 53 patients who had already been diagnosed as diabetic according to the standard ADA criteria and had regular follow up in the pediatric endocrinology outpatients' clinic, Minia University children's hospital, Egypt. Group II comprised 15 apparently healthy subjects, age and sex matched to the diseased group. Subjects were collected during the period from September 2013 to December 2013. According to the onset of diabetes, we divided the diabetic group into two sub-groups. Group Ia (newly diagnosed; duration of disease < 1 year) comprised 20 patients; 10 were males (50 %), and 10 were females (50 %), with ages ranging from 6-18 years (mean 11.4 ± 4.2). Group Ib(their duration of disease >1 year) comprised 33 patients; 17 were males (49 %), and 18 were females (51%), with ages ranging from 6 to 17 years (mean 12.3± 5.1). Informed consent was obtained from every subject enrolled in this study. The studied groups were subjected to the following: history taking, clinical examination and laboratory investigations. Random blood glucose levels (Colorimetric, Human, Germany) were assayed using a fully automated clinical chemistry auto-analyzer system Konelab 20i (Thermo Electron Incorporation, Finland) (9). HbA1c % was determined, as a parameter for glycemic control, by using resin column chromatography. Kit contents were supplied by TECO DIAGNOSTICS, California; USA (10). Five mL of blood samples from the diabetic children were collected and centrifuged at 5000 rpm. Serum samples were collected in sterile tubes; then DNA was extracted using the QIAamp Min elute kit protocol.

Principle Real time PCR

Procedure: CMV Quantitative Real Time PCR was used according to literature (OD-0002-02) from Life River. 17.5 µL of Reaction Mix CMV was used. Reaction was mixed with 0.2 µL of PCR enzyme mix and 0.5 µL of internal control then 6.8 µL of extracted DNA for a total volume of 25 µL. A positive control was defined as 10^7 copies/mL. To generate a standard

curve, a four-dilution standard was used. The real-time PCR instrument was operated according the thermal profile in the manual Ref (OD-0002-02). Each sample was spun down briefly in order to collect the Master Mix in the bottom of the reaction tubes, then the following protocol was performed in the instrument: 37°C for 2 min 1 cycle, 94 °C for 2 min 1 cycle at 94°C for 15 sec and at 60 °C for 1 min 40 cycles

Statistical Methods: The data were coded and verified prior to data entry. All statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS/Windows Version 19.0.0, SPSS Inc., an IBM Company). Microsoft excel 2013 was used for drawing figures. Continuous variables were presented as mean followed by standard deviation (SD), and categorical variables were presented as frequency and percentage. For qualitative data, chi-square (κ^2) was used: For quantitative data: Independent-Samples T test (for two groups) used in person correlation for correlation between two quantitative variables. Two-tailed partial correlation coefficients (r) adjusted for age, sex and BMI were used to assess the relationships between 25-hydroxy vitamin D and other variables. P-value < 0.001 was considered significant, and P-value > 0.001 was considered in significant. Kendl's test: was used for correlation between quantitative and qualitative variables.

RESULTS

Diabetic children had significantly higher weight and lower height than the control group (P < 0.001 for both). On the contrary, the differences between the diabetic and control groups as regards BMI and waist circumference was insignificant (P =0.4 and 0.3 respectively; Table 1). Table 2 shows that diabetic children, compared to the control group, had significantly higher fasting blood sugar, HbA1c %, and positivity of cytomegalovirus (45% *vs*7%).

***Duration*:** The current study found that newly diagnosed diabetic children, compared to those with longer duration of diabetes, had significantly higher frequency of cytomegalovirus (70% *vs* 30%), lower fasting blood sugar, and higher HbA1c% (P< 0.001, Table 3).

TABLE 1.COMPARISON BETWEEN THE DIABETIC CHILDREN AND THE CONTROLS AS REGARD SOME CLINICAL PARAMETERS

Clinical Parameter	Group I (Diabetic children)(N=53)	Group II (Control)(N=15)	P-Value
Age:(year)			
6-12 years. No (%)	**17 (32 %)**	**5 (33%)**	**0.9**
12-18 years. No (%)	**36 (68 %)**	**10 (67 %)**	
Sex:			
Male No (%)	**27 (51 %)**	**10 (67 %)**	**0.2**
Female No (%)	**26 (49 %)**	**5 (33 %)**	
Wt t(kg) mean±(SD)	**40.4±16.2**	**37.9±12.6**	**<0.001***
Height(cm)Mean±SD)	**142.8±21.1**	**149.3±13.1**	**<0.001***
BMI(kg/m²)(Mean±SD)	**18.9±4.3**	**16.8±2.7**	**0.4**
Waist circumference (cm)(Mean±SD)	**65±10.9**	**68.1±8.4**	**0.3**

***Significant**

TABLE 2.COMPARISON BETWEEN THE DIABETIC CHILDREN AND THE CONTROLS AS REGARD SOME LABORATORY PARAMETERS

Laboratory parameter	Group I, No=53(Mean±SD)	Group II, No=15 (Mean±SD)	P Value
Cytomegalovirus positivity No (%)	24(45 %)	1(7 %)	0.003*
Fasting blood sugar(mg/dl)	229.5±92.6	92.8±7.6	<0.001*
HbA1c (%)	8.7±1.8	5.7±0.6	<0.001*

- *Significant*

TABLE 3.COMPARISON BETWEEN NEWLY DIAGNOSED AND THOSE WITH DURATION > 1 YEAR AS REGARD SOME CLINICAL AND LABORATORY PARAMETERS

Parameter	Group Ia (duration < 1 year) No=20/cases	Group Ib (duration > 1 year) No = 33 (+ve CMV)	P value
Age (year)			
6-12. No (%)	4(20%), 3 (75%)	10 (33%), 4 (40%)	0.41
12-18 No (%)	16(80%), 11(68.7)	23 (67%), 6 (26%)	
Gender			
Male No (%)	10 (50%), 3 (30%)	16 (48%), 3 (18%)	0.915
Female No (%)	10 (50%), 10 (100%)	17 (52%), 7 (43%)	
Dose of insulin:(IU/kg/day) (Mean±SD)	0.9±0.14	0.9±0.12	0.8
Family history of DM:			
Positive No (%)	4(20%)	6(22%)	0.9
Negative No (%)	16 (80%)	27(78%)	
Cytomegalovirus positivity No (%)	14(70%)	10(30%)	<0.002
HbA1c% Mean±SD)	10.7±1.3	7.7±0.4	<0.001*
Fasting blood sugar(mg/dl) (Mean±SD)	121.2±7.9	259.6±75.5	<0.001*

- *Significant*

On the contrary, there were insignificant differences between these groups as regards age, dose of insulin, and family history of DM. Table 4 shows that there were significant positive correlations of cytomegalovirus positivity with fasting blood sugar and HbA1c%, and significant negative correlations with duration of disease and dose of insulin.

TABLE 4. CORRELATIONS BETWEEN POSITIVITY OF CYTOMEGALOVIRUS AND SOME CLINICAL AND LABORATORY PARAMETERS AMONG THE DIABETIC CHILDREN

Parameter	positivity of cytomegalovirus	
	R	Pvalue
Duration of diabetes (months)	-0.24	0.02*
Fasting blood sugar (mg/dl)	0.76	<0.001*
HbA1c (%)	0.86	<0.001*
Insulin dose (IU/kg/day)	-0.38	0.02*

DISCUSSION

T1D is an autoimmune disease, which implies a role of immune response effectors in the pathogenic processes and a failure of tolerance towards β-cell antigens. There is interplay between immune response, genetic and environmental factors. Several teams paid attention to the relationship between viruses and type 1 diabetes, and their role in the pathogenesis of the disease.[11]In the current study, the diabetic children (group I)were found to have higher weight and shorter height than the control group (group II; Table 1). This result was in agreement withPaulinoMFVM et al (2006)[12]who found that diabetic children were higher in weight and shorter in height in comparison to healthy children. Concerning BMI and waist circumference, there were insignificant difference between group I and group II (P> 0.05). This result was in agreement with that reported in lit.[13]Regarding laboratory parameters. Table 2 showed that diabetic children (group I) had significant higher fasting blood sugar and HbA1c % than the control group (group II). This was in agreement with CiechanowskiPC (2002)[14]Cytomegalovirus tested positive in 45 % of the diabetic patients versus (7%) of the control group, a statistically significant difference (P = 0.003). This finding was in agreement withAhmad-AbakurEH(2014), (15) who found that the positive rate of IgG against cytomegalo virus in the study group (diabetic patients) was 37% while it was 14.8% among a control group, indicating statistically significant association between IgG antibodies of cytomegalovirus and diabetes mellitus type I (*P* value 0.025). The study reveals significant relation (*P* value 0.003) of cytomegalovirus IgG antibodies with type I diabetes mellitusin age group (5-9 years) in Sudanese children. (15)

Regarding the duration of diabetes, we found that newly diagnosed diabetic children had significantly higher frequency of cytomegalovirus, compared to those with longer duration of diabetes, by 70% over 30%. This can be explained in that Human CMV (HCMV) plays an important role in diabetogenesis, where it was postulated that there is T-cell cross-reactivity between Human CMV (HCMV) and GAD 65 in pancreatic islet β-cells. HCMV-derived epitope could be naturally processed by dendritic cells and recognized by GAD65 reactive T-cells. Thus, HCMV may be involved in the loss of T-cell tolerance to autoantigen GAD65 by a mechanism of molecular mimicry leading to autoimmunity.[7]In 2008, Aarnisaloet al (16) analyzedspecific anti-CMV IgG antibodies in 169 serum samples from children who had developed the first T1D-associated autoantibody by the age of 2 years, and, in parallel, in 791 serum controls from healthy children. Serological, immunological, histological signs of autoimmunity and allograft rejection appeared concomitantly with early CMV infections in type 1 diabetic patients receiving pancreas allograft. This observation suggests that persistent CMV infections might be relevant to the pathogenesis of type 1 diabetes. Chen et al, (2012) (16) indicated that cells are damaged directly by viral infection, as the pancreas is a target organ of viral infection through induction of pro-inflammatory cytokines, or the cytotoxic activated effects or lymphocytes.

Regarding the duration of diabetes, the current study found that newly diagnosed diabetic children had significantly higher levels of cytomegalovirus than those with duration >1 year (P=002). This could be explained by the finding by Hjelmesaeth et al (2004) (17) who foundthat asymptomatic cytomegalovirus infection is associated with increased risk of new-onset type I diabetes and impaired insulin release after renal transplantation. As regards other parameters, the newly diagnosed had significantly lower fasting blood sugar and higher HbA1c% than those with long duration of diabetes (P < 0.001, Table 3). On the contrary, there were insignificant differences between these groups as regards age, dose of insulin, and family history of DM. Table 4 shows that there were significant positive correlations between cytomegalovirus levels with fasting blood sugar and HbA1c% and significant negative correlations with the duration of disease and insulin dose. This could be explained in that CMV infection apparently did not cause direct destruction of β-cells but was leading to changing in production of insulin.

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