academicJournals

Vol. 8(16), pp. 1730-1734, 16 April, 2014
DOI: 10.5897/AJMR2013.5891
Article Number: 2C492B744057
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Associations of cytomegalovirus with type I diabetes mellitus among children in Khartoum State

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Received 24 April, 2013; Accepted 24 March, 2014

Cytomegalovirus is one of the most common microorganisms that cause opportunistic infection that complicate the clinical care and progress of immunecompromised patients. The virus can cause severe diseases with multiple complications including type I diabetes mellitus. The present study is a case control study aimed at determining cytomegalovirus among type I diabetic mellitus in Sudanese children. Sera of eighty one (81) children were collected, 27 (33.3%) from diabetic which represent the study group and 54 (66.7%) from apparently healthy children (control group). The samples were tested for IgG anti-cytomegalovirus using enzyme-linked immunosorbent assay (ELISA) technique. 18 (22.2%) of the total population of study were sero-positive for cytomegalovirus IgG, most of them, 10 (55.6%) were diabetic patients, the results indicate significant association (*P* value 0.025) of cytomegalovirus IgG antibodies with type I diabetes mellitus in children. The study reveals significant relation (*P* value 0.003) of cytomegalovirus IgG antibodies with type I diabetes mellitus in age group (5-9 years).

Key words: Cytomegalovirus, type I diabetes mellitus, children.

INTRODUCTION

The incidence of diabetes mellitus is rising continuously all over the world, and this may be due many reasons that can collectively or separately lead to the disease. These factors include genetic factors, obesity, autoimmunity disorder and infection (Brickell et al., 2007). Certain viruses can infect human and may cause diabetes mellitus through different mechanism such as pancreatitis or hepatitis and their subsequent complications (WHO, 1999; Elhawary et al., 2011). Cytomegalovirus (CMV) is one of the most important factors that is thought to be associated with type I diabetes mellitus owing to its ability to induce immunological beta cells (β-cells) damage (Aarnisalo et al., 2008). CMV is an ubiquitous herpes group virus causing chronic life-long infection in affected participants. It is a widely distributed virus, belonging to betaherpesvirinae, subfamily of herpesviridae. The virus is enveloped and double stranded deoxyribonucleic acid (DNA) with icosahedral

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coat (Aarnisalo et al., 2008; Jawetz et al., 2007).

Molecular mimicry is one of the principal immunological mechanisms that lead to destruction of the pancreatic β-cells. This mimicry could be involved in cytomegalovirus-induced diabetes by inducing islet cell autoantibodies. The loss of T-cell tolerance to self (GAD65) may be due to processing and presentation of molecularly mimic cytomegalovirus protein pUL57 by dendritic cells (Hiemstra et al., 2001).

Interferon (INF) and natural killer cells (NK) play an important role in defense and clearance of cytomegalovirus during primary infection. The virus possesses several proteins called unique long protein (UL) such as UL16, UL40, UL140, UL141 and UL142; all of them play roles in NK cells down modulation (Knipe and Howley, 2007; Tomasec et al., 2005). Moreover, cytomegalovirus encodes others genes for glycoprotein called unique short protein (US) such as US2, US3, US6 and US11 that down regulate cell surface expression of major histocompatibility antigens class I (MHC class I), also US2 and US3 have the same impact on MHC class II expression.

Besides these genes, the virus can produce two types of interleukin 10 (IL-10); cytomegalovirus interleukin-10 (cmvIL-10) that during replication, and cytomegalovirus interleukin-10 that during latency (LAcmvIL-10), and both have the ability to down regulate the T-cells function (Jenkins et al., 2004). However, nucleoside analogue with a modified pentose such as foscarnet, a pyrophosphate analogue and ganciclovir have anti-cytomegalovirus activity and are used in clinical care for both prophylaxis in transplantations and in suppressive treatment in active infections (Jawetz et al., 2007).

In previous studies, results on the association of CMV infection with type 1 diabetes have been contradictory; Nicoletti et al. (1990) reported a significant association between high titer of anti-cytomegalovirus and anti-islet cell antibodies. Hjelmesaeth et al. (2004) showed that asymptomatic cytomegalovirus infection is associated with increased risk of new-onset type I diabetes and impaired insulin release after renal transplantation, whereas Chen et al. (2012) found that the CMV seropositivity is significantly associated with various indicators of glucose regulation and therefore CMV infection might be a risk factor for the development of type 2 diabetes in the elderly.

In contrast to these findings, Hiltunen et al. (1995) did not find any correlation between the presence of anti-cytomegalovirus IgG antibodies and anti islet cell antibodies in children with newly diagnosed type I diabetes mellitus. Several unpublished studies were carried in Sudan to determine Cytomegalovirus among different groups, these studies indicated high rate of immunoglobulin class G (IgG). It was 72.2% in pregnant women in western Sudan (Hamdan et al., 2011), while Redwan et al. (2011) conducted study in Jeddah city, Saudi Arabia to determine the prevalence of cytomegalovirus (CMV) infection among foreign manpower and it was 87.8% among Sudanese. However, the present study aimed to determine the associations of Cytomegalovirus infection with type I Diabetes mellitus among children in Khartoum state, Sudan.

MATERIALS AND METHODS

This was an analytical biomedical case control study, the sample size was calculated by Open Epi statistical program for case control study, and it was found to be 81 candidates, 27 diabetic patients representing the study group and 54 healthy people representing control group.

The samples of study group were collected from the candidates diagnosed with diabetes mellitus type I, aged between five and fifteen years, regardless of the history of diabetes mellitus of their families, whereas the samples of control group were collected from the apparently healthy children of the same age group, regardless of the family history of diabetes mellitus. The candidate who had a history of cytomegalovirus infection after being diabetic was excluded; the study population was asymptomatic.

Using sterile disposable vacationer container, about 5 ml of blood were drawn from the anticubital vein under aseptic conditions. The blood samples were poured in sterile plain containers, and left to clot at room temperature. The clotted samples were centrifuged at 1500 rpm for 5 min in order to separate the sera, the obtained sera samples were kept frozen at -20°C until used. ELISA technique (EUROIMMUN™) was applied in this study to detect the IgG anti-cytomegalovirus.

The information related to the study such as age, gender and family history of diabetes mellitus were collected via direct interview. Chi squire test and frequencies was used to analyze the data by statistical package for social studies (SPSS) program.

The ethical considerations and conformity to individuals were considered by agreement and signature of children's parent and the study was approved by the Ethics Committee, Faculty of Graduate Studies, Alzaeim Alazhari University, Sudan.

RESULTS

Eighty one (81) children participated in this study, 27 (33.3%) represented study group (diabetic children) and 54 (66.7%) were apparently health children representing the control group, 32 (39.5%) of study population were male and 49 (60.5%) were female, 42 (51.9%) of the them were categorized in age group of 5-9 years, while the rest 39 (48.1) were sorted in group of 10-15 years (Table 1).

The IgG antibodies against cytomegalovirus were detected in 18 (22.2%) of the study population; the seropositive of cytomegalovirus was slightly higher in female, 11 (22.4%) than male 7 (21.9%). However, the majority of positive cases 10(55.6%) were diabetic patients, that is, belonging to study group. The positive rate of IgG against cytomegalovirus in the study group (diabetic patients) was 37% while it was 14.8% among control group, this result indicated statistically significant association between IgG antibodies of cytomegalovirus and diabetes mellitus type I (*P* value 0.025; Table 1). Also significant association (*P* value of 0.003) was

Table 1. showed statistical relation of study population (study group and control group), gender, age groups, and family history of Diabetes Mellitus against sero-diagnosis IgG of anti-cytomegalovirus.

Variable		Frequency	CMV (IgG)		P value	Odd ratio	CI (95%)	
			Positive	Negative			Lower	upper
Study population	Study group	27(33.3%)	10(37%)	17(63%)	0.025	3.382	1.145	9.994
	Control group	54(66.7%)	8(14.8%)	46(85.2%)				
Gender	Male	32(39.5%)	7(21.9%)	25(78.1%)	0.588	0.976	0.331	2.830
	Female	49(60.5%)	11(22.4%)	38(77.6%)				
Age group (year)	5-9	42(51.9%)	7(16.7%)	35(83.3%)	0.163	0.509	0.175	1.484
	10-15	39(48.1%)	11(28.2%)	28(71.8)				
Family history of DM	Yes	3(3.7%)	2(66.7%)	1(33.3%)	0.123	7.750	0.660	90.945
	No	78(96.3%)	16(20.5%)	62(79.5%)				
CMV (IgG)	Positive	18(22.2%)	-	-	-	-	-	-
	Negative	63(77.8%)	-	-				

The median age was 7.1 and 7.9 years for study and control groups, respectively. Chi squire test and frequencies was used to analyze the data; P value <0.05 considers significant. Odd ratio >1 indicates strong association. CI 95% means confidence interval at level of 95%.

Table 2. Statistical relation of study group, gender, age groups, and family history of diabetes mellitus against sero-diagnosis IgG of anti-cytomegalovirus.

Variable		CMV IgG		Total	P value	Odd ratio	CI (95%)	
		Positive	Negative				Lower	Upper
Age group 5-9 years	Study group (diabetic)	5(11.9%)	4(9.5%)	9(21.4%)				
	Control group	2(4.8%)	31(73.8%)	33(78.6)	0.003	19.375	2.777	135.163
Total		7(16.7)	35(83.3%)	42(100%)				
Age group 10-15 years	Study group (diabetic)	5(12.8%)	13(33.3%)	18(46.2%)				
	Control group	6(15.4%)	15(38.5%)	21(53.8%)	0.620	0.962	0.237	3.899
Total		11(28.2%)	28(71.8%)	39(100%)				
Gender	Male	2(7.4%)	7(25.9%)	9(33.3%)				
	Female	8(29.6%)	10(37%)	18(66.7)	0.244	0.357	0.058	2.217
Total		10(37%)	17(63%)	27(100%)				
Family history of DM	With family history	2(7.4%)	1(3.7%)	3(11.1%)	0.303	4	0.314	51.027
	Without family history	8(29.6%)	16(59.3%)	24(88.9%)				
Total		10(37%)	17(63%)	27(100%)				

Chi square test and frequencies was used to analyze the data; P value <0.05 is considered significant. Odd ratio >1 indicates strong association. CI 95% means confidence interval at level of 95%.

founded between sero-diagnosis of cytomegalovirus of diabetic children and younger age group (Table 2).

The present study showed no association between seropositive IgG anti-cytomegalovirus among diabetes mellitus type I and gender (*P* value 0.244) and family history of diabetes mellitus (*P* value 0.303).

DISCUSSION

Cytomegalovirus is one of the most common viruses in the world, the virus can cause severe disease with multiple complications. A chronic stressor for the immune system is the common herpes virus cytomegalovirus (CMV) which establishes persistent, life-long infections and can become reactivated periodically (Hiemstra et al., 2001; Hamdan et al., 2011). However, type 1 diabetes is an autoimmune disease resulting from a complex interplay between genetic and environmental factors. CMV infection is one of the environmental factors implicated in the development of type 1 diabetes, although the association remains unproven (Aarnisalo et al., 2008). The present study aimed to determine the pos-

sible correlation between CMV infections and type 1 diabetes among children in Khartoum State, Sudan.

The study showed that the positive rate of IgG against cytomegalovirus in diabetic (37%) was higher than that of normal individuals (14.8%). This result is similar to that reported by Guo and Jia (1998) who studied cytomegalovirus infection in patients with diabetes mellitus and concluded that the positive rate of IgG against cytomegalovirus in diabetic is higher than that of normal individuals.

Our results reveal significant correlation between IgG of cytomegalovirus and type I diabetes mellitus in children (*P* value 0.025). This finding support the hyposis of association between cytomegalovirus and diabetes mellitus type I, and agreed with results of Hjelmesaeth et al. (2004), who found significant association between a symptomatic cytomegalovirus infection and increase risk of new onset diabetes mellitus, Nicoletti et al. (1990) also found a significant association between high titers of anti-cytomegalovirus IgG antibodies and anti islet cell antibodies (ICA), whereas Pak et al. (1988) reported strong correlation between cytomegalovirus genome and islet cell autoantibodies.

CMV might be involved in accelerating pancreatic failure to compensate for insulin resistance via at least two possible mechanisms. First, it could influence the pancreatic cells directly; secondly, it might act indirectly by influencing the immune system which in turn affects the pancreas. This is consistent with the first possibility which reported that CMV may infect and reside in pancreatic cells without causing cytopathic effects but nonetheless influencing insulin production directly after repeated reactivations (Reeves et al., 2005). Additionally, infection of human pancreatic β-cells with CMV induced the release of proinflammatory cytokines and increased cellular immunogenicity (Boppana et al., 1992).

The indirect effects of CMV could be exerted via infected monocyte production of IL-1β which induces TNF-α production in human pancreatic duct cells, driving cells into apoptosis and thus compromising β-cell function (Reeves et al., 2005). CMV seropositivity is associated with accumulations of potentially senescent late differentiated T-cells and elevated numbers of CD^{4+} and CD^{8+} effector cells (Doyle et al., 1996) which are more likely to produce pro-inflammatory cytokines (Aarnisalo et al., 2008). However, recently it was shown that human pancreatic β-cells are susceptible to CMV infection (Chen et al., 2012)

The present study displayed insignificant relation (*P* value 0.660) between cytomegalovirus IgG and family history to diabetes mellitus type I. Several studies were carried out to find the relation between cytomegalovirus and genetic susceptibility. Santos et al. (2000) did not get evidence of statistical interaction between cytomegalovirus antibodies and the DQB10201 allele or the DQB10302 allele. Nicoletti et al. (1990) reported insignificant relation between the presence of any HLA-A-B-C, DR antigens and the prevalence of anti-cytomegalovirus IgM and IgG antibodies and/or ICA.

Contrary to our finding, De Mattia et al. (1991) found significant increase of IgG anti-cytomegalovirus in female, where we reported slightly increased IgG anti-cytomegalovirus in female (22.4%) than male (21.9%) which was similar to findings of Kinpe et al. (2007). At the level of study population (study and control groups), the present study found increased seropositive in 10-15 years age group; similar observation was reported by Kinpe et al. (2007) and De Mattia et al. (1991).

The findings of the present study supported the hypothesis of association between CMV infection and diabetic mellitus type 1, however the number of studies on the possible connection between CMV and type 1 diabetes still remains limited and the results are controversial.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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