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Research Article

Coxsakie B virus among type 1diabetic Sudanese children

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ABSTRACT

Background: Type 1 diabetes is characterized by autoimmune destruction of pancreatic beta cells. Infection by Coxsackievirus B virus has been linked to the onset of type 1 diabetes; however its precise role has not been elucidated yet.

Objective: The aim of this study was to detect the seroprevalance of group B CoxsackievirusIgG antibodies, and to determine the relationship between the presence of antibodies and certain factors such as (Duration, family history, a report of symptoms).

Methods: A total of 54 blood samples (29 paediatric diabetic cases and 25 non diabetic children as controls) who attending to Omdurman Children Hospital, Ahmed Gasim Children Hospital, during the period from September – October 2014, were enrolled in this study. 54 serum specimens were collected from Paediatric diabetic and non-diabetic children, and analysed by ELISA technique.

Results: The results showed that in the whole study sample, there were 61% (n=33) group B CoxsackievirusIgG positive; there were 39% (n=21) group B CoxsackievirusIgG negative. Of the 29 diabetic cases, there were 68.97% (n=20) positive for group B CoxsackievirusIgG, and 31.03% (n=9) negative for group B CoxsackievirusIgG. On the other hand, of the 25 non diabetic controls, we found 52% (n=12) positive for group B CoxsackievirusIgG, and 48% (n=13) negative for group B CoxsackievirusIgG.

Conclusion: Statistical analysis showed that no association between a positive ELISA test in a diabetic child and a report of fever, reporting a family history, or longer duration of illness. Further research is needed on serological specimens with larger sample size.

Keywords: Type 1 diabetes; Coxsackievirus B virus; children; IgGantibodies

INTRODUCTION:

Diabetes mellitus (DM) is a chronic progressive metabolic disorder characterized by hyperglycaemia mainly due to absolute (Type 1 DM) or relative (Type 2 DM) deficiency of insulin hormone (1). DM virtually affects every system of the body mainly due to metabolic disturbances caused by hyperglycaemia, especially if diabetes control over a period of time proves to be suboptimal (2) . Until recently it was believed to be a disease occurring mainly in developed countries, but recent findings reveal a rise in number of new cases of type 2 DM with an earlier onset and associated complications in developing countries (3, 4) . Diabetes is associated with complications such as cardiovascular diseases, nephropathy, retinopathy and neuropathy, which can lead to chronic morbidities and mortality (5, 6) . World Health Organization (WHO) estimates that more than 346 million people worldwide have DM. This number is likely to more than double by 2030 without any intervention. Almost 80% of diabetes deaths occur in low and middle-income countries (7). Diabetes mellitus type 1 (T1D) is a form of diabetes mellitus. It is an autoimmune disease that results in the permanent destruction of insulin producing beta cells of the pancreas. An

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association between enterovirus (EV) infection especially coxsackie B virus and T1D, has been suspected for a long time(8). The aetiology of type I (insulin-dependent) diabetes mellitus is multi-factorial, comprising both genetic and environmental factors. Environmental risk factors include certain virus infections and dietary factors, but the mechanisms by which these may trigger B-cell damage are not known. Cow's milk proteins are among the strongest dietary risk factor candidates [9–11], while among all potentially diabetogenic viruses, enteroviruses have been the most suspected ones. Serological studies have indicated increased enterovirus antibody levels in patients with type I diabetes [12], and enterovirus RNA has been detected in the blood of subjects with type I diabetes more frequently than in control subjects [13–15]. Recent prospective studies have suggested that enterovirus infections can initiate and accelerate the B-cell damaging process years before the manifestation of clinical type I diabetes [16–20],and that in some cases this may happen already in utero [16,17,21].

In Sudan there was an increase in the incidence of diabetes in the recent years ; there were 3 million cases of diabetes in Sudan in 2014 according to International Diabetes Federation (IDF) (22).This study was done to explore the expected role of coxsackievirus in causing T1D as one of the environmental factor that might contribute in causing T1D among children in Sudan. This study aims to explore the serological evidence for the presence of group B coxsackievirusIgG antibodies.

Methodology

This was observational case _ control study which had been conducted in Khartoum state during period from September to October 2014, 54 samples were collected (29 paediatric diabetic cases and 25 non diabetic children as controls) , Data was collected by using direct interviewing questionnaire with children and their Parent ; ethical clearance was obtained from research ethical committee of Graduate College ; Al_Neelain University and ministry of health (Khartoum state_ Sudan), Parent of participants had signed their approval for the study.

Samples collection

Serums Blood samples were collected from 54 study population, under direct medical supervision by medial vein puncture was kept in -20°C till serological study was performed.

Specimens were processed by Enzyme linked immune sorbent assay ((ELISA) (SERION ELISAclassic) (Gentaur-Bilgum) Code: ESR134G, for detection of coxsakie B virus IgG.Enzyme linked immune sorbent assay for detection of anti coxsackie B virus IgG according to manufacturer's instructions: All reagents and samples were allowed to reach room temperature before use. The results were calculated by relating each sample optical density (OD) value to the Cut off value of plate.

Interpretation of Results

Negative results: samples giving absorbance equal to or less than lower Cut-off value are negative for this assay. Positive result: sample giving absorbance equal to or greater than upper Cut-off considered initially reactive. Borderline: sample with absorbance between the upper and lower Cut-off value are considered borderline and retesting of these samples in duplicate is recommended. Data analysis: Data was analyzed by R software, we use chi-square test in the analysis of categorical data (e.g. comparison of genders) and t test in the analysis of continuous data (e.g. the difference in term of age).ANOVA was used in the analysis of between groups differences (e.g. comparing the age classes in term of duration of diabetes).To calculate confidence intervals we used normal approximation of the natural logarithm of the odds ratio.

Results

A total of 54 blood samples (29 paediatric diabetic cases and 25 non diabetic children as controls) who attending to Omdurman Children Hospital, Ahmed Gasim Children Hospital, during the period from September – October 2014, were enrolled in this study.The mean age in the diabetes cases was 13.72 years, whereas the mean age for the non-diabetic controls was 9.88 years. The aim of this study was to detect the seroprevalance of group B CoxsackievirusIgGantibodies, and to determine the relationship between the presence of antibodies and certain factors such as Duration, family history, a report of symptoms).

Samples examined for the presence of IgG antibodies using ELISA technique. The results showed that, there were 61% (n=33) group B

CoxsackievirusIgG positive; whereas, there were 39% (n=21) group B CoxsackievirusIgG negative (fig.1). Of the 29 diabetic cases, there were 68.97% (n=20) positive for group B CoxsackievirusIgG, and 31.03% (n=9) negative for group B CoxsackievirusIgG. On the other hand, of the 25 non diabetic controls, we found 52% (n=12) positive for group B CoxsackievirusIgG, and 48% (n=13)negative for group B CoxsackievirusIgG (fig2). The odds for a positive group B CoxsackievirusIgG is 2.222 for a diabetic child, this is to be compared with odds of 1.083 for a healthy child. Therefore the observed odds ratio (OR) in our study sample for a positive group B CoxsackievirusIgG for diabetic children compared with healthy controls is 2.051. For further statistical analysis we assume normal distribution of the natural logarithm. The 95% confidence interval (CI) for the OR was calculated to be (0.675 to 6.231) and the P value = 0.205. This clearly accommodates the 'null OR' of 1. We have no sufficient evidence to reject the null hypothesis that diabetic children produce more positive IgG ELISA for group B Coxsackievirus than their healthy non-diabetic controls. We conclude that group B CoxsackievirusIgG are not different statistically between diabetic and non-diabetic children. (Table 1: tow by tow contingency table for odds ratio (OR) calculation).

Statistical analysis showed that no association between a positive ELISA test in a diabetic child and a report of fever (TABLE: 2 tow by tow contingency table for odds ratio (OR) calculation of fever) P = 0.4533, also statistically non-significant.Statistical analysis also showed that no association between a positive ELISA test in a diabetic child andreporting a family history (fig: 3) Amongst the 29 diabetic patients, 48.3% reported positive family history of diabetes (n=14), whereas 51.7% reported no such history (n=15). P = 0.3535) {Table: 5 tow by tow contingency table for odds ratio (OR) calculation according to family history}, also statistically non-significant, or longer duration of illness (TABLE 2: We reanalyse the data using only the odds of those children with long-term diabetes against the healthy controls. The OR was 2.769. P = 0.6167), clearly non-significant.

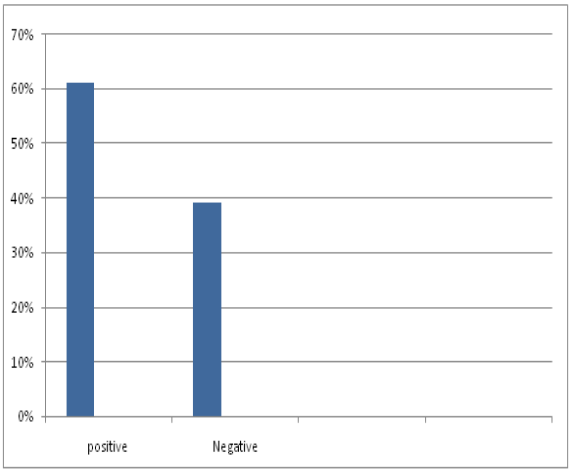

FIGURE1: SEROFREQUENCY OF COXSAKIE B VIRUS AMONG STUDY POPULATION (n=54)

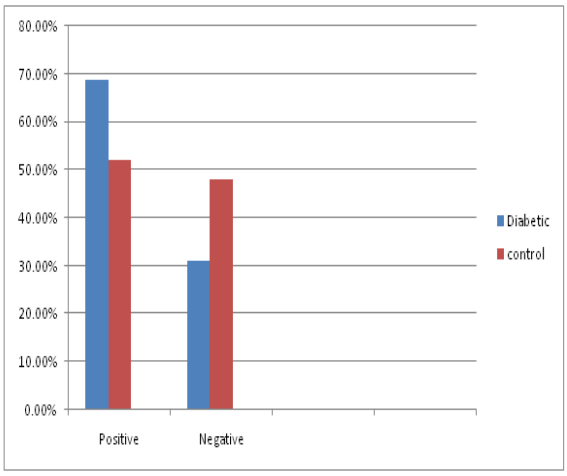

FIGURE 2: SEROFREQUENCY OF COXSAKIE B VIRUS AMONG DIABETIC PATIENT (n=29)

AND CONTROLS (n=25)

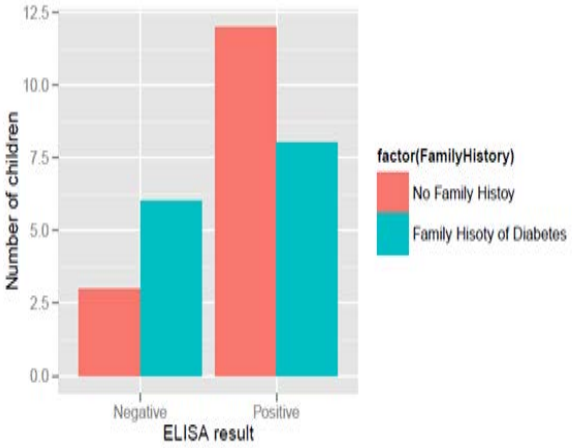

FIGURE 3: SEROPOSITIVITY OF COXSAKIE B VIRUS IN RELATION TO FAMILY HISTORY

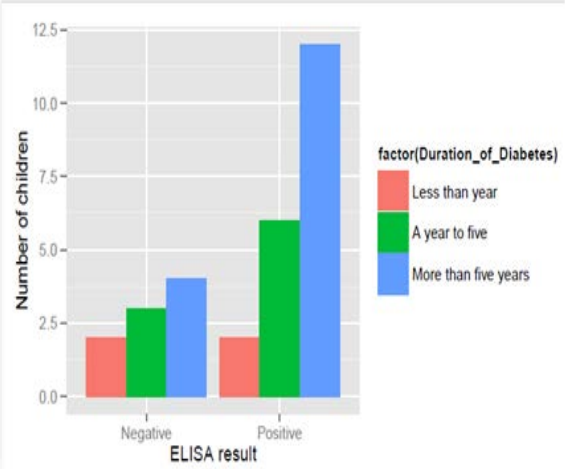

FIGURE 4: SEROPOSITIVITY OF COXSAKIE B VIRUS IN RELATION TO DURATION OF DIABETICS

Table 1: ODDS OF ELISA POSITIVE IN CASES COMPARED TO CONTROLS

	Positive	Negative	Total
Diabetics	20	9	29
Controls	13	12	25
Total	33	21	54

P value = 0.205(STATISTICALLY NOT SIGNIFICANT)

Table 2: DURATION OF ILLNESS AND POSITIVE ELISA RESULT

	Negative	Positive	Odds Positive
Less than 1 year	2	2	1
1 to 5 year	3	6	2
More than 5 year	4	12	3

Table 3: CALCULATION FOR LONG-TERM DIABETICS COMPARED WITH CONTROLS

	Positive	Negative	Total
Long-term Diabetics	12	4	16
Controls	13	12	25
Total	25	16	41

Tow by tow contingency table for odds ratio (OR) calculationfor long term diabetics p value =0.6167

Table 4: FEVER AND ELISA POSITIVE TEST

	Positive	Negative	Total
Fever	1	2	3
Asymptomatic	19	7	26
Total	20	9	29

Table 5: FAMILY HISTORY AND ELISA POSITIVE TEST

	Positive	Negative	Total
Family History	8	6	14
No Family History	12	3	15
Total	20	9	29

DISCUSSION

In our work coxsackievirus antibodies had been detected with ELISA, for IgG 68.97% were found positive while 31.03% were negative for paediatric diabetics. While for controls 52% were found positive and 48%were negative. This indicates the wide spread of coxsackievirus in Sudan and it may have a significant role in causation of T1D. similar results were found in previous study carried also in Sudanshowed 45% positive for IgG(23).which indicate high prevalence of coxsackievirus within T1D. another study conductedin Sweden in 1982 ; Found that 33% positive cases for IgM in T1D children (10). It was noticed from the results obtained in this study that out of 19 samples tested for IgG and IgM, some samples were positive only for IgG, some were positive for IgG while IgM was at the border line, only one sample was positive for IgM but negative for IgG, this is expected as IgM usually appears early in infection and disappear after few months while IgG appears later and persists for a long time. Another international study done at Department of Virology, University of Tampere, Tampere, Finland that evaluates the association between specific enteroviruses (EV) subtypes and type 1 diabetes by measuring type-specific antibodies against the group B coxsackievirus (CVBs), which have been linked to diabetes in previous surveys. Altogether, 249 children with newly diagnosed type 1 diabetes and 249 control children matched according to sampling time, sex, age, and country were recruited in Finland, Sweden, England, France, and Greece between 2001 and 2005 (mean age 9 years; 55% male). Antibodies against CVB1 were more frequent among diabetic children than among control children (odds ratio 1.7 [95% CI 1.0-2.9]),the results support previous studies that suggested an association between CVBs and type 1 diabetes, highlighting the possible role of CVB1 as a diabetogenic virus type (24).

The results of thiscurrent study indicated the high prevalence of coxsackievirus within T1D patients at

Khartoum State and also among non-diabetics children.The proportion of diabetic children who tested positive was 69% a little but not significantly larger than the 52% of non-diabetic children who tested positive on the same test that mean no association between the diabetes status and a positive ELISA test.

This is one of few reports for the detection of coxsackievirusIgG antibodies in T1D children patients in Sudan, further studies for the detection and characterization of coxsackievirus antigen as well as its association with T1D is highly recommended. Advance in molecular and genomic studies may facilitate the identification of association at earlier stage of autoimmunity.

Conclusion

The wide spread of coxsackievirus in Sudan it may suggest a link between enterovirus infections and type 1 diabetes. Further research is needed on serological specimens with larger sample size for the identification of coxsackievirus as a potentially diabetogenic virus to explore the mechanisms of enterovirus-induced diabetes and may also open the door for the development of an enterovirus vaccine against the disease.

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Conflict of interest

The authors have no conflict of interest to disclose.

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