# Epidemiology of Type 1 Diabetes Mellitus Among Children in Sudan: Serological Evidence of Coxsackievirus Infection

## Emad M.A[1]., Ali Y.H[2], Enan, K.A.[3]

[1]Arkawit Medical Center, Khartoum, Sudan
[2]Central Veterinary Research Laboratory, Virology Department, P.O. Box 8067, Khartoum, Sudan
[3] Central Laboratory, Ministry of Science and Technology Khartoum, Sudan.

**ABSTRACT**: Epidemiological study on Diabetes mellitus in Sudan was conducted through the collection of data for three years (2005-2007) about the incidence of diabetes mellitus. It was noticed that there was a marked increase in the numbers of affected patients, the reported cases for 2005 were 83702 and for 2007 were 97399. Diabetes mellitus was found to be one of the top 10 diseases which leads to hospital admission. Type 1 diabetes mellitus cases among children is increasing, where it was 2839,3152 and 3524 during 2005,2006 and 2007, respectively. Detection of coxsackievirus antibodies IgG and IgM are studied in a group of T1D children patients at Khartoum state. The samples were collected during July and September 2008, from different five medical centers and hospitals in Khartoum state. A total of 101 Plasma samples were collected. The test result for IgG have shown 45% positives and 8% positives for IgM. The positive results were found among a group of patients with very low socio-economic status. This is the first report for the detection of coxsackievirus IgG and IgM in children with type 1 diabetes mellitus in Sudan.

**KEYWORDS**: T1D, infants, IgM, IgG, Sudan.

## INTRODUCTION

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. T1D is lethal unless treatment with exogenous insulin via injections replaces the missing hormone (Dooner et al., 2007).

An association between enterovirus (EV) infection especially coxsackie B virus and T1D, has been suspected for a long time (Jaïdane and Hober, 2008). Increased prevalence of EV IgM antibodies have been reported in newly diagnosed TID patients (King et al., 1983, Frisk et al., 1992). A few case studies reported that TID developed after EV infection (Lonnrot et al.,1998,

Yin et al., 2002,). EV infection have been shown coincide with the appearance of auto- antibodies against islet cell protein (Hyoty et al., 1995 ). It has been suggested that EV infection may initiate the process leading to islet cell destruction several years before clinical diabetes appears (Hyoty et al.,1995). EV have also been isolated from newly diagnosed T1D patient in a few cases (Yoon et al., 2005)  and some of the isolated virus strains have been shown to cause diabetes in animal models (Yoon, 1995). More evidence for the involvement of the EV infection in T1D is the presence of EV RNA in the pancreatic islet of a few T1D patients (Ylipaasto et al., 2004).

In Sudan there was an increase in the incidence of diabetes in the recent years (Anon 2007).  During 2006 the

reported diabetes mellitus cases were 14677, the number was increased during 2007 to 17905 (Anon, 2007). No 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 prevalence rate of T1D in Sudan and to investigate the serological evidence for the presence of coxsackievirus infection.

## MATERIALS and METHODS

**Area of study:** Five areas in Khartoum state has been selected for this study. These areas are Jabir AbulEz Diabetic Specialist Center, Dr.Jaffar Ibn Oaf Children Specialist Hospital, Omdurman Children Hospital, Ahmed Gasim Children Hospital and Buluk Children Hospital.

**Data collection:** The data about the reported cases of diabetes mellitus in Sudan was obtained from the annual reports of the Ministry of Health during the year 2005 up to 2007, the data was statistically analyzed.

By the aid of questionnaire applied to diabetic patients, information about the incidence of the disease has been collected. Data included history of the diseases associated with family history of T1D, symptoms and treatment and how they noticed about their child illness and seasonality of the infection along with previous viral infection prior to diabetes infection. The presence of T1D child patient in the same family or within the area or locality they live was highlighted.

A total of 82 questionnaires were collected, data were statistically analyzed using SPSS software programme. Additional 50 questio-nnaires without sample collection were also collected for epidemiological analysis.

**Sample collection and storage:** This work has been approved by the Ethical Committee of the Tropical Medicine Research Institute. All participants has signed their approval for the study.

A total of 82 samples of blood serum or plasma (EDTA or Heparin or citrate) have been collected from children at different children hospitals and health Centers. These blood samples were centrifuged at 2000 rpm for 3 minutes and then separated by the use of automatic pipette into new plain container with proper labeling for the sample identification. All these samples were then stored at -20 C$^{°}$ till examination.

The samples collected were divided into IgG and IgM groups according to the duration of the disease where newly diagnosed T1D were selected for IgM test, six months and up to one year were selected for both IgM and IgG test and more than one year has been selected only for IgG test, the total numbers for IgG tests were 75 and 26 for IgM.

**Detection of coxsackievirus IgG/IgM antibodies:** Serion cosackievirus IgG/IgM are quantitative and qualitative tests for detection of human antibodies in serum or plasma against Coxsackievirus. The Kits were obtain-ned from Virion\Serion GmbH, Würzburg, Germany. The test was performed according to the manufa-cturer instructions.

## RESULTS
### Incidence of diabetes mellitus in Sudan:
According to the system of disease reporting in Ministry of Health, the top ten diseases leading to hospitalization are listed. Comparative analysis of the data for the year 2007 has shown that diabetes mellitus was found to be one of the ten leading diseases of hospital

admission with increased prevalence rate (2.1%) when compared to 2005 and 2006 which indicate an increase in the prevalence of the disease in the recent year.

The results showed that there was high incidence of the disease in certain states such as Khartoum which shows high case number and prevalence, followed by Northern State and Red sea, the results are shown in Table 1. Statistically there was a significant difference between the states (P = 0.00).

**Laboratory confirmed cases of diabetes mellitus in Sudan:** The reported positive cases of diabetes in Sudan slightly increased in 2007 (97399) compared to 2005 (83702). Most of the cases had been reported at Khartoum state then Gezira and River Nile, the details are presented in Table 2.

**Epidemiology of T1D among children in Sudan:** Data obtained from the annual reports of the Ministry of Health has showed that 1313 cases of diabetes were among males in the age group less than 1 to 14 years with 32 deaths in 2005 when compared with females of the same year which showed 1477 with 17 deaths. In 2006 the number of cases raised up to 1427 with 23 deaths. Female cases during the same year showed 1632 cases with 25 deaths. In 2007 the number jumped to 1652 cases with 26 deaths, female cases raised to 1822 cases with 24 deaths, this data are clearly represented in Table 3. Although the number of the affected females was more than the males, there was no significant difference between them in getting the infection by coxsackie virus (p = 0.780)

**Epidemiological parameters of T1D in the studied group:** Data obtained from medical statistics departments at the studied areas about the registered cases for routine follow up of T1D among children in the age group 1 to 18 years showed that 2234 cases are registered, of those 751 cases were registered at Jabir Abu- Eliz diabetic specialist Center.

The entire participants are type one diabetes mellitus under insulin treatment. According to the data collected from the questionnaired T1 patients in this study, it was noticed that 54.2% were Females while 45.8 were Males. The age range was from 2 to 18 years. The onset of the disease varies from new cases less than 1 month, cases less than 6 months, and more than 6 months less than 3 years which show 10%, 21% and 69%, respectively (Table 4).

**The socio- economic status of T1D studied patients:** The classified 4 groups of socio- economic status of diabetic patients which are, very good, middle, low and very low were found to be 5%, 10%, 17% and 67%, respectively (Table 5). Most of the positive results are among low and very low groups, meanwhile there was no significant differences between the socio-economic status and coxsackie virus infection (p = 0.283).

**Seasonality of the T1D:** Seasonality of the T1D has showed 39% cases in summer, 54% in winter and 7% in autumn (Table 6).

**Family history of T1D:** Almost 55% of the participants stated that there was no family history of both T1D and type 2 diabetes mellitus against 45% whom they had family history of diabetes mellitus. The type of relationship for

those who had history of the disease in their families was showed 5% for fathers, 8% for mothers, 45% for

relatives (Table 7). However we found a significant relationship between IgG antibodies with family history (p = 0.016).

**Past viral infection prior to T1D:** Investigation about past viral infection for measles and mumps prior to T1D showed 15% and 3%, respectively. Questions for other viral infection showed 9% for varicella (chicken box) (Table 8). Some of them complained about chronic tonsillitis, fever with unknown origin and malaria.

10% of the participants declared the existence of children with T1D either in their families or in their localities.

100% of these participants are presented by polyuria, thirst, weight loss and fatigue, 18% of them got admitted to the hospital with diabetic keto acidosis (DKA). Most of these cases were diagnosed immediately according to their clear clinical sign and feature of diabetes and few of them were diagnosed late. 90% of them complained about the deteriorated hygiene system, garbage, undrinkable water and plenty of house flies.

**Detection of coxsackievirus antibodies:** Cosackievirus IgG and / or IgM antibodies were examined in plasma samples collected from T1D patients. 18 males were found positive against 19 females for IgG. Only 1 female sample was positive for IgM. The age group for positive cases ranged from 4 to 19 years. The test results for IgG have shown 45% positives where 6% were at the border line. In IgM testing the results were 8% positive and 46% were at the border line (Table 9).

A total of 19 samples were tested for IgM and IgG , no samples were positive for IgM only , 3 samples were positive only for IgG , while no samples were positive for both. 3 samples were strongly positive for IgG where IgM were on the border line. 3 samples were negative for IgG where IgM were on the border line; only one case was positive for IgM but negative for IgG.

**DISCUSSION**
The data collected from the Ministry of Health over the last three years (2005-2007) has showed a marked increase in the prevalence of diabetes mellitus in Sudan in the recent years which is coincide with the prevalence of the disease all over the globe (Huizinga and Rothman, 2006).

Many factors can contribute in increasing the rate of diabetes, viral causes are one of them; bad hygiene observed may be the main cause of this increase. It was observed that Khartoum state usually reporting the highest figures of diabetes mellitus in Sudan, the high incidence at Khartoum state could be due to the high rate of immigration from rural areas to big cities and most people usually visits Khartoum seeking medical care; most of diabetic patients investigated were from the low socioeconomic status group in which high rate of enterovirus infection is expected. The high prevalence of diabetes in northern states and red sea should be investigated, statistically there was a significant differences between the states (p = 0.00).

From the questionnaired patients we observed that female patients are more than male patients which coincided with the Ministry of Health reports, however there was no significant difference between them in getting the

infection by coxsackie virus (p = 0.780).

The socio-economic status of the majority of the study group was very low and this can clearly reflects the surrounding bad hygienic situation among these children where a fertile environments for entroviruses is suitable, where the viruses is easily got transmitted through fecal oral rote. Although most of the positive results are among this group there is no significant differences between the socio-economic and coxsackie virus infection (p = 0.283).

The seasonality in tropical areas of T1D is coinciding with enteroviruses infection internationally in different studies as well in our study where most of these children got the disease in winter season (Terri et al., 2002).

The family history of the disease in the studied groups reflected the minor role of genetic association of T1D within the study group where most of these patients are not genetically associated with the disease; this led us to investigate about the environmental factor especially viral diseases which is the ignored area in clinical investigation in Sudan.

The association of past viral infection in the studied groups (Mumps and Measles) showed the possibility of the association of other viral disease with T1D, as the previous infection of measles and mumps within the T1D affected group was15% and 3%, respectively. We high lights on measles and mumps duo to a considerable results we found in this study, other viruses should be investigated. Few of these patients got chiken box prior to T1D where research should be carried out to explore its role in this disease.

Chronic tonsillitis is a very common complain among children as well as in our study group where coxsackievirus as well as enteroviruses infection plays an important role in this respect, as this virus can cause chronic pharyngitis or tonsillitis when they got misdiagnosed as bacterial infection.

Viruses have long been considered as a major environmental factor in the etiology of Type I (insulin-dependent) diabetes mellitus and recent work has greatly confirmed and extended this role.

In addition to the enteroviruses, there are several other viruses which, from time to time, have been considered as potential causal agents for human diabetes. With the exception of rubella, their role is not clear.

The relation of enteroviruses with Type I diabetes has only been properly clarified by the use of new technologies, especially those based on polymerase chain reaction methods to identify them in blood. It is now evident from studies in several countries that enterovirus infection accompanies or precedes the onset of diabetes in many children. It is less certain whether this is true for older persons or for other types of diabetes (Hyöty and Taylor, 2002). Enterovirus infection in pregnancy has also been suggested to cause diabetes in children.

The infection with enteroviruses seems to be linked to the induction of islet-cell auto-antibodies as well as to the expression of interferon-α. Both of these events are connected with islet-cell destruction.

It has become increasingly important to establish the nature of the infecting virus in the early stages of diabetes. It seems likely that a number of viruses of the coxsackie or echovirus types are involved, although the nature of the nucleotide sequences responsible for diabetogenicity remains elusive (Hyöty and Taylor, 2002).

In our work coxsackivirus antibodies had been detected with ELISA, 45% were found positive for IgG while 6% were at the border line. In IgM detection, 8% were found positive whereas 46% were at the border line. This indicates the wide spread of coxsackievirus in Sudan and it may have a significant role in causation of T1D.

The same work was conducted by King and his co workers in Sweden in 1982 they

Found 33% positive for IgM in T1D children (King et al., 1983). 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.

The results of this study indicated the high prevalence of coxsackievirus within T1D patients at Khartoum State, the detection of IgM antibodies may indicate the association of this virus with T1D.

This is the first report for the detection of coxsackievirus IgM and IgG antibodies in T1D children patients in Sudan, further studies for the detection and characterization of coxsackivirus antigen as well as it's association with T1D is highly recommended.

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Table 1: The incidence of diabetes mellitus in hospitalized patients in Sudan during 2006-2007.

| States | 2006 | %* | 2007 | %* |
|--------|------|-----|------|-----|
| Khartoum | 8089 | 2.00 | 8576 | 2.2 |
| Gazera | 1975 | 1.6 | 2125 | 1.6 |
| B.Nile | 280 | 1.2 | 246 | 1.1 |
| W.Nile | 306 | 1.1 | 642 | 1.7 |
| Sinnar | 340 | 0.9 | 449 | 0.9 |
| R.Nile | 461 | 1.7 | 587 | 1.9 |
| Northern | 923 | 3 | 1051 | 2.9 |
| Red sea | 563 | 1.8 | 1560 | 4.7 |
| Gadarif | 604 | 1.4 | 558 | 1.4 |
| Kasala | 218 | 0.9 | 635 | 1.8 |
| N.Kordfan | 572 | 1.7 | 811 | 1.5 |
| S.kordfan | 22 | 0.2 | 107 | 0.8 |
| N.Dafor | 84 | 0.8 | 75 | 0.5 |
| S.Darfor | 135 | 1.3 | 286 | 1.8 |
| W.Darfor | 105 | 1.2 | 149 | 0.8 |
| Total Sudan | 14677 | 1.8 | 17905 | 1.9 |

**\* Percentage of diabetic patients out of total hospitalized patients**

Table 2: Reported laboratory confirmed diabetic cases in Sudan during 2005 and **2007.**

| State | Year 2005 | Year 2007 |
|---|---|---|
| Khartoum | 32510 | 35337 |
| Gazera | 25099 | 26926 |
| B.Nile | 1922 | 1014 |
| W.Nile | 753 | 3106 |
| Sinnar | 3202 | 6337 |
| R.Nile | 11359 | 5446 |
| Northern | 3537 | 7732 |
| Rd sea | 584 | 4325 |
| Gadarif | 2306 | 4919 |
| Kasala | 1934 | 610 |
| N.Kordfan | 37 | 378 |
| S.kordfan | 17 | 282 |
| N.dafor | 442 | 587 |
| S.Darfor | - | 143 |
| W.darfor | - | 257 |
| T.Sudan | 83702 | 97399 |

Table 3: Reported cases and deaths of diabetic children in Sudan (2005-2007).

| year | Total diabetic | | Deaths | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| 2005 | 1313 | 1477 | 32 (2.4%) | 17 (1.1%) |
| 2006 | 1427 | 1632 | 23 (1.5%) | 25 (1.5%) |
| 2007 | 1652 | 1822 | 26 (1.5%) | 24 (1.3%) |

Table 4: Distribution of T1D patients according to medical centers, hospitals and number of samples collected from each.

| Hospital / Medical center | Patients Registered for follow up | Patients sampled | Sex of sampled patients | |
|---|---|---|---|---|
| | | | Male | Female |
| Jabir Abo-eliz diabetic specialist center | 751 | 45 | 20 | 25 |
| Dr.Jaffar Ibn Oaf children hospital | 500 | 9 | 4 | 5 |
| Omdurman children hospital | 433 | 16 | 8 | 8 |
| Buluk children hospital | 200 | 9 | 4 | 5 |
| Ahmed Gasim children hospital | 350 | 3 | 1 | 2 |
| Total | 2234 | 82 | 45% | 55% |

Table 5: The socio-economic status of T1D studied patients

| Number Tested | | Scio-economic status | % |
|---|---|---|---|
| 4 | | Very good | 5 |
| 8 | | Middle | 10 |
| 54 | | Low | 67 |
| 16 | | Very low | 18 |
| Total | 82 | | 100 |

Table 6: Seasonality of T1D in Sudan (2008).

| Season | No. | % |
|---|---|---|
| Summer | 32 | 39 |
| Winter | 44 | 54 |
| Autumn | 6 | 7 |
| Total | 82 | 100 |

Table 7: Family history among T1D Patients

| Family history of diabetes mellitus | % |
|---|---|
| Father | 4 |
| Mother | 8 |
| Strong relation | 45 |
| No relation | 43 |

Table 8: Past viral infection prior to TID infection

| Past viral infection | % |
|---|---|
| Measles | 15 |
| Mumps | 3 |
| Varicella (chicken box) | 9 |

Table 9 : Coxsackievirus antibody detection by indirect ELISA method in T1D patients in Sudan.

| Coxsackievirus IgG | | | | Coxsackievirus IgM | | | |
|---|---|---|---|---|---|---|---|
| Total tested | Positive | % +ve | %Border line | Total tested | Positive | % +ve | %Border line |
| 76 | 37 | 46% | 6% | 25 | 2 | 8% | 46% |