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Helicobacter pylori infection and the onset of type 1 diabetes mellitus in Sudanese children

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ABSTRACT

Type 1 diabetes mellitus (T1DM) is a chronic T cell mediated autoimmune disease that results in destruction of pancreatic islet cells. Helicobacter pylori (HP) was recently thought to be a triggering factor for T1DM. This is a prospective case control study at Gaafar Ibnauf Children's Hospital and three other diabetic clinics in Khartoum, during the period January-September 2012. Ninety newly diagnosed T1DM children and a similar number of a control group were compared. Assessment of HP specific serum immunoglobulin was performed using Eliza test. There were 40(44.4%) female and 50(55.6%) male diabetic children. Diabetic children tested positive for HP constituted 56/90 (62.2%) compared to 59/90 (65.6%) from the control group. Diabetic children aged 11-18 years represented 46 (51%), 32/46 (57%) of them were seropositive for HP. A similar number of the same age in control group 30/46 (50.8%) were seropositive. Of 41newly diagnosed diabetic children (44.4%) of newly who complained of symptoms, 30 (53.6%) were seropositive for HP compared to 34 (37.7%) among the healthy children, out of whom 24 (40.7%) tested positive for HP. Diabetic children with moderate anemia were 35 (45.5%) compared to 54 (60.0%) in the control group. Seropositive children for HP in the 2 groups were, respectively, 20(40.8%) and 38(64.4%). Those with a poor family background were 28 (56%), 20 (40.0%) tested positive for HP, compared to 38 (64.4%), of whom 20 (64.5%) were seropositive in the controls. In conclusion, HP infection does not seem to play a role in triggering T1DM in children.

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion or its action or both. The first known mention of diabetes symptoms was in 1552 B.C by an Egyptian physician. In 150 A.D, Arateus described the disease as melting down of flesh and fat into urine. It wasn't until the 1800s that scientists developed chemical tests to detect urine sugar [1].

The incidence of childhood T1DM disease is rising worldwide, with reported annual increases of 2-5 % in Europe, the Middle East and Australia [2,3]. The rise is primarily in young children. Prevalence of cases in children under 15 is expected to rise by 70% in the coming years until 2020 [4,5].

Helicobacter pylori is a spiral shaped, microphilic, gram negative bacterium measuring approximately 3.5 micron in length and 0.5 microns in width. In vitro it is a slowly growing organism that can be cultured on blood agar or selected media such as skirrow. It specifically colonizes the gastric epithelium producing urease, catalase and oxidase, which are known to play a role in the pathogenesis of chronic gastritis and peptic ulcer disease, they may be present in more than one half of the people in the world according to the Mayo Clinic [6]. It was first described in the human stomach 100 years ago by a polish Clinical Researcher Prof W. Jaworaski at the Krakow Jwagielonian University. Later, in late 1970s, Robin Waren, a pathologist in Perth noticed pathogenic changes in gastric epithelium on histological specimens [7]. In Europe, the prevalence of HP seems to be lower in Northern countries than in Southern and Eastern countries, and an estimated prevalence of 32% was reported [8]. Asian studies published in 2013-showed high prevalence rates of 54% -76%, however lower incidence of 28% was reported among healthy individuals in Saudi Arabia [9,10]. New data have also been published from African countries reporting a prevalence of HP of 65% -75% [11,12]. The prevalence of HP is about 30% in developed and up to 80% in developing countries [13].

In Sudan, a study done by Abu-Median and Mirghani [14] in 100 children reported 51 % to be seropositive. The risk of acquiring HP infection is related to socioeconomic status, density of housing, overcrowding, family education and lack of running water [15]. The disease is transmitted through fecal oral route, and children who regularly swim in rivers, streams, pools drink stream water or eat uncooked vegetables are more likely to be infected [16]. Infected individuals are more likely to have infected spouses and children than uninfected individuals [17,18]. Organisms have also been identified in dental plagues [19]. Moreover, Infection has been documented following the use of a variety of disinfected gastric devices and endoscopes [20].

Pathophysiology of HP is induced through bacterial attachment with subsequent tissue injury and release of enzymes, which can cause cellular damage by direct or indirect mechanisms [21-24].

A study done in Sacramento in 1998-1999 demonstrated for the first time that HP leads to an increased rate of incidence of diabetes in a prospective cohort study, their findings were implicated that a potential role for antibiotics and gastrointestinal treatment in preventing diabetes [25].

In addition to the known role of HP in causing chronic gastritis, the pathogen has also been considered a potential trigger in autoimmune gastritis. However, a considerable number of reports has attempted to link pylori infection with the development of over hundred extra -gastrointestinal autoimmune disorders including Sjogren syndrome, rheumatoid arthritis, autoimmune liver diseases, systemic lupus erythematosus, vasculutides, thrombocytopenic purpura, thyroiditis and diabetes mellitus [26]. Moreover, emerging data now indicate a strong relationship between HP and the development of type 2 diabetes mellitus [27].

In a study in Egypt to evaluate antithyroid peroxidase (anti TPO) and antithyroglobulin (antiTg) auto antibodies with anti HP IgG and IgA in young patients with T1DM, the seroprevalence of HP was significantly higher in patients with T1DM than in healthy controls; 79% vs. 51.2% (p< 0.001) [28]. In a study in Iran, seropositivity for HP was detected in 22.7 % of diabetic patients compared to 17.3% of the controls (P > 0.05). It was concluded that T1DM is not associated with an increased risk for HP infection [29]. Another study conducted in Pediatric University of Catholic, Italy, concerning HP and gastrointestinal symptoms, metabolic control in young T1DM patients using c-urea breath test in 121 T1DM patient, they found that glycosylated HbA1c, insulin dose and disease duration are not affected by infection and they concluded that HP infection doesn't affect metabolic control in T1DM [29]. In Louisiana State University, investigating the prevalence of HP in diabetic patients and possible role of infection in their metabolic control, it was found that HP infection is associated with increased levels of HbA1c in diabetic patients [30]. In another study among diabetic children in Turkey they used Elisa for anti IgG, the result was that the rate of HP seropositivity was significantly different (p, 0.05) between the diabetics (47.5%) and the controls (18.9%). There were no statistical differences between HP positive and negative diabetics for HbA1c and daily insulin dose. But seropositivity increased with disease duration [31].

MATERIALS AND METHODS

This is a prospective hospital based case- control study conducted in the period from January 2012- July 2012. Children were selected from 3 diabetic clinics in Khartoum Province: Jabber Aboaliz, Omdurman and Ahmed Gasim diabetes clinics. We included all children in the age group from 1 – 18 years with newly discovered T1DM (duration < 6 month), children with other types of diabetes or with other chronic diseases were excluded from the study.

A predesigned questionnaire was completed including personal data name, age, gender race, residence and socioeconomic status, clinical information, weight, height, BMI, Tanner staging, pallor, abdominal distention and epigastric tenderness. RBS and urine for sugar and acetone at time of diagnosis, HB and Eliza IgG antibody test for Helicobacter pylori infection. Results were then presented.

We included 90 newly discovered T1DM children and 90 healthy children matched for age and sex as control group. Verbal consent was taken from each child and his family to be included in the study and children who were found to be seropositive received treatment and their families were counseled. The job of filling the questionnaires, conducting clinical examination and collecting blood samples was fully done by the author. The collected data was analyzed by computer using Statistical Package for Social Sciences (SPSS) version 17. Level of significance was considered if P value < 0.05.

Ethical Approval: Ethical approval was obtained from Sudan Medical Specialization Board. Confidentiality was maintained throughout. Informed verbal consent was obtained from parents or caregivers.

RESULTS

Ninety newly discovered T1DM children and the same number as control group were investigated, serum immunoglobulin antibody for HP among diabetic children was positive in 56(62.2%); seronegative children were 31(34.4%). While in the control group seropositive and seronegative children were 59(56.6%) and 31(34.4%) respectively, three children of the diabetics (3.3%) were borderline (Table 1), the variation between study and control group was not found to be statistically significant (P= 0.21). In

the study group males were 40(44.4%) out of them 20(50%) were seropositive, females constituted 50(55.6%) with 30(50%) seropositive. The same percentage was found in the control group,

The most affected age group in the study group was 11-18 years 46(51.1%) children, among them 32 (69.5%) were seropositive. Diabetic children aged 7-10.9 years were 21(23.3%) with 13(61.9%) seropositive subjects, while those in the age group from 3-6.9 years 14 (15.6%); seven of them (50%) were seropositive. Diabetic children in the age group less than 3 years were nine and four of them (44.4%) had positive titers.

Similarly in the control group, 46 (50.8%) were in the age group 11 -18 years, and thirty of them (65.2%) were seropositive. The age groups 7-10 years and 3- 6.9 years constituted 21(23.3%) and 14 (15.6%) respectively. Seropositive subjects represented 14(66.6%) of the former and 10(71.4%) of the latter. The age group less than 3 years was 9(10%) children, five (55.6%) of them were seropositive. variation between study and control group was not found to be statistically significant (P: case = 0.25), (P: control=0.8).

Khartoum Province was the residence of 62(68.8%) of the diabetic children, out of them 38(61.2%) significant were seropositive. On the other hand 60(66.6%) from the control group were from Khartoum, 38(63.3%) of them proved to be seropositive for H.pylori infection (Figure 1). The difference between the 2 groups was not found to be statistically significant (P: case =0.95), (P: control= 0.53)

Abdominal distention was found in 5(5.5%) of the diabetic children, out of them 2 children (40%) were positive for HP infection. The same number from control group had abdominal distention, 3(60%0) of them were seropositive.

Poor family background was found in 50(55.5%) of the diabetic children, seropositive titers were found among 28(56%) of them (P=0.05) clearly shown in (Table 2a). On the other hand, 58(64.4%) of the controls had low socioeconomic status and 38(65.5%) had significant titers for HP infection, statistical difference was not found to be significant, (P=0.9) [Table 2b].

Pallor was detected in 32(35.6%) of the diabetics and 30(33.3%) of the controls; positive serology was significant in 22(68.75%) and 19(63.3%), respectively. Hemoglobin levels among the diabetic children varied from 5-7 g/dL in 23 (25.5%) out of them 15(65.2%) were seropositive; 8-10 g/dL in 35(38.8%) with seropositive titers in 20(57.1%) and levels more than 10 g/dL in 32(35.5%) patients among which 14 (43.7%) had seropositive titers, (P= 0.2). In the control group similar levels were prevalent in 10(11.1%), 54(60%) and 26(28.9%), seropositive titers were found in 4(40%), 38(70.3%) and 17(65.38%) respectively, (P= 0.21).

Random blood glucose readings (RBS) at presentation was less than 200 mg /dL in one patient (1.1%) who proved to be seropositive, levels of 200-300 g/dL were reported in 20 (22.2%) with seropostive titers In 14(70%) patient and those with readings more than 300 g/dL were 68(75.5%) patient out of them 41(60.29%) were found to have significant titers for HP infection.

Table 1 - Serum immunoglobulin G antibody to Helicobacter pylori among the study and control group

Result	Study populationTotal		Total
	Case	**Control**	
Positive	56(62.2%)	59(65.6%)	115(63.9%)
Negative	31(34.4%)	31(34.4%)	62(34.4%)
Borderline	3(3.3%)	0(0.0%)	3(01.7%)
Total	**90(50.0%)**	**90(50.0%)**	**180(100.0%)**

P value = 0.21

Table 2a - Distribution of the diabetic children according to socioeconomic status

	Serum IgG antibodies			
Socioeconomic	**Positive**	**Negative**	**Borderline**	**Total**
Moderate	21(52.5%)	19(47.5%)	0(0.0%)	40(44.4%)
Low	28(56%)	22(44%)	0(0.0%)	50(55.5%)
High	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Total	**49(54.4%)**	**41(45.5%)**	**0(0.0%)**	**90(100.0%)**

P value = 0.05

Table 2b - Distribution of the control group according to socioeconomic status

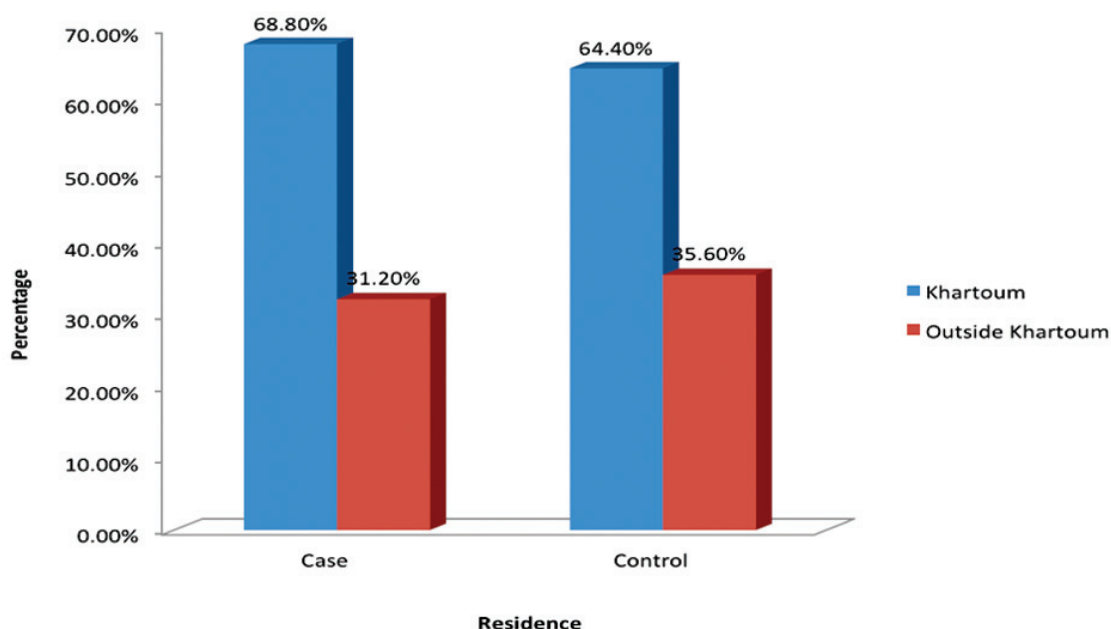|	Serum IgG antibodies			
Socioeconomic	**Positive**	**Negative**	**Borderline**	**Total**
Moderate	21(65.6%)	11(34.4%)	0(0.0%)	32(35.6%)
Low	38(65.5%)	20(34.4%)	0(0.0%)	58(64.4%)
High	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Total	**59(65.5%)**	**31(34.4%)**	**0(0.0%)**	**90(100.0%)**

P value =0.9

Figure 1 - Distribution of the diabetic children and the control group according to residence

P value: Case = 0.95
P value: Control =0.5

DISCUSSION

Helicobacter pylori (HP) is one of the most common human bacterial pathogens and infection causes a wide array of gastric disorders, including simple gastritis, peptic ulcers and gastric malignancies. Gastrointestinal inflammation caused by HP can influence the absorption of glucose and lipids, which are also abnormal in diabetes mellitus. Production of proinflamatory cytokines may exert effects in remote tissues and organic systems and result in extra gastric manifestations.

This study is a prospective hospital based study that represents the first study in Sudanese diabetic children, which evaluated HP as triggering factor for the development of T1DM. Ninety newly discovered T1DM children and an equal number of children for control were investigated for HP infection using Eliza immunoglobulin G- antibody (sensitivity 92% / specificity 83%).

Almost two thirds of both diabetic and control groups were found to be seropositive for HP, this prevalence was lower than records from developing countries and higher than previously reported in Sudanese subjects [7,8]. Our study showed insignificant difference between serologically positive and serologically negative subjects for HP among both test and control groups, the same result was concluded in an Italian study, also similar results were found in Iranian diabetic patients [29,30].

However, our results were contradictory to that found by Egyptian and Turkish researchers who observed higher titers for HP among diabetic children compared to controls [28,30]. These differences can be attributed to the fact that in this study newly discovered patients were enrolled, while they included patient with longer disease duration which can cause delayed gastric emptying and motility with eventual increased colonization of HP organism. Moreover, they used urea breath test as confirmatory test with 95%sensitivity and 96% specificity, upper

GI endoscopy and serological IgA antibody test. The discrepancies reported concerning the association of HP infection and T1DM are likely due to inconsistencies in the methods used to define HP positivity and patient status. The accuracy Bof self reported data on medical history is mainly depending on patient's knowledge, understanding of relevant information, ability to recall and willingness to report. The most affected age group was 11-18 years representing nearly half of patients with seropositive titers for HP, the same frequency was observed among controls, a result that was in agreement with the literature explained by the fact that eating outside is more among older children than young children who are usually fed by their care takers. However it was found that younger patients were affected more in Egypt [28].

Gender was insignifintly variable among both test and control groups, these observations were similar to the literature [29,31]. However contradictory results were obtained in the Egyptian study that described predominance of females.

Most of the patients in this study had more than one symptom, however no significant difference was found between symptomatic and asymptomatic subjects, moreover the majority of the asymptomatic controls proved to be serologically positive. Epigastric pain was the predominant symptom among the diabetic patients possibly because of the associated delayed gastric emptying. Gastric mucosal damage and epithelial remodeling is due to the active chronic inflammation caused by colonization of the gastric epithelium by H. pylori and the subsequent gastric submucosal infiltration by neutrophils and monocytes. Duration of symptoms of 2 weeks to 2month in serologically positive patients may support the hypothesis of that chronic infection with HP could trigger T1DM.

Insignificant difference was found between test and control groups regarding   dyspepsia, these results

were in agreement with the Eygyptian study [28], but there is no comparison with the Turkish and Italian studies because patients with gastrointestinal symptoms were excluded from the study [28,32].

Pallor has been detected in more than two thirds of serologically positive subjects, in line with the fact that HP is associated with anemia, most probably iron deficiency anemia. Also nearly half of diabetics and controls serologically positive for HP were found to have hemoglobin concentrations of 8-19 g/dL.

More than half of the patients seropositive for HP and control groups were of low socioeconomic status; this fact was proved by previous studies .The relationship between poverty, illiteracy, poor sanitation and poor hygiene was well known feature of the underdeveloped world.

CONCLUSIONS

It was concluded that the role of Helicobacter pylori (HP) infection in triggering the onset of T1DM among Sudanese diabetic children does not seem to be significant. However, further research among larger study populations, using more specific laboratory tests, are needed to assess the correlation between HP and the development of T1DM.

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