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Helicobacter pylori Infection in Type 1 Diabetes Children and Adolescents Using 13C Urea Breath Test

AGATA CHOBOT[1]*, KATARZYNA BĄK-DRABIK[2], ELIZA SKAŁA-ZAMOROWSKA[3],
AGNIESZKA KRZYWICKA[1], JAROSŁAW KWIECIEŃ[2] and JOANNA POLAŃSKA[4]

[1] Clinical Hospital No1, Zabrze, Poland; [2] Medical University of Silesia, Katowice, Poland
[3] Upper Silesian Center of Child's Health, Katowice, Poland
[4] Data Mining Group, Silesian University of Technology, Gliwice, Poland

A b s t r a c t

There is a 10–30% prevalence of HP infection in the general pediatric population in Poland. This study aimed to determine its prevalence in T1DM children in Upper Silesia, Poland and estimate its influence on metabolic control of patients. We studied 149(82♀) children with T1DM (duration > 12 months, mean HbA1c) and 298(164♀) age-matched controls. In all cases height and weight z-scores and Cole's index were assessed. In T1DM patients additionally glycated hemoglobin A1c and T1DM duration were analyzed. Presence of HP infection was determined using 13C-isotope-labeled urea breath test (UBT) (fasting and 30min after ingestion 75 mg of 13C urea). HP infection was present in 17 (11.4%) T1DM patients and in 49 (16.4%) controls (p > 0.05). T1DM patients presented higher values of anthropometric parameters than healthy controls (weight SDS 0.25[−0.46÷0.84] vs. −0.25[−1.06÷0.26], height SDS 0.09[−0.60÷0.69] vs. −0.31[−1.17÷0.48] and Cole's index 103%[93÷111%] vs. 97%[86÷106%]; for all p < 0001). Within both groups – T1DM children and controls – no differences regarding sex, age and any of the anthropometric parameters were determined. T1DM duration and HbA1c showed no relation to prevalence of HP infection. Prevalence of HP infection in pediatric T1DM patients is similar to that of healthy peers and shows no relation to glycemic control.

K e y w o r d s: *Helicobacter pylori*, HbA1c, type 1 diabetes, urea breath test

Introduction

Helicobacter pylori (HP) causes one of the most common chronic infections worldwide. This is a gram-negative, spiral-shaped pathogenic bacterium that specifically colonizes the gastric epithelium and is related to chronic gastritis and duodenitis, peptic ulcer disease and functional gastrointestinal motor disorders (Marshall and Warren, 1984; Wotherspoon *et al.*, 1991). The remote consequences of a chronic infection, which appear especially in adulthood, include: peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer.

The prevalence of HP infection varies between countries and depends on the age and socio-economical status. It is estimated that in developed European countries 10–30% of the population is infected, however most cases remain asymptomatic. In developing countries the prevalence in the adult population raises even up to 70% (Goh *et al.*, 2011). In previous research the prevalence of HP infection in the general pediatric population in Poland was assessed to be 30% (Iwańczak *et al.*, 2004). Nowadays the frequency of infection seems to be lower than formerly reported. An assessment by Żabka *et al.* (2010) revealed a 10% prevalence and a most recent publication showed that the frequency of HP infection in healthy children in Poland was 15.7% (Krusiec-Świdergoł *et al.*, 2010).

It is known that patients with diabetes are more prone to infection than healthy people. This is probably because of the fact that both cellular and humoral immune systems are perturbed, especially in the context of poor glycemic control with severe complications (de Luis *et al.*, 1998; Mustapha *et al.*, 2005). Many studies have evaluated the prevalence of the HP infection in diabetic patients, but the epidemiological relationship remains controversial (Ariizumi *et al.*, 2008; Ciortescu *et al.*, 2009; Ojetti *et al.*, 2001, 2010; Otto-Buczkowska,

* Corresponding author: A. Chobot, Clinical Hospital No1, 3 Maja 13–15, 41-800 Zabrze, Poland; phone: 0048 323704267; **e-mail:** agata.chobot@gmail.com

2012; Stępkowski and Grzeszczak, 2009). Furthermore, publications investigating HP infection in children and adolescents with type 1 diabetes (T1DM) are sparse and their conclusions contradictory (Arslan *et al.*, 2000; Colombo *et al.*, 2002; Khalil *et al.*, 2007; Ojetti *et al.*, 2010; Toporowska-Kowalska *et al.*, 2006, 2007). Some researchers revealed higher prevalence of HP infection in diabetic patients in general as well as in the pediatric population with T1DM (Ariizumi *et al.*, 2008; Arslan *et al.*, 2000; Ciortescu *et al.*, 2009; de Luis *et al.*, 1998; Ojetti *et al.*, 2001, 2010; Otto-Buczkowska, 2012; Stępkowski and Grzeszczak, 2009). Nevertheless, the results of other authors have not confirmed such observations (Ciortescu *et al.*, 2009; Colombo *et al.*, 2002; Khalil *et al.*, 2007; Ojetti *et al.*, 2001; Toporowska-Kowalska *et al.*, 2006, 2007).

The studies in T1DM subjects yielded also additional data on the relationship between HP infection and glycemic control, although their conclusions are also not unequivocal. On one hand, there are reports of a negative impact of HP infection on HbA1c levels (Toporowska-Kowalska *et al.*, 2006, 2007), but discordant conclusions have also been published (Candelli *et al.*, 2003; Colombo *et al.*, 2002; Khalil *et al.*, 2007). A supplementary interesting assessment revealed that the eradication of HP infection did not improve the metabolic control in a short-term follow-up (Candelli *et al.*, 2004, 2012; Khalil *et al.*, 2007). Although a study by Begue *et al.* (1999) demonstrated that HP eradication might decrease HbA1c.

The aim of the present study was to determine the prevalence of HP infection in children and adolescents with T1DM in Upper Silesia, Poland and to estimate the influence of HP presence on metabolic control of the patients.

Experimental

Material and Methods

Patients. The study group comprised of 149 (82 female and 67 male) T1DM children and adolescents, aged 13.4 ± 3.4 years. Volunteers from the regional out-patient diabetes clinic were enrolled using the following criteria: DM duration > 12 months, no history of antibiotic or proton pump inhibitor therapy in the past 4 weeks. The patients were examined between July 2011 and June 2012. Table I. shows the detailed characteristics of the studied patients. The control group consisted of 298 age- and sex-matched children and adolescents (164 female and 134 male) with no known glucose metabolism disorders – characteristics are presented in Table I. This group was enrolled from a large cohort (n = 1253) of children investigated

Table I
Characteristics and comparison of the study and control groups.

UBT result N	T1DM Patients Total 149	Controls Total 298	P
Age [yrs]	13.4 ± 3.4	13.3 ± 3.3	ns
Weight Z-score	0.25 ± 0.93 [−0.46 ÷ 0.84]	−0.25 ± 1.07 [−1.06 ÷ 0.26]	<0.001
Height Z-score	0.09 ± 0.97 [−0.60 ÷ 0.69]	−0.31 ± 1.23 [−1.17 ÷ 0.48]	<0.001
Cole's index [%]	103 ± 13 [93 ÷ 111]	97 ± 15 [86 ÷ 106]	<0.001
T1DM Duration [yrs]	4.6 ± 3.5		
HbA1c [%]	7.69 ± 1.63		

Legend: N – number of children, ± SD, [± 95% CI], SDS – standard deviation score, T1DM – Type 1 diabetes mellitus, UBT – urea breath test, HbA1c – hemoglobin A1c

during the program "Good diagnosis-Treatment-Live" carried out at the Clinical Hospital No 1 in Zabrze, Poland between March 2010 and June 2012.

Methods. In all T1DM patients Z-score for height and weight, Cole's index and glycated hemoglobin A1c (HbA1c) were assessed. Height and weight in T1DM patients were measured using a standardized scale and the Martin stadiometer. HbA1c measurements were carried out in the same laboratory using the recommended high pressure liquid chromatography (HPLC), which is a DCCT reference method. The normal range for this laboratory is < 6%.

The presence of HP infection was determined in all subjects by means of a breath test (UBT) with 13C isotope-labeled urea, which was performed fasting. Breath samples – at baseline and 30 minutes after ingestion of 75 mg of 13C isotope-labeled urea – were collected in 650 ml aluminized bags with one-way valves. An infrared spectrophotometer (IRIS, Wagner GMBH, Germany) was used to measure the 12CO2/13CO2 ratio. The results of the test were considered positive if C13 concentration (delta over baseline, DOB [o/oo]) raised in the exhaled air by more than 4.0‰ (Żabka *et al.*, 2010).

Statistical analysis. Statistical analysis was performed with the R software (www.bioconductor.org). Descriptive statistics were calculated for all analyzed parameters, and adequate figures were generated. To detect the outlying values Tukey's criterion was used. To check distribution normality Lilliefors test was employed. The verification of the variance homogeneity hypothesis was carried out using the F or Bartlett statistic. ANOVA algorithm and Student's t test were performed for comparative analysis of normally distributed variables. In the case of a distribution other than normal, we used the non-parametric ANOVA

Kruskal-Wallis test and Mann-Whitney U test to verify the concordance between groups. Pearson's or Spearman's correlation coefficients were used to estimate the associations between two variables – depending on the distribution of the analyzed continuous variables. Storey's multiple testing correction algorithm of the p value was used. For the analysis of discrete variables the χ^2 test or likelihood-ratio G test was performed. In each case an appropriate contingency table was constructed. The results were considered as significant at $p < 0.05$.

Ethical considerations. This study was approved by the institutional ethical committee of the Medical University of Silesia, Katowice, Poland.

Results

HP infection was present in 17 (11.4%) of T1DM patients. No differences between genders were found. Additionally no relation of the UBT result to age as well as any of the anthropometric parameters – height z-score, weight z-score, Cole's index – was determined (Table II). Parameters associated to T1DM – disease duration as well as HbA1c – showed no relation to the prevalence of HP infection (Table II).

Within the control group a positive UBT result was obtained in 49 (16.4%) cases. Similar as in the T1DM group no differences regarding sex, age and no relation to the investigated anthropometric parameters were found (Table II).

The comparison of the frequency of HP infection in T1DM patients and non-diabetic children revealed no discrepancy. All anthropometric parameters – height and weight z-scores as well as the Cole's index – were significantly higher in T1DM patients (Table I).

Discussion

This study was performed using the UBT as a gold standard method (Koletzko *et al.*, 2011). It was conducted in a large number of T1DM pediatric patients presenting quite good metabolic control (HbA1c 7.69%). The observed frequency of HP infection among the non-diabetic cohort (approximately 16%) was similar to the newest reports regarding the general polish pediatric population, which demonstrated a decrease of the prevalence in comparison to the past decade (Iwańczak *et al.*, 2004; Krusiec-Świdergoł *et al.*, 2010; Żabka *et al.*, 2010).

The results of former studies investigating HP infection in patients with T1DM are contradictory and there are only few publications concerning the pediatric population (Arslan *et al.*, 2000; Ciortescu *et al.*, 2009; Colombo *et al.*, 2002; de Luis *et al.*, 1998; Ojetti *et al.*, 2001; Toporowska-Kowalska *et al.*, 2006, 2007). Some of them revealed a higher prevalence of HP infection in children with T1DM (Arslan *et al.*, 2000; de Luis *et al.*, 1998). However, our findings and the results of other authors have not confirmed such observations (Candelli *et al.*, 2003; Ciortescu *et al.*, 2009; Colombo *et al.*, 2002; Khalil *et al.*, 2007; Ojetti *et al.*, 2001; Toporowska-Kowalska *et al.*, 2006, 2007). Given the facts mentioned in the former paragraph it seems justified to make an assumption that the prevalence of HP infection in T1DM children is the same as in healthy peers. The frequency of HP infection in the studied patients is lower than in earlier publications (Candelli *et al.*, 2003; Colombo *et al.*, 2002; Toporowska-Kowalska *et al.*, 2006, 2007). This might be explained by the fact that some of the studies were based on a serological screening that does not distinguish past and present infections

Table II
T1DM patients and non-diabetic subjects – comparison between subgroups according to the UBT result.

UBT result N	T1DM Patients			Controls		
	Positive 17	Negative 132	P	Positive 49	Negative 249	P
Age [yrs]	13.3 ± 3.3	13.9 ± 3.6	Ns	13.9 ± 3.2	13.2 ± 3.3	Ns
Weight Z-score	0.34 ± 0.97 [−0.21 ÷ 0.94]	0.24 ± 0.93 [−0.46 ÷ 0.83]	Ns	−0.39 ± 1.02 [−1.22 ÷ 0.16]	−0.22 ± 1.08 [−1.01 ÷ 0.30]	Ns
Height Z-score	−0.01 ± 1.09 [−0.60 ÷ 0.69]	0.10 ± 0.96 [−0.60 ÷ 0.69]	Ns	−0.48 ± 1.48 [−1.27 ÷ 0.60]	−0.28 ± 1.18 [−1.16 ÷ 0.46]	Ns
Cole's index [%]	105 ± 12 [96 ÷ 109]	103 ± 13 [92 ÷ 111]	Ns	97 ± 16 [86 ÷ 107]	97 ± 17 [86 ÷ 106]	Ns
T1DM Duration [yrs]	5.3 ± 3.9 [2.8 ÷ 7.8]	4.4 ± 3.4 [1.9 ÷ 6.4]	Ns			
HbA_{1c} [%]	7.82 ± 1.42 [6.90 ÷ 8.70]	7.60 ± 1.66 [6.60 ÷ 8.15]	Ns			

Legend: N – number of children, ± SD, [± 95%CI], SDS – standard deviation score, T1DM – Type 1 diabetes mellitus, UBT – urea breath test, HbA_{1c} – hemoglobin A1c, ns – not significant

as well as by the general decrease of HP infection in Polish and European children during the last decade (Colombo *et al.*, 2002; Iwańczak *et al.*, 2004; Krusiec-Świdergoł *et al.*, 2010; Żabka *et al.*, 2010).

In our study T1DM children with a confirmed HP infection did not differ significantly in terms of metabolic control and disease duration. No association between HP infection and HbA1c was also found by other authors (Candelli *et al.*, 2003; Colombo *et al.*, 2002; Khalil *et al.*, 2007). In addition HP eradication was shown to have no impact on metabolic control (Candelli *et al.*, 2004, 2012; Khalil *et al.*, 2007). However, in the former study in T1DM polish children, those infected with HP presented higher HbA1c levels (Toporowska-Kowalska *et al.*, 2007). Also Begue *et al.* (1999) suggested a positive relation between HP eradication and metabolic control. These discrepancies might be related to the differences between the study groups, especially regarding age, race and socioeconomic status. Moreover, the link between HP infection and metabolic control might be indirect (*i.e.* HP is known to influence the gastric motility and was shown to affect insulin resistance and may in this way influence blood glucose and HbA1c levels) and therefore difficult to determine clearly (Gen *et al.*, 2010; Toporowska-Kowalska *et al.*, 2006). The relation between HP infection and diabetes duration was investigated only by few other researchers, but all findings are similar to our results (Candelli *et al.*, 2003; Colombo *et al.*, 2002; Khalil *et al.*, 2007; Toporowska-Kowalska *et al.*, 2006, 2007).

Epidemiological studies suggest that the prevalence of HP infection should rise with age (Krusiec-Świdergoł *et al.*, 2010). Despite that fact research conducted in T1DM children showed no significant relation between HP infection and age (Khalil *et al.*, 2007; Toporowska-Kowalska *et al.*, 2006, 2007). Our results revealed also no dissimilarities in age between HP positive and negative children in both groups – T1DM and controls.

Because HP was in the past described to potentially impair growth in the pediatric population, our study also assessed the standardized anthropometric parameters of T1DM patients and non-diabetic controls (Krusiec-Świdergoł *et al.*, 2010). All mean values for both groups remained well within the normal ranges (90–110% for Cole's index and –1.0–1.0 for Z-scores), although both cohorts differed significantly by all of the anthropometric measurements. As expected T1DM children had slightly higher mean weight and height Z-scores as well as a little higher mean Cole's index. Nonetheless, within both groups HP positive and negative children presented no discrepancies regarding the analyzed anthropometric parameters.

The strong aspects of this study are – except for the ones mentioned in the beginning of the discussion – the race and ethnical homogeneity of the studied groups.

Nonetheless, because patients were not randomly chosen for this study, the presented data cannot be considered to represent the incidence of HP infection in the whole pediatric T1DM population in Poland.

The observed prevalence of HP infection in children and adolescents with T1DM is similar to that of healthy peers. No evidence for a relationship between the glycemic control and presence of HP infection in patients with T1DM could be determined.

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