

## HELICOBACTER INFECTION AND ALLERGY IN MAJORITY AND ROMA POPULATION IN THE CZECH REPUBLIC

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### ABSTRACT

In our paper we decided to evaluate the hypothesis about the relation between living conditions, infection with *Helicobacter pylori* and development of allergic problems. Two groups of children, one from the Roma population and one from the majority population, were chosen for this study. Comparing the levels of total IgE and specific IgE antibodies, IgA and IgG *Helicobacter pylori* antibodies in both populations, we found significant differences in all tested aspects. We conclude that changes in lifestyle together with changes in improvements in living conditions and reduction of risk factors can significantly influence the health conditions of the population and overall quality of life.

**Keywords:** *Helicobacter*, Immunity, Infection, Roma, Allergy

### 1. INTRODUCTION

The incidence of allergic atopy and asthma severely increased in the last decades, but the reasons for this occurrence are still unclear. Among suggested associations is exposure to tobacco smoke (Arnold *et al.*, 2011; Hancox *et al.*, 2004; Laszewicz *et al.*, 2014; Moayyedi *et al.*, 2002; Roesler *et al.*, 2014; Wang *et al.*, 2012), quality of indoor conditions (Laszewicz *et al.*, 2014), quality of environment (Hancox *et al.*, 2004; Krumbiegel *et al.*, 2000; Roesler *et al.*, 2014; Rothenbacher *et al.*, 1998; Vasar *et al.*, 2011; Wang *et al.*, 2012; Wex *et al.*, 2010), genetic and race influences (10,17,22,26,29,32), social-economic effects and education (Amedei *et al.*, 2010; Bode *et al.*, 2002; Chen and Blaser, 2008; Hancox *et al.*, 2004; Laszewicz *et al.*, 2014; Leal *et al.*, 2008), exposure to allergens from domestic animals (Amedei *et al.*, 2010) and lately the exposure to nanoparticles from car exhausts, mainly with diesel engines (Amedei *et al.*, 2010). Another theory speculates about the possibility that the use of antibiotics given either therapeutically or

in small amounts in food influences composition of the microbiome and triggers the allergies (Arnold *et al.*, 2011; Blaser 2012; Fu 2014; Holster *et al.*, 2012). On the other hand, there are known mechanisms leading to reduction of the risk of allergic diseases. Among them, effects of infections such as *Bordetella pertussis*, flu, enteroviruses, hepatitis A or *Toxoplasma gondii* were found (Amedei *et al.*, 2010; Reibman *et al.*, 2008). The most studied effects seem to be persisting infection with *Helicobacter Pylori* (HP) (Amedei *et al.*, 2010; Arnold *et al.*, 2011; 2012; Blaser, 2012; Bode *et al.*, 2002; Bridge and Merrell, 2013; Chen and Blaser, 2008; Karimi *et al.*, 2013; Kato and Sherman, 2005; Krumbiegel *et al.*, 2000; Oertli and Muller, 2012; Pacifico *et al.*, 2014; Perez-Perez *et al.*, 2003; Reibman *et al.*, 2008; Rothenbacher *et al.*, 1998). HP survives in the human gastrointestinal tract and, in addition to clinical manifestations such as lymphoma, adenocarcinoma or gastric ulcers, it can cause iron and vitamin B12 deficiencies, idiopathic thrombocytopenic purpura or growth retardation in children (Amedei *et al.*, 2010). HP colonizes stomach

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at an early age, causes medical problems in adulthood and persists for the entire life (Amedei *et al.*, 2010).

In 1989, Strachan hypothesized that persisting exposure to infectious agents and long-term living in unhygienic conditions can “educate” the immune system and therefore leads to the defense against development of allergic diseases. A balance between Th1 and Th2 lymphocyte population is the main foundation of this hypothesis. It is possible that HP infection resulting in predominant activation of Th1 cells with subsequent production of IFN- $\gamma$ , IL-12, IL-18, IL-23 and TNF- $\alpha$  leads to the inhibition of allergic problems regulated by Th2 (Amedei *et al.*, 2010). Another possible mechanism might be the ability of HP infection to induce regulatory T lymphocytes. This might explain why HP-negative patients are more predisposed to allergic diseases. We hypothesize that higher exposure to microorganisms at a young age results in achieving an immune system balance and a subsequently lower appearance of health problems resulting from immune imbalance.

We decided to use the Roma pediatric population as a model for our evaluation of the effects of high exposure to HP from early childhood. This group of children differs from the majority population in the Czech Republic in lifestyle and nutrition habits and is characterized by a high concentration of persons sharing one room (an average of 4 persons per room). In addition, this population suffers from high exposure to tobacco smoke (both passive and active), as 80% of Roma women smoke during pregnancy. When compared to the majority population, the Roma population significantly differs in morbidity rates (Hajioff and McKee, 2000; Nesvadbova *et al.*, 2000). Scabies prevalence reached 265 cases per 10,000 in Roma population compared to just 7.7 in majority population. Similarly, there are large differences in the incidence of viral hepatitis A (58.3:1), shigellosis (57:1), or salmonellosis (144:38). A variety of socio-economic factors such as lifestyle, low level of education, bad nutrition and other factors clearly results in a shorter lifespan of the Roma population, which is app. 10-15 years shorter when compared to majority population. A low interest in their own health observed among Roma people also adds to the short lifespan (Nesvadbova *et al.*, 2000). These facts led us to this study.

## 2. MATERIALS AND METHODS

### 2.1. Protocol

From a total of 353 children aged 7-14 years, we tested 152 Roma children (mean age 10.01), 98 males

with a mean age of 10.4 years and 54 females with a mean age of 9.7 years). The control group consisted of 211 aged 7-11 years children from the majority population (a mean age of 10.4; 107 males and 104 females of the same mean age). All children were from the same region of North-West Bohemia, an area with extreme environmental pollution caused by heavy concentration of surface mines, chemical industry and coal-powered power stations. In addition, this area suffers from a low level of socio-economic conditions, the lowest level of education and the highest unemployment in the Czech Republic. The study was approved by the Ethics committees of the Public Health Institute. This study was performed in agreement with the Helsinki Declaration (revised version 2000.09.01) and in full compliance with the rules for clinical testing in the Czech Republic. Parental consent was given in all cases.

We used blood drawn for comprehensive evaluation, the antibody response to *Helicobacter pylori* and general levels of total and specific IgE were evaluated for our study.

### 2.2. Tests

Specific IgE was tested by high-affinity fluorescent enzyme immunoassay (CAP FEIA, Pharmacia Upjohn, Peapack, NJ, USA). Levels of IgE higher than 0.35 kU/L were considered positive. In addition, we measured specific antibodies against inhalation allergens CAP Phadiatop (complex of inhalation allergens), against cat dander (e1), dog dander (e5), k82 (*Hevea brasiliensis*) and food allergens (mixture of egg white, milk, fish [cod], wheat, peanuts and soya beans). The total IgE was evaluated using Immulite 2000 analyzer using standards and diagnostics recommended by the manufacturer.

IgG and IgA antibodies to *H. pylori* were measured by an Enzyme-Linked Immunosorbent Assay (ELISA). Commercial sets (Meridian Diagnostics) were used according to the manufacturer’s recommendations. Concentration of antibodies above 20 U/mL was treated as positive result of *H. pylori* infection (Laszewicz *et al.*, 2014).

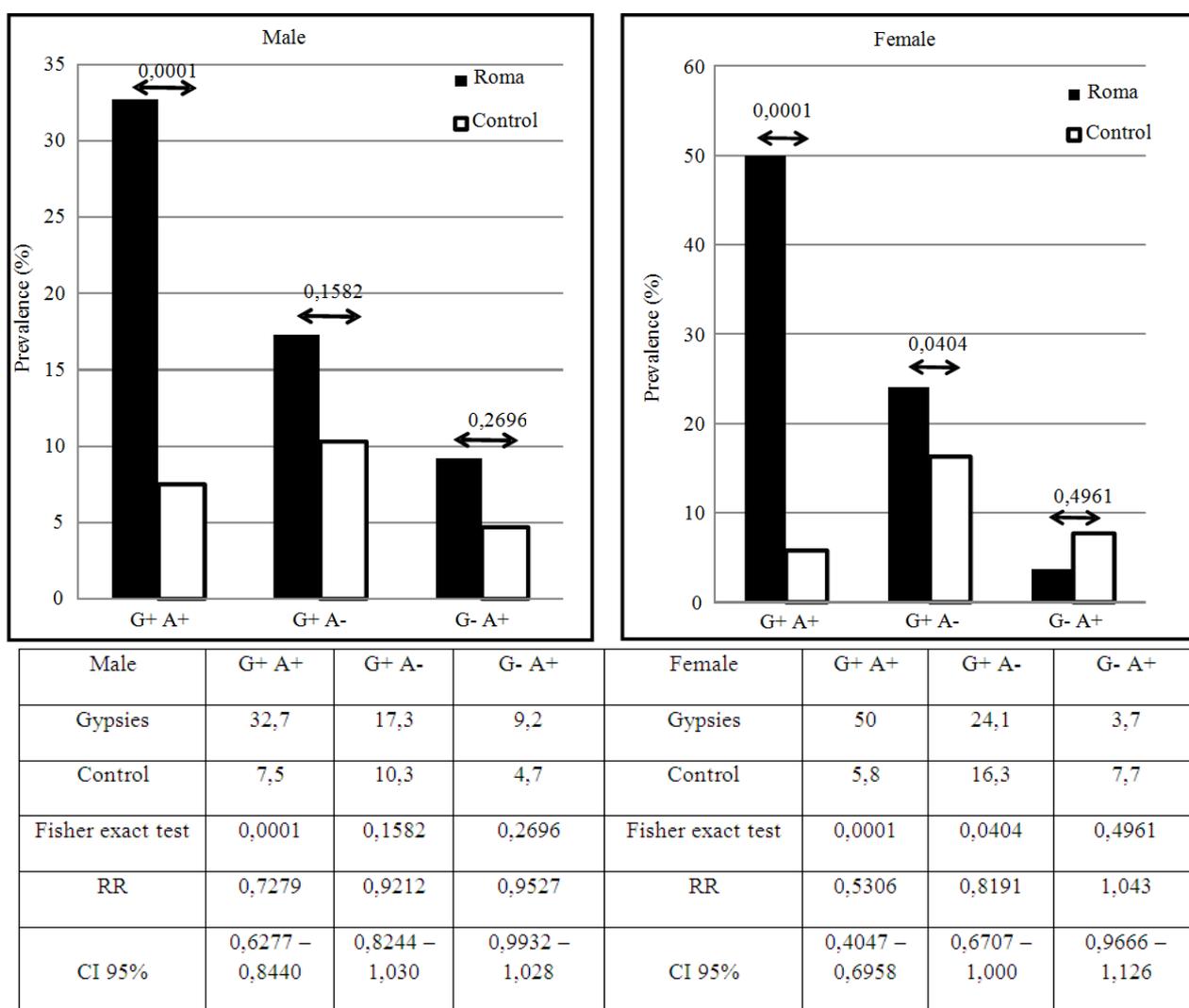
### 2.3. Statistical Analysis

Statistical significance was evaluated by a paired t-test using GraphPad Prism 5.04 software (GraphPad Software, USA). A mean and standard deviation were evaluated after determination of composition of standard values. For evaluation of prevalence differences, we used Fisher’s exact test.

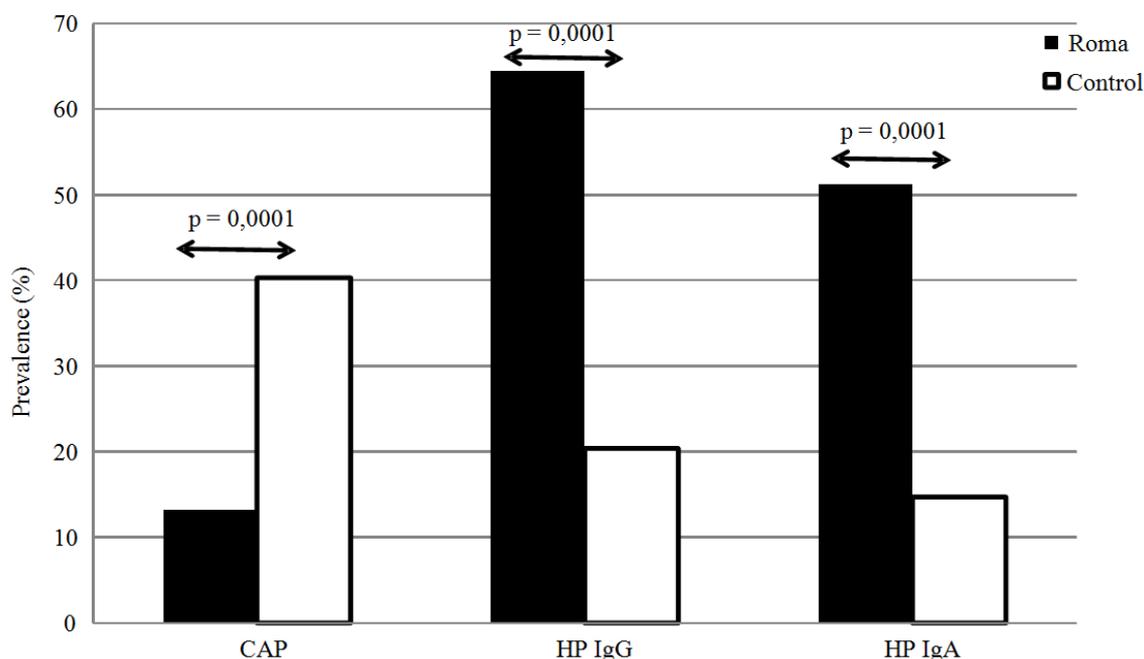
### 3. RESULTS

Anti-HP antibodies of the IgG class were found in 64% of Roma children and only in 20.4% of control population. Similar findings were found in case of anti-HP IgA antibodies-51.3% in Roma population Vs 17.4% in the control group. **Figure 1** summarized the appearance of antibodies in individual groups. It shows the differences in various isotypes of immunoglobulins between the tested groups. Our findings of differences in specific antibodies are statistically highly significant

( $p < 0.0001$ ). Positivity of specific IgE antibodies (CAP Phadiatop) was detected in 13.2% of the tested Roma population with no significant differences based on sex (13.3% in males, 13.0% in females). The findings of specific antibodies in the control group were also significant ( $p < 0.001$ ), with 43.9% of positive males and 36.5% of positive females (40.2% total). The prevalence rates of specific IgE and anti-HP antibodies are summarized in **Fig. 2**. The total levels of IgE were higher than 100 UI/L in 26.3% of the Roma subjects and in 34.6% of the controls.



**Fig. 1.** The types of IgG and IgA *Helicobacter* antibody combinations found in the serum of children from the Roma and majority population in the Czech Republic (RR-relative risk, CI-95% confidence interval).



	CAP	HP IgG	HP IgA
Gypsies	13,2	64,5	51,2
Control	40,3	20,4	14,7
Fisher act test	0,0001	0,0001	0,0001
RR	1,454	0,4512	0,5904
CI 95%	1,281 - 1,651	0,359 - 0,566	0,466 - 0,703

**Fig. 2.** Seroprevalence of *Helicobacter pylori* infection IgG and IgA antibody level (above 20 IU/mL) and specific IgE antibodies in the Roma children and controls (male and female). (RR-relative risk, CI-95% confidence interval)

#### 4. DISCUSSION

The study focused on the prevalence of IgA and IgG antibody response against HP. We used commercial kits employing ELISA and guaranteeing high specificity and sensitivity, which allowed us to compare the infection prevalence in different tested groups, varying in regions and even countries (Gosciniak, 1997; Sunnerstam *et al.*, 1999). In our groups of children, we found significant differences in anti-HP antibody response. The group of Roma children was 65.8% positive, whereas the group made up of children from the majority population was only 26.1% positive. The level of positivity significantly differs among individual countries. Our findings in the control group are slightly higher than those published by German authors, ranging from 4.8 to 14.6% (Bode *et al.*, 2002; Krumbiegel *et al.*, 2000; Rothenbacher *et al.*, 1998; Wex *et al.*, 2010). On the

other hand, a group of Turkish children living in Germany has much higher positivity of 37.9 to 44.8% (Bode *et al.*, 2002; Rothenbacher *et al.*, 1998). In Poland, 32% of positive cases among children were reported, whereas the Dutch population reached only 9% positivity (Holster *et al.*, 2012). The average level of positivity in the US studies is 27.3% (Perez-Perez *et al.*, 2003), but the majority population of New York has lower positivity of 16.8% (Reibman *et al.*, 2008). It was suggested that the positivity in children in developed countries was app. 16 to 20%, whereas in developing countries this can reach 80 to 90% of tested children (Kariya *et al.*, 2014). It is clear that these findings are significantly influenced by lifestyle, nutrition, social conditions, quality of living, education level of parents and other factors (Amedei *et al.*, 2010; Gold *et al.*, 2001; Hancox *et al.*, 2004; Krumbiegel *et al.*, 2000; Laszewicz *et al.*, 2014; Mitchell *et al.*, 2003;

Moayyedi *et al.*, 2002; Perez-Perez *et al.*, 2003; Reibman *et al.*, 2008; Rothenbacher *et al.*, 1998; Vasar *et al.*, 2011). The Roma population originates in Northern India and has lived in Europe for more than 1,000 years (Hajioff and McKee, 2000). In the last 100 years, they have migrated throughout Europe, forming closed communities with persisting inadequate lifestyle, low education and substandard level of hygiene, leading to low interest in their own health. The low standards of living results in high risk of infections, manifested from an early age (Hajioff and McKee, 2000). Close communication of individuals subsequently leads to early and frequent infection with HP. In addition, infectious diseases (namely hepatitis A and E) can help to modulate the immune system. Reports suggesting that HP infections can help to reduce risk of allergic diseases and asthma (Amedei *et al.*, 2010; Arnold *et al.*, 2001; 2012; Chen and Blaser, 2008; Holster *et al.*, 2012; Karimi *et al.*, 2013; Oertli and Muller, 2012; Reibman *et al.*, 2008; Wang *et al.*, 2012) led us to our study evaluating groups of children originating in the majority and Roma populations with the aim to find possible differences in specific and general antibody response to inhaled and food allergens. Our findings seem to confirm the hypothesis of a positive role of HP infection in the development of allergic diseases. Persisting HP infection can also be connected with additional extragastric problems such as low iron anemia, growth problems and other health disorders (Kariya *et al.*, 2014; Kato and Sherman, 2005; Pacifico *et al.*, 2014). It is possible to speculate that higher exposure to chemical agents and antibiotics (compared to the situation 100 years ago) results in reduction of HP infection and subsequent increase of allergic diseases and asthma (Blaser, 2012), which further supports the hypothesis of HP infection-induced protection against allergies. Current important changes in microbiome quality and composition results in generation and higher frequency of additional diseases (Blaser, 2012; Gold *et al.*, 2001). The hypothesis of possible restoration of the gastrointestinal microbiome either via reduction of continuous exposure to chemical pollutant or by prevention of allergic diseases (e.g., by anti-HP vaccination) is becoming more and more relevant (Annesi-Maesano *et al.*, 2012; Bode *et al.*, 2002). The benefits of using HP Neutrophil Activating Protein not only for diagnostic and therapeutic purposes, but also for prevention by vaccination has been experimentally proved (Fu, 2014). We conclude that changes in lifestyle together with changes in improvements in living conditions and reduction of risk factors can significantly

increase the health conditions of the population and their overall quality of life.

## 5. ADDITIONAL INFORMATION

### 5.1. Author's Contributions

All authors equally contributed in this work.

### 5.2. Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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