

COW'S MILK ALLERGY IN THE POPULATION OF INFANTS AND EARLY PRESCHOOLERS FROM THE VUKOVAR – SRIJEM COUNTY

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Summary

Introduction: Cow's milk allergy is the most common allergic reaction present among infants and early preschoolers. The early diagnosis of the condition is crucial for adequate treatment of the affected child.

Aim: To determine prevalence of cow's milk allergy in the population of infants (0-1 years) and early preschoolers (1-4 years) from Vukovar–Srijem County and demographical characteristics of affected children.

Materials and methods: This cross-sectional study was conducted from 1st May 2013 to 30th April 2014 in Vukovar General County Hospital and was approved by the responsible Ethical Committee. It included 59 children - suspected of having cow's milk allergy (38/59, 64.4% boys and 21/59, 35.6% girls; mean age 20.8±16.2 months). The serum concentrations of the specific IgE antibodies to cow's milk protein were determined and demographic data for each patient were collected (gender, age, place of living). Standardized UniCAP fluorescence-enzyme immunoassay (Phadia AB, Uppsala, Sweden) was used to determine serum concentration of the specific IgE antibodies for cow's milk protein.

Results: In observed population there were 35.6% (21/59) of children affected with cow's milk allergy. The allergy was more common with boys (39.5%), and children who lived in villages (56.5%). Average value of serum concentration of the specific IgE antibodies to cow's milk protein among affected children was 9.9±23.8 kU/L, and it was higher for boys (12.6 kU/L) in comparison to girls (3.2 kU/L).

Conclusion: This study indicates that the cow's milk allergy is relatively frequent within the observed population thus there is a need for further evaluation of this issue. In addition, the study has shown that the diagnosis of cow's milk allergy in Croatia is still relatively late and because of that there is a need to make additional efforts for earlier diagnosis of such condition that would also improve the therapeutic approach to affected children.

Key words: cow's milk allergy, child, food allergy, Croatia.

Introduction

The clinical response to cow's milk proteins was first described by the Galen and Hippocrates and at the beginning of the last century, Finkelstein described the first case of anaphylactic shock after intake of milk (Jurčić and Oberiter, 1996). Cow's milk allergy is a hypersensitivity reaction initiated by specific immunologic mechanisms. In most children with cow's milk allergy, the condition can be immunoglobulin E (IgE)-mediated and is thought to manifest as a phenotypical expression of atopy, together with (or in the absence of) atopic eczema, allergic rhinitis and/

or asthma. A subset of patients, however, have non-IgE mediated (probably cell-mediated) allergy and present mainly with gastrointestinal symptoms in reaction to the ingestion of cow's milk (Fiocchi et al., 2010).

The perception of milk allergy is far more frequent than confirmed cow's milk allergy. In a large European survey of above 44,000 telephone contacts, 5 million European respondents claimed to be milk-allergic, with adult women as the group making most of these claims. There were also wide national differences ranging from 13.8% of reports from Greece to 52.3% from Finland. In this survey milk was the most often reported offending food in children (38.5% of reports) and the second food most often implicated

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by adults (26%) (Steinke and Fiocchi, 2007). In a group of 600 children less than 4 years, cow's milk allergy was reported by the parents of 18 children (3%) (Kilgallen and Gibney, 1996). Milk reactions were reported by the parents of 2% of children without wheeze and by 16% of wheezers (Sandin et al., 2005). According to age, the prevalence of self-reported cow's milk allergy varies between 1 to 17.5% in preschoolers, 1 and 13.5% in 5 to 16-year-olds, and between 1 and 4% in adults (Roehr et al., 2004). Cow's milk-specific IgE sensitization point prevalence progressively decreased from about 4% at 2 years to less than 1% at 10 years of age (Maticardi et al., 2008). The most reliable data in epidemiology are those from birth cohorts that are free from selection bias (Fiocchi et al., 2010). There are five such challenge-confirmed studies. The cow's milk allergy prevalence during infancy ranged from 1.9% in a Finnish study, 2.16% in the Isle of Wight, 2.22% in a study from Denmark, 2.24% in the Netherlands, and up to 4.9% in Norway (Saarinen et al., 1999; Høst, 2002; Høst et al., 2002; Venter et al., 2006; Kvenshagen et al., 2008). To our knowledge, in Croatia there are no epidemiological data concerning the exact prevalence of cow's milk allergy in children, but according to the reports of pediatricians one can say that the cow's milk allergy is the most frequent allergic reaction in Croatian children aged two years or less (Vrdoljak, 2014).

Cow's milk contains several proteins that could each in principle elicit an allergic reaction in a sensitized individual. The most common cause of cow's milk allergy in children is alpha-lactalbumin, rarely casein, and in adults beta-lactoglobulin. Individuals with cow's milk allergy may present with a wide variety of symptoms. Consequently, knowledge of the various cow's milk allergic disorders and a detailed medical history are essential for the clinician to arrive at the correct diagnosis and the early diagnosis of the condition is crucial for adequate treatment of the affected child (Vrdoljak, 2014).

Allergic (immune-mediated) reactions to cow's milk may be classified as "immediate" (typically IgE-mediated) or "late onset" (typically non-IgE or cell-mediated) reactions. Immediate reactions to cow's milk may present as generalized systemic reactions (anaphylaxis) or IgE-mediated

gastrointestinal, cutaneous, and/or respiratory reactions. Patients presenting with IgE-mediated disorders will typically have positive skin tests and/or serum IgE antibodies to milk (Fiocchi et al., 2010; Vrdoljak, 2014). Symptoms of late-onset cow's milk allergy are not IgE-mediated and typically develop one to several hours or after several days of ingesting cow's milk. There are no reliable laboratory tests to diagnose late-onset cow's milk allergy and tests for IgE antibodies are negative. The majority of disorders involving late-onset cow's milk allergy are localized to the gastrointestinal tract, but disorders involving the skin and respiratory tract also occur (Fiocchi et al., 2010; Vrdoljak, 2014).

The aim of this study was to determine prevalence of cow's milk allergy in the population of infants (0-1 years) and early preschoolers (1-4 years) from Vukovar-Srijem County and demographic characteristics of affected children.

Materials and Methods

This cross-sectional study was conducted from 1st May 2013 to 30th April 2014 in Vukovar General County Hospital. Participation in the study was voluntary, and the study was approved by the Ethics Committee of the County General Hospital Vukovar.

The final sample consisted of 59 children from the Vukovar-Srijem County - suspected of having cow's milk allergy (38/59, 64.4% boys and 21/59, 35.6% girls; mean age 20.8±16.2 months). The serum concentrations of the specific IgE antibodies to cow's milk protein were determined and demographic data for each patient were collected (gender, age, place of living).

Standardized UniCAP fluorescence-enzyme immunoassay (Phadia AB, Uppsala, Sweden) was used to determine serum concentration of the specific IgE antibodies to cow's milk protein. According to the UniCAP fluorescence-enzyme immunoassay manufacturer the measuring range of the specific IgE antibodies to cow's milk protein in serum was 0.1 to 100.0 kU/L. According to the manufacturer, the expected normal values of the specific IgE antibodies for cow's milk protein in serum were all values below 0.35 kU/L and these were considered negative, while all values equal or above 0.35 kU/L were considered

positive. All positive values were further classified into classes as follows: Class I: 0.35–0.69 kU/L; Class II: 0.70–3.49 kU/L; Class III: 3.50–17.49 kU/L; Class IV: 17.50–49.99 kU/L; Class V: 50.00–99.99 kU/L and Class VI: 100.0 kU/L or above (Phadia AB, 2012).

Statistical analysis

Statistical analysis included data obtained by the laboratory analysis of participants' blood and collected demographic data. Normality of data distribution was tested by the use of Shapiro-Wilkinson test. All data were processed by the methods of descriptive statistics. The proportions were calculated and compared by the use of Fisher's exact test. The quantitative variables were compared by the use of Mann Whitney U-test. $P < 0.05$ was considered statistically significant. Statistical analysis was done by the SPSS Statistical Package for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The study sample consisted of 59 children from the Vukovar–Srijem County, 64.4% (38/59) boys mean age 21.1 ± 16.8 months and 35.6% (21/59) girls mean age 20.2 ± 15.4 months. According to the age group all subjects were divided into four groups. In group one (aged 1-12 months) there were 39.0% (23/59) of subjects; in group two (aged 13-24 months) there were 22.0% (13/59) of subjects; in group three (aged 25-36 months) there were 13.6% (8/59) of subjects and in group four (aged 37-48 months) there were 25.4%

(15/59) of subjects. According to the place of living in the study sample there were 61.0% (36/59) of children who lived in town and 39.0% (23/59) of children who lived in village.

The study revealed that among observed children there were 35.6% (21/59) of children affected with cow's milk allergy, i.e. with the measured serum concentrations of the specific IgE antibodies to cow's milk protein equal or above 0.35 kU/L. The average value of serum concentration of the specific IgE antibodies to cow's milk protein among affected children was 9.9 ± 23.8 kU/L, with the minimal measured value of 0.5 kU/L and maximal measured value of 100.0 kU/L. When looking the measured serum concentrations of the specific IgE antibodies to cow's milk protein according to the gender it was evident that boys had higher average value of these antibodies (12.6 kU/L) in comparison to girls (3.2 kU/L) but this difference was not statistically significant ($Z = -0.982$; $P = 0.331$).

All children who were, according to the measured values of serum concentrations of the specific IgE antibodies to cow's milk protein, found positive for the presence of cow's milk allergy were further classified into classes of positivity proposed by the manufacturer of the used immunoassay. It was evident that 33.3% (7/21) of allergic children belonged to the class I, 42.9% (9/21) of allergic children belonged to the class II, 14.2% (3/21) of allergic children belonged to the class III, none of the allergic children belonged to the class IV and 4.8% (1/21) of allergic children belonged to the classes V and VI (Table 1).

Table 1. Children from the Vukovar–Srijem County with the positive values of serum concentrations of the specific IgE antibodies to cow's milk protein (that imply the existence of cow's milk allergy) according to the classes of positivity proposed by the manufacturer of the used immunoassay.

Classes of positivity to the specific IgE antibodies for cow's milk protein according to the values of the specific IgE antibodies for cow's milk protein in patients' serum (kU/L)	Children from the Vukovar–Srijem County with the positive values of serum concentrations of the specific IgE antibodies to cow's milk protein N (%)
Class I: 0.35–0.69	7 (33.3)
Class II: 0.70–3.49	9 (42.9)
Class III: 3.50–17.49	3 (14.2)
Class IV: 17.50–49.99	0
Class V: 50.00–99.99	1 (4.8)
Class VI: 100.0 kU/L or above	1 (4.8)
Total	21 (100.0)

When looking the all children from the Vukovar–Srijem County suspected of having cow's milk allergy according to their gender it was found that among all of them there were 39.5% (15/38) of boys with the confirmed cow's milk allergy and 28.6% (6/21) of girls with the confirmed cow's milk allergy, but this difference was not statistically significant (Fisher's exact test; $P=0.571$). According to the age of diagnose, the study showed that the mean age of the confirmation of the existence of cow's milk allergy in the study

population was 27.2 ± 15.9 months, 25.5 ± 16.5 in boys and 31.5 ± 14.9 months in girls.

When looking the age of diagnose of the existence of cow's milk allergy according to the age groups of the children it was evident that the diagnose of this allergy was confirmed in 13.0% (3/23) of children aged 1-12 months, 53.8% (7/13) of children aged 12-24 months, 50.0% (4/8) of children aged 25-36 months and 46.7% (7/15) of children aged 37-48 months (Table 2).

Table 2. The age of diagnose of the existence of cow's milk allergy among children from Vukovar–Srijem County allergic to cow's milk according to the age group.

Age group of children	Children from the Vukovar–Srijem County with the positive values of serum concentrations of the specific IgE antibodies to cow's milk protein N (%)	Total
1-12 months	3 (13.0)	23
13-24 months	7 (53.8)	13
25-36 months	4 (50.0)	8
37-48 months	7 (46.7)	15
Total	21 (35.6)	59

When looking the all children from the Vukovar–Srijem County suspected of having cow's milk allergy according to the place of their living the study revealed that 22.2% (8/36) of children with confirmed cow's milk allergy lived in town and 56.5% (13/23) of children with confirmed cow's milk allergy lived in village, and this difference was statistically significant (Fisher's exact test; $P=0.012$).

This study has shown that cow's milk allergy was confirmed in 35.6% of subjects with suspected allergy, accordingly, this study also confirmed the well-known fact, according to which the prevalence of allergy established by the diagnostic testing is always three to four times lower than those claimed by patients themselves or their parents (Kilgallen and Gibney, 1996; Roehr et al., 2004; Sandin et al., 2005; Rona et al., 2007; Steinke and Fiocchi, 2007; Kolaček, 2011). Data from cross-sectional studies conducted elsewhere in the world demonstrated the prevalence rates of cow's milk allergy from 0.6 to 2.5% in preschoolers, 0.3% at 5 to 16 years of age, and of less than 0.5% in adults (Madrigal

and Alfaro, 1996; Osterballe et al., 2005; Rona et al., 2007). Comparing the results of this study regarding the established prevalence of cow's milk allergy with the above mentioned results of similar studies conducted elsewhere it can be said that the prevalence of this allergy in study population from the Vukovar–Srijem County is much higher.

This study has further shown that the average value of the serum concentration of specific IgE antibodies to cow's milk protein in all children allergic to cow's milk was 9.9 kU / L, where boys had higher average value of these antibodies (12.6 kU/L) in comparison to girls (3.2 kU/L). According to the gender the existence of cow's milk allergy was confirmed in 39.5% of boys and 28.6% of girls. These results are in compliance with the results of the similar studies conducted in Finland, Lithuania and Iran, which also showed how cow's milk allergy is much more common in boys than in girls (Pyrhönen et al., 2009; Kavaliūnas et al., 2013; Teymourpour et al., 2013). In addition, in a study conducted in Iran that examined the incidence of anaphylaxis

caused by cow's milk allergy in the population of children aged five years, it was found that the average value of serum concentrations of specific IgE antibodies to cow's milk protein in patients with anaphylaxis was 19.3 kU/L, where in 59.2% of cases of anaphylaxis occurred among boys. If this value is compared with the values determined in this study, in which the boys had a significantly higher average value of serum concentrations of specific IgE antibodies to cow's milk compared to girls, it can be concluded that in this study, clinical manifestations of allergy in boys were much severe and that boys are consequently more prone to develop anaphylaxis (Teymourpour et al., 2013). With regard to the higher incidence of allergy to cow's milk and all other nutritional allergies in boys, in the available literature there is no unique or exact explanation. As possible causes narrower airways in small boys than girls, increased bronchial tone or possibly higher concentrations of IgE antibodies in the blood of the boys are mentioned and all of them lead to changes in the bronchial lumen in response to exposure to a variety of potentially allergenic stimuli from food (Kavaliūnas et al., 2013).

Results of the study showed that the mean age of diagnosis of cow's milk allergy in the study population was 27.2 months, while it was slightly lower in boys (25.5 months) than in girls (31.5 months). When comparing these results with the results of a similar study conducted in Thailand, where the mean age of diagnosis of cow's milk allergy was 14.8 months, it may be concluded that the confirmation of cow's milk allergy in the studied population of children from the Vukovar–Srijem County was relatively late (Ngamphaiboon et al., 2008).

The study further showed that 56.5% of subjects with confirmed allergy to cow's milk lived in the village and 22.2% in the town, which is consistent with the results of a similar study conducted in Poland in the population of children aged up to three years, which showed that the prevalence of nutritional allergies, including those to cow's milk was higher among children who lived in the village (Malinowska et al., 2002). Possible reasons for the frequent occurrence of cow's milk allergy in children from rural areas compared to

children from the town is the earlier introduction of unprocessed cow's milk in the diet of infants and young children in the village compared to the town which consequently leads to more frequent development of allergic reactions (Vrdoljak, 2014).

This study has several limitations that should be considered when evaluating the obtained results. One of the limitations refers to the small number of children from the Vukovar–Srijem County - suspected of having cow's milk allergy and consequently small number of children with confirmed allergy to cow's milk. Second limitations refers to the lack of application of the specially designed questionnaire that would serve as a tool for collecting additional data concerning the subjects' personal and family history and information about possible risk factors for the occurrence of cow's milk allergy among each of them.

Conclusions

This study indicates that the cow's milk allergy is relatively frequent within the observed population of children from the Vukovar-Srijem County pointing to the need for further evaluation of this issue. In addition, the study has shown that the diagnosis of cow's milk allergy in Croatia is still relatively late. Bearing in mind that early diagnosis of the condition is crucial for the adequate treatment of the affected child one must make additional efforts for earlier diagnosis of such condition that would also improve the therapeutic approach to affected children.

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