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BRIEF ARTICLE

Milk protein IgG and IgA: The association with milk-induced gastrointestinal symptoms in adults

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Abstract

AIM: To study the association between serum levels of milk protein IgG and IgA antibodies and milk-related gastrointestinal symptoms in adults.

METHODS: Milk protein IgG and IgA antibodies were determined in serum samples of 400 subjects from five outpatient clinics in Southern Finland. Subjects were randomly selected from a total of 1900 adults undergoing laboratory investigations in primary care. All 400 participants had completed a questionnaire on abdominal symptoms and dairy consumption while waiting for the laboratory visit. The questionnaire covered the nature and frequency of gastrointestinal problems, the provoking food items, family history and allergies. Twelve serum samples were disqualified due to insufficient amount of sera. The levels of specific milk protein IgG and IgA were measured by using the ELISA technique. The association of the milk protein-specific antibody level was studied in relation to the milk-related gastrointestinal symptoms and dairy consumption.

RESULTS: Subjects drinking milk (n = 265) had higher levels of milk protein IgG in their sera than non-milk drinkers (n = 123, P < 0.001). Subjects with gastrointestinal problems related to milk drinking (n = 119) consumed less milk but had higher milk protein IgG levels than those with no milk-related gastrointestinal symptoms (n = 198, P = 0.02). Among the symptomatic subjects, those reporting dyspeptic symptoms had lower milk protein IgG levels than non-dyspeptics (P < 0.05). However, dyspepsia was not associated with milk drinking (P = 0.5). The association of high milk protein IgG levels with constipation was close to the level of statistical significance. Diarrhea had no association with milk protein IgG level (P = 0.5). With regard to minor symptoms, flatulence and bloating (P = 0.8), were not associated with milk protein IgG level. Milk protein IgA levels did not show any association with milk drinking or abdominal symptoms. The levels of milk protein IgA and IgG declined as the age of the subjects increased (P < 0.004).

CONCLUSION: Milk protein IgG but not milk IgA seems to be associated with self-reported milk-induced gastrointestinal symptoms.

Key words: Abdominal symptoms; Cow's milk; Food hypersensitivity

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INTRODUCTION

More than 40% of adults in primary care suspect that milk ingestion is causative of their gastrointestinal symptoms[1]. Furthermore, patients suffering from irritable bowel syndrome (IBS) often relate their symptoms to milk[2]. To assess the impact of milk in

abdominal complaints is challenging. It is often difficult to distinguish the symptoms of milk hypersensitivity from other types of milk- or food-related gastrointestinal symptoms. Milk hypersensitivity in early childhood is mostly milk protein IgE-mediated[3,4] causing immediate-type hypersensitivity reactions. The frequency of IgE-mediated cow's milk allergy decreases with increasing age and a high level of cow's milk-specific IgE is rare in adults[5-8]. The impact of other types of immune reactions to cow's milk and, more specifically, the association of antibodies of IgG and IgA isotypes with cow's milk-induced adverse gastrointestinal symptoms in adults, is presently controversial[9-13]. Ou-Yang *et al*[14] have reported that an elimination diet based on the elevated level of food-specific IgG improved chronic diarrhea in children. Another recent study from China showed no association between symptom severity and food antigen-specific IgG in patients suffering either from IBS or functional dyspepsia (FD) although the levels of food-specific IgG titres were higher both in IBS and FD patients compared to healthy controls[15].

The purpose of our study was to evaluate the association of serum level of milk protein IgG and IgA antibodies with gastrointestinal symptoms experienced by cow's milk ingestion in working age adults and to evaluate the milk IgA and IgG levels in relation to dairy consumption.

MATERIALS AND METHODS

We screened adults during spring 2004 in five different primary care centres for food-related symptoms, focusing on milk-related problems[1,16]. Ethical approval was received from the Ethics Committee for outpatient clinics in Helsinki and surrounding areas (567/E1/03). Subjects who were referred to the laboratory for blood tests were invited to give a blood sample for the study purposes and to complete a questionnaire on gastrointestinal symptoms and dairy consumption as described recently[1]. Of the 1900 adults who agreed to a blood sample within the three month study period, an exceptionally high proportion, 99%, returned the questionnaire. Randomly, serum samples from 400 of these subjects (198 women and 202 men) were selected for the measurement of milk protein IgG and IgA levels. Twelve samples were excluded due to an insufficient amount of sera. Thus, the study group comprised 388 adults (aged 18-64 years, mean age 40 years) of whom 119 informed us that they experienced gastrointestinal symptoms from consuming milk and 198 reported having no milk-related symptoms. The non-response rate was, in general, low per question. However, 71 (18%) did not answer the question on the presence of subjective milk-related symptoms, although they responded to questions on dairy consumption and gastrointestinal symptoms.

The reasons for laboratory testing were; gastrointestinal symptoms in 69/388 (18%), health check-up in 209/388 (54%), and follow up of an earlier diagnosed disease in 90/388 (23%). In 23/388 (6%) the indication for blood test was not reported. All subjects had been genotyped for adult-type hypolactasia[1] and screened for celiac disease[16]. IgG antibodies to *Helicobacter pylori* (*H pylori*) were determined with an in-house enzyme immunoassay as previously described[17]. The lower limit for raised titres was 700 with a sensitivity of 99% and specificity of 93% as compared to histology[17].

The milk protein IgG and IgA antibodies were measured by the ELISA technique using an adapted infant formula to coat the microtitre plates. Values are expressed as % of the standard with a very high titre of cow's milk antibodies[18]. The major antigen in the formula was casein.

Statistical analyses

Kruskall-Wallis test, Spearman Rank Correlation, Mann-Whitney, Fisher's exact test, and ANOVA were used for analyzing the results. Significance was set at $P < 0.05$.

RESULTS

The levels of milk protein IgA and IgG antibodies declined as the age of the subjects increased, being lowest in the oldest age group, and the age-related decline was statistically significant with regard to milk protein IgG (ANOVA, $P < 0.004$; Table 1). Age and personally estimated milk-related gastrointestinal problems showed no correlation (P = NS, Spearman Rank). Men had higher milk protein IgA but not milk IgG levels in their sera than women (Mann-Whitney, $P = 0.04$; Table 1). Subjects drinking milk daily had higher levels of milk protein IgG in their sera than non-milk drinkers (Mann-Whitney, $P < 0.001$; Table 1). The daily consumption of milk was less frequent among subjects reporting gastrointestinal problems after drinking milk, but they had higher milk protein IgG levels than those who experienced no gastrointestinal symptoms (Table 1). Milk protein IgA levels did not show any association with milk drinking or abdominal symptoms (Table 1).

The association of high milk protein IgG levels with constipation was close to the level of statistical significance (Table 1). Diarrhea had no association with milk protein IgG level ($P = 0.5$). Regarding minor symptoms, flatulence and bloating ($P = 0.8$, Mann-Whitney), were not associated with milk protein IgG level. Subjects reporting dyspeptic symptoms had lower milk protein IgG levels than non-dyspeptics ($P < 0.05$). Furthermore, dyspepsia was not associated with milk drinking ($P = 0.5$, Fisher's exact test) or age ($P = 0.19$, Spearman Rank).

Milk protein IgG level was lower in subjects positive for antibodies to *H pylori* ($n = 76/386$, $P < 0.05$ Mann-Whitney) although they drank milk more often than *H pylori*-negative subjects ($n = 62/76$, $P < 0.006$ Mann-Whitney). However, the *H pylori*-positive group was somewhat older (mean age 46 years) than the *H pylori*-negative group (mean age 40 years, $P = 0.004$, ANOVA), which may explain the result. Accordingly, the presence of *H pylori* antibodies in serum was associated

Table 1 Data of the study group, the experienced symptoms and correlation of milk protein IgG and IgA levels with different parameters

Subjects	*n* (% of the study group)	Mean IgG% (arbitrary units) 95% CI (lower-upper)	*P*	Mean IgA% (arbitrary units) 95% CI (lower-upper)	*P*
Study group (yr)	388	13.5 (11.5-15.5)		7.3 (55-9.2)	
18-34	139 (35)	16.6 (13.1-20.2)	< 0.004[1]	9.4 (5.5-13.2)	0.16
35-49	123 (32)	14.7 (10.7-10.8)		7.4 (4.1-10.6)	
50-64	126 (33)	8.8 (6.5-11.1)		5.1 (3.1-6.9)	
Male	195 (50)	14.0 (11.2-16.7)	0.5	7.7 (5.1-10.3)	0.04[2]
Female	193 (50)	13.0 (10.2-15.9)		7.0 (4.4-9.6)	
Drinking milk daily	265 (68)	15.4 (12.8.-18.0)	< 0.001[3]	7.8 (5.6-10.0)	
Not drinking milk	123 (32)	9.3 (6.5-12.1)		6.4 (3.2.-9.7)	
Subjective symptoms from milk	119 (31)	10.5 (7.6-13.5)	0.02[4]	6.4 (2.5-10.3)	0.5
No subjective symptoms from milk	198 (51)	16.0 (12.8-19.1)		8.0 (5.7-10.4)	
Constipation	47 (12)	19.2 (11.7-26.8)	0.05	11.3 (3.7-18.8)	0.06
No constipation	282 (73)	13.3 (11.0-15.6)		7.5 (5.4-9.7)	
Diarrhea	133 (34)	14.4 (11.0-17.8)		8.0 (4.4.-11.6)	0.7
No diarrhea	196 (51)	14.0 (11.0-17.0)		8.1 (5.5.-10.8)	
Dyspepsia	127 (33)	11.9 (8.5.-15.3)	< 0.05[5]	4.8 (300-668)	0.1
No dyspepsia	202 (52)	15.6 (12.6-18.5)		10.1 (6.8-13.3)	

[1]The level of IgG decreased according to the age statistically significantly. [2]The IgA level was statistically significantly higher in men than in women. [3]The milk drinking was associated with IgG level statistically significantly. [4]The IgG level was statistically significantly higher in subjects with symptoms from milk. [5]The level of IgG was statistically significantly lower in subjects with dyspepsia.

in a statistically significantly manner with a lower level of milk protein IgA antibodies ($P = 0.03$, Mann-Whitney).

There was no correlation between milk protein IgG or IgA antibodies and C/T-13910 genotype associated with adult type hypolactasia. Unexpectedly, none of these randomly picked subjects was screen-positive for celiac disease[16]. There was no association of milk-specific IgG or IgA with a reported history of a diagnosed gastrointestinal disorder [irritable bowel syndrome $n = 12/388$ (3.0%) or inflammatory bowel disease $n = 4/388$ (1.0%)], since none of these patients had high levels of cow's milk-specific IgG or IgA. Irritable bowel syndrome was reported less in the study group than in an average western population (5%-10%) and inflammatory bowel disease more often than in an average western population (0.1%)[19,20].

DISCUSSION

Milk protein IgG but not milk IgA seems to be associated with self-reported milk-induced gastrointestinal symptoms. The nature of these symptoms, however, is unclear. There was a clear association of milk protein IgG with milk drinking in our study, supporting the view that the presence of milk protein IgG-specific antibodies may, to a certain level, be a normal physiologic reaction to ingested milk protein. Regarding the gastrointestinal symptoms, dyspepsia but not diarrhea or constipation showed a statistically significant association with milk protein IgG level. However, the association with milk protein IgG and dyspepsia was confounding as it was negative and not attributed to milk drinking or age.

The serum samples and questionnaires of the 388 patients included in this study were randomly picked from a group of 1900 volunteers attending a larger study of milk-induced gastrointestinal symptoms[1]. The blood samples were initially obtained during a short period of three months. Thus, seasonal variation had minimal effect on the results. An exceptionally high proportion, 99% of the 1900 participants, returned the questionnaire on abdominal symptoms and dairy consumption. The questionnaires were well completed and no samples needed to be excluded because of missing questionnaire data. However, as the number of samples included was limited there may be bias in the results. The information about milk-related gastrointestinal problems was derived from the questionnaires, not from milk challenge and subsequent observation of the participants. However, questionnaires were well-formulated and the participants were all working age people capable of understanding the questions. Furthermore, the most common causes for milk-related symptoms such as adult-type hypolactasia or celiac disease had been screened out by blood tests in the study group[1,16].

There are only a few studies which have been conducted in adults regarding milk protein antibodies, especially involving IgG and IgA. Although there are a number of studies in children, extrapolation of results from pediatric populations requires caution. There are some recent pediatric studies which imply that determination of milk-specific IgG4 might have a role in this field[13,21,22]. In this study we did not have the opportunity to measure milk-specific IgG4. However, the results of the earlier studies conducted in adults provided controversial results[9-13].

In our previous study, we showed that IgE antibodies to milk did not correlate with the self-reported, milk-related symptoms[5]. Consistent with this, Pelto *et al*[11] have shown that hypersensitivity to cow's milk does also occur in adults but the mechanism is most likely not milk-specific antibody-mediated but rather due to an increase in serum reactivity to milk protein. Moreover, the positive association between milk protein IgG level

and reported gastrointestinal symptoms from milk are in accordance with earlier findings showing that bowel irritation had a correlation with high mucosal IgG levels to food antigens[10,18].

The importance of milk protein IgG and IgA antibodies in the etiology of gastrointestinal symptoms following cow's milk ingestion remains uncertain. At present however, it would appear that measurement of these antibodies in routine clinical practice is of limited value and cannot be recommended.

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COMMENTS

Background

Cow's milk-related gastrointestinal symptoms are often complained about by primary care patients. In adult populations "lactose intolerance" or, more precisely, adult-type hypolactasia, is the most common cause of cow's milk induced gastrointestinal symptoms. Celiac disease, the most common cause of secondary hypolactasia, is encountered in 1%-2% of western populations and thus should be considered when milk-related symptoms are being diagnosed. Allergy to cow's milk protein is relatively rare in adults and the mechanisms by which it is mediated are not yet known. Milk protein-specific immunoglobulin (Ig)E, the most common mediator of milk hypersensitivity in children, is hardly ever the mediator in adults. There are a number of studies regarding milk protein hypersensitivity in children, but only a few conducted among adults and the results achieved from children cannot be extrapolated to adults without caution. This study was conducted in order to evaluate the roles of milk protein specific IgG and IgA in cow's milk hypersensitivity in adults.

Applications

Milk protein IgG is associated with self-reported milk-related gastrointestinal symptoms, whereas milk protein IgA has no such association. Milk protein IgG antibody levels also correlate with drinking milk. These findings imply that milk protein-specific IgG has a role in the humoral reaction to ingested milk. However, the measurement of milk protein IgG provides no accurate information on milk hypersensitivity.

Peer review

This is an interesting work that is concerned with a controversial area of gastroenterology.

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