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Influence of Early Feeding Practices on Celiac Disease in Infants

Nedeljko P. Radlović¹,
Marija M. Mladenović²,
Zoran M. Leković¹, Zorica
M. Stojšić³, Vladimir N.
Radlović¹

¹Department of Gastroenterology and Nutrition, University Children's Hospital, Belgrade, Serbia

²Pediatric Department, Health Center, Valjevo, Serbia

³Institute of Pathology, University Medical School, Belgrade, Serbia

Aim To investigate whether duration of breastfeeding and timing of gluten introduction influence the age at diagnosis and severity of celiac disease.

Methods Medical records of 89 infants (59 girls and 30 boys; mean age of 14.2 months, standard deviation 4.80) diagnosed with classic celiac disease at the University Children's Hospital in Belgrade from 2000 to 2008 were retrospectively analyzed to determine the duration of breastfeeding and timing of gluten introduction. The severity of celiac disease was assessed based on weight loss, longitudinal growth retardation, anemia, and secondary lactose intolerance.

Results Longer breastfeeding significantly reduced the risk that celiac disease would manifest in the first year of life (odds ratio, 0.655; 95% confidence interval, 0.481-0.891; $P=0.007$), and duration of breastfeeding was the most significant predictor of developing celiac disease ($B=0.49$; 95% confidence interval, 0.131-0.768; $P=0.007$). There were no significant differences in age at diagnosis between infants who had started consuming gluten before the fourth month and those who had started between the fourth and sixth month. Neither breastfeeding nor timing of gluten introduction affected the severity of the disease.

Conclusion Longer breastfeeding and continuation of breastfeeding after gluten introduction delay the onset of classic celiac disease. On the other hand, neither breastfeeding nor the timing of gluten introduction affects the severity of celiac disease.

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Correspondence to:

Marija Mladenović
Health Center, Valjevo
Str. Sindjeliceva 62
14000 Valjevo, Serbia
drmladenovic@beotel.net

Celiac disease, also known as gluten-sensitive enteropathy, is an autoimmune disorder caused by ingestion of gluten in genetically sensitive individuals (1). It is a disease of complex etiology and its onset and clinical features are determined by interaction between genetic and environmental factors, ie, a combination of adaptive and innate immune responses to gluten (2). The disease may present in various forms and clinical features vary depending on the age of onset. Its classification into classic or non-classic form depends on whether gastrointestinal symptoms are present or not. The classic form refers to presentation in children aged 9-24 months with chronic diarrhea, vomiting, abdominal distention, and systemic malnutrition (3). This form is strongly associated with the genes in the HLA class II complex, though other genes also contribute to it (4). Non-classic celiac disease comprises atypical (mono or oligosymptomatic), silent, and latent forms, which are usually diagnosed in older children with anemia, short stature, and unusual gastrointestinal complaints or through serological screening (3).

Gluten, a highly complex protein present in wheat, rye, and barley, is well-known to trigger celiac disease. According to the latest guidelines, gluten should be introduced into the diet of infants 4-6 months old, but no particular quantity is recommended. Studies have claimed that infant feeding practices, especially breastfeeding and timing of gluten introduction, may delay the onset of the disease or modify its symptoms (5-10). In addition, they have suggested that breastfeeding at the time of gluten introduction can protect against celiac disease. However, none of these studies has described in detail the effects of breastfeeding and the time of gluten introduction on the development of celiac disease, particularly on its severe form.

In this study, we investigated whether the duration of breastfeeding and timing of gluten introduction delayed the onset of the disease or influenced its severity in infants with classic form of the disease.

METHODS

Patients

We retrospectively analyzed medical records of 89 infants (59 girls and 30 boys) diagnosed with classic form of celiac disease at the University Children's Hospital, Belgrade, a tertiary pediatric hospital in Serbia, between 2000 and 2008. Ethical approval for the study was obtained from the ethics committee of the hospital.

The youngest patient in the study was 7 months old and the oldest was 24 months old (median age, 14 months; interquartile range, 7 months). Of the 89 infants, 39 (44%) were diagnosed with celiac disease in the first year of life and 50 (56%) in the second. At the time of diagnosis, all of them had gastrointestinal symptoms. When their condition had stabilized, they underwent small intestine enterobiopsy, which showed severe mucosal damage, and were placed on a gluten-free diet. Upon completion of a median of 36 months of gluten-free diet (interquartile range, 25 months), clinical and histological remission was confirmed in all infants, based on criteria of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (11). Infants were then challenged with gluten as described in the guidelines (11) and disease relapse occurred in all cases.

Clinical features and pathohistological findings

The following data were recorded at the time when the diagnosis of celiac disease was made: duration of breastfeeding before the diagnosis, timing of gluten introduction, the type and duration of symptoms, and the age at diagnosis. Data about breastfeeding were self-reported, and we assumed that the infant had been breastfed if mothers had produced enough milk for a minimum of 3 complete nursings per day. Exclusively breastfed infants were defined as those having received no other milk formula, food, or drink.

All the patients had typical gastrointestinal symptoms with a gradual onset and progressive course. The most common clinical feature, present in all patients, was chronic diarrhea, followed by weight loss ($n=75$, 84%), anorexia ($n=74$, 83%), apathy, and irritability ($n=72$, 81%). Less frequent features were occasional vomiting ($n=45$, 51%), dehydration ($n=20$, 22%), and edema ($n=12$, 13%). Celiac crisis occurred in 3 (3%) infants and the disease was precipitated by gastrointestinal infection in 5 (6%). The cause of gastrointestinal infection in these 5 infants was rotavirus ($n=2$), Salmonella enteritidis ($n=2$), and Campylobacter ($n=1$). In all 5 cases, pathohistological examination of the small intestinal mucosa indicated destructive enteropathy.

The type and degree of mucosal damage in the small intestine was assessed in all infants during the initial biopsy. Based on the Marsh classification, 43 (48%) showed total (IIIc), 36 (41%) subtotal (IIIb), and 10 (11%) partial damage (IIIa) (12).

Assessment of disease severity

The assessment of disease severity was based on the occurrence of weight loss, longitudinal growth retardation, anemia, and secondary lactose intolerance. Body weight and body length measurements at diagnosis were documented. Body weight was expressed as the percentage of divergence from the ideal weight for length, and body length in percentiles for the particular age and sex (13). Hemoglobin values were noted and interpreted according to standards, and if they were lower than 110 g/L, the child was considered to have anemia (14). A diagnosis of lactose intolerance was based on the occurrence of osmotic diarrhea, low stool pH (<5.5), and the presence of reductive substances in stool after lactose loading (2 g/kg body weight in 10% solution) (15).

Statistical analysis

Conditional binomial logistic regression was used to determine the risk of delaying the disease onset until the second year of life by early feeding practice modification. After having checked the normality, linearity, and correlation of the data, the multiple linear regression method was used to determine whether there was an association between the age at diagnosis on one hand, and the duration of breastfeeding and timing of gluten introduction on the other. Differences between the groups of infants in terms of age at diagnosis, percentage of divergence from the ideal weight for age and length, percentiles for the corresponding age and the weight, frequency of anemia and lactose intolerance were determined using *t* test, χ^2 test, and one-way analysis of variance, where appropriate. The effect of duration of breastfeeding on the timing of gluten introduction was checked by parametric correlation, whereas the effect of the timing of gluten introduction and the duration of breastfeeding on both weight loss and longitudinal retardation were tested with multiple analysis of covariance. For all statistical analyses OpenStat software for Windows, version 11.9.08 (<http://openstat.en.softonic.com/>), was used.

RESULTS

Participants' basic descriptive statistics are shown in Table 1. The infants were divided into a group ($n=33$) that had been exclusively breastfed at the time of gluten introduction and a group that had not been breastfed at the time of gluten introduction ($n=56$). The infants were also divided into groups based on the timing of gluten intro-

duction: prior to the fourth month ($n=22$), between the fourth and sixth month ($n=63$), and after the sixth month ($n=4$). We conducted conditional logistic regression to estimate the impact of early feeding practices, ie, duration of breastfeeding, timing of gluten introduction, and breastfeeding at the time of gluten introduction, on the risk of disease onset in the first year of life (Table 2). The model with statistical significance influenced the risk ($\chi^2=16.02$; $N=1$; $P<0.001$) and explained 26.5%-35.4% of variance. Finally, duration of breastfeeding reduced the risk of disease manifestation in the first year (odds ratio, 0.665, 95% confidence interval, 0.481-0.891). Multiple linear regression was conducted to analyze the simultaneous effect of duration of breastfeeding and timing of gluten introduction on the age at celiac disease diagnosis. Only duration of breastfeeding was significantly associated with the age at diagnosis (Table 3).

TABLE 1. Descriptive statistics of 89 infants diagnosed with celiac disease at the University Children's Hospital, Belgrade, from 2000 to 2008*

Parameter	Value
Age (months) at celiac disease diagnosis, mean \pm SD,	14.19 \pm 4.80
Duration (months) of symptoms, median (IQR) [†]	2.00 (1.00-3.00)
Age (months) at gluten introduction, mean \pm SD	4.59 \pm 1.24
Duration (months) of breastfeeding, median (IQR) [†]	2.00 (1.00-4.00)
Percent change in body weight, mean \pm SD	-12.57 \pm 10.16
Percentile of body length, median (IQR)	37.5 (10.0-50.0)
Hemoglobin (g/L), mean \pm SD	108.92 \pm 16.15

*Abbreviations: IQR – interquartile range; SD – standard deviation.

[†]When these data were recorded, mothers were asked to report values to the nearest month or half-month.

TABLE 2. Conditional logistic regression for the analysis of association between duration of breastfeeding and occurrence of celiac disease after the first year in 89 infants diagnosed with celiac disease at the University Children's Hospital, Belgrade, from 2000 to 2008*

Variable	B	SE	P	OR	95% CI for OR
Duration of breastfeeding	-0.424	0.157	0.007	0.655	0.481-0.891
Constant	1.307	0.479	0.006	3.696	

*Abbreviations: OR – odds ratio; CI – confidence interval; SE – standard error.

Since the regression model indicated that timing of gluten introduction was not a significant explanatory variable, we adopted a different statistical approach by dividing the infants into groups based on timing of gluten introduction. In the group of infants that had started

consuming gluten prior to the fourth month, the mean age at diagnosis was 11.94 ± 4.26 months, compared with 14.65 ± 4.75 months in the group that had started between the fourth and sixth month, and 22.00 ± 2.00 in the group that had started after the sixth month. The age at diagnosis in the last group was significantly higher than in the two other groups (analysis of variance, $P=0.002$ between the first and third group, $P=0.025$ between the second and third group). The age at diagnosis was not significantly different between the first and second group ($P=0.095$).

Longer breastfeeding delayed the timing of gluten introduction ($r=0.345$; $P=0.01$). Of all 89 infants, 56 (63%) had not been breastfed at the time of gluten introduction and their mean age at diagnosis was 13.04 ± 5.01 months, whereas in 33 infants who had been exclusively breastfed, it was 16.21 ± 3.31 . The difference in the age of diagnosis between the two groups was significant ($t=-2.255$; $df=87$, $P=0.029$).

Anemia was found in 45 of 89 infants (51%) and secondary lactose intolerance in 18 (20%). The majority of infants (75 of 89, 84%) showed weight loss as a symptom, and in 27 (36%) the divergence from ideal weight was greater than -20%. Four (4%) infants were below the fifth percentile of length for the corresponding age and sex. Multiple

TABLE 3. Linear regression for the analysis of association between duration of breastfeeding and delaying the age at celiac disease diagnosis in 89 infants diagnosed with celiac disease at the University Children's Hospital, Belgrade, from 2000 to 2008*

Variable	B	SE	P	r ²	95% CI for B
Duration of breastfeeding	0.49	0.159	0.007	0.129	0.131-0.768
Constant	12.175	0.821	<0.001		10.529-13.822

*Abbreviations: CI – confidence interval; SE – standard error.

TABLE 4. Association between early feeding practices and severity of celiac disease in 89 infants diagnosed with celiac disease at the University Children's Hospital, Belgrade, from 2000 to 2008

Clinical feature	Infants grouped according to						
	breastfeeding status during gluten introduction			age when gluten was introduced into their diet			
	with breastfeeding (n=66)	no breastfeeding (n=33)	P	<4 mo (n=22)	4-6 mo (n=63)	>6 mo (n=4)	P
Anemia (n=45)	10	35	0.693*	14	31	0	0.534*
Lactose intolerance (n=18)	3	15	0.565*	5	13	0	0.610*
Percent of body weight divergence (mean±SD)	13.94±10.84	-15.59±9.00	0.65†	-13.56±8.04	-11.97±1.44	-12.24±9.77	0.890‡
Percentile of body length (mean±SD)	38±19.43	42.31±28.91	0.585†	30.03±22.62	39.18±26.86	54.17±19.09	0.240‡

* χ^2 test.

† t test.

‡ One way analysis of variance.

analysis of covariance showed an association between the percentile of length, percentage of divergence from the ideal weight, and the timing of gluten introduction ($F=3.49$, $P=0.036$). However, neither growth parameter on its own was associated with the timing (percentile of length and timing, $P=0.053$; percentage of weight divergence and timing, $P=0.292$). Similarly, breastfeeding at the time of gluten introduction was not associated with either growth parameter (percentile of length and breastfeeding, $P=0.703$; percentage of weight divergence and breastfeeding, $P=0.756$).

Neither breastfeeding during gluten introduction nor timing of gluten introduction was associated with severity of the disease (Table 4). In addition, we found no association between sex and age at diagnosis ($t=0.711$; $df=87$; $P=0.479$).

DISCUSSION

The main finding of our study was that breastfeeding delayed the onset of celiac disease and that breastfeeding at the time of gluten introduction had a significant protective effect against the disease. Early introduction of gluten, before the fourth month, did not result in an earlier onset of the disease. Neither breastfeeding nor the timing of gluten introduction affected the severity of the disease.

We found that breastfeeding, although generally short, reduced the risk of celiac disease occurrence in the first year of life. Some observational studies claimed that breastfeeding at the time of gluten introduction and longer breastfeeding were associated with delay and prevention of the disease (5). However, Norris et al, in a study including children with high risk for autoimmune diseases, found no protective effect of breastfeeding (6). D'Amico et al

showed that children with celiac disease who had been exclusively breastfed had a delayed onset and less severe disease symptoms than those who had not been exclusively breastfed (7). All of these studies, including ours, clearly point to the importance of breastfeeding for the delay of celiac disease in infancy.

Another important environmental factor is timing of gluten introduction, as well as the amount of gluten in weaning food (6,8,9). In our study, gluten introduction before the fourth month was not associated with an earlier onset of celiac disease, but the introduction after 6 months delayed the disease. These older infants had also been breastfed longer. According to the literature, the optimal period in which food antigens, such as gluten, should be introduced in order to maximize tolerance is between the fourth and sixth month (3). It is unclear whether the predominant risk factor for celiac disease is infant's age at the time of gluten introduction or the amount of gluten consumed (6,9). Some studies point to the importance of continuing breastfeeding at the time of gluten introduction (7,10). It is not clear from either our or other studies whether the timing of gluten introduction is the sole explanatory variable that predicts age at celiac disease onset. Nevertheless, it is safe to conclude that continuing breastfeeding at the time of gluten introduction is important for protection against the disease.

According to our research, longer breastfeeding, especially breastfeeding at the time of gluten introduction delays the onset of the disease. This effect occurs through various mechanisms. A small amount of gluten in breast milk helps induce oral tolerance, as is the case with all other food allergens. Due to protective factors in human milk, gastrointestinal infections are rare and less severe in breastfed infants than in those who are not breastfed (16). This is a very important point because gastrointestinal infections can additionally increase permeability of the gut by causing inflammation or by other mechanisms. In this way, gastrointestinal infections allow that large amounts of gluten fragments cross the intestinal wall and exceed the oral tolerance capacity. In addition, human milk provides many bioactive factors, including antimicrobial and anti-inflammatory agents, enzymes, hormones, and growth factors, many of which are involved in gut maturation and development of the infant's innate and acquired immunity (17,18). Our results further suggest that breastfeeding indirectly delays the age at diagnosis of celiac disease by delaying gluten introduction. One-fourth of the infants in our study had been breastfed at the time of gluten intro-

duction, and they developed the disease later than the infants who had not been breastfed at the time of gluten introduction, which speaks in favor of the protective effect of consuming human milk while introducing other food antigens.

Our results showed that gluten introduction before the fourth month did not affect the onset of disease, but gluten introduction after the sixth month delayed it. We cannot be sure that the effect is solely based on delaying the introduction of gluten, because this group of infants was breastfed longer and was breastfed during gluten introduction. Another possible factor, which we did not measure, was the amount of gluten consumed by the infants.

None of the early feeding practices that we measured was associated with the severity of the celiac disease, as assessed based on the occurrence of anemia, secondary lactose intolerance, weight loss, and longitudinal retardation. All our patients had had symptoms for a long time, and the age at onset was critical for growth, especially for weight loss. Weight loss is an indicator of recent nutritional disturbances, and serious weight loss was detected in nearly one-fourth of our patients. Length in 4 infants was below the fifth percentile, indicating prolonged malnutrition. The frequency of anemia in our study (51%) was higher than the 17% reported in previous research (19). The reason for this may be the younger age of our participants and the long duration of symptoms before the diagnosis. All our patients had severe villous atrophy, which led to the malabsorption of important nutrients and may be the reason why early feeding practices did not influence the occurrence of anemia or secondary lactose intolerance.

The role of environmental factors in the expression of celiac disease, especially early feeding practices, warrants further investigation. In the present study, we analyzed their influence on a homogeneous group of infants diagnosed with classic form of celiac disease. The relationship between certain genetic backgrounds and celiac disease expression is well explained in the study by Mearin et al, especially with regard to the age at onset (20). Although we did not look at their genetic predisposition, all our patients were most likely genetically predisposed to early clinical expression of the disease. In the light of current recommendations for infant feeding, it is important to point out the favorable role of breastfeeding at the time of introduction of new food antigens, including gluten, in helping to establish oral tolerance.

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