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Alteration of secretory IgA in human breast milk and stool samples after the intake of a probiotic – report of 2 cases

Case Report

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Abstract: Only a few studies reveal immunological changes in breast milk after the intake of probiotic and none focus on secretory IgA (sIgA). The aim our report was to investigate the levels of sIgA in human breast milk and stools before and after 4 weeks of probiotic intake in a patient with ulcerative colitis (UC) and a control. The study included 2 lactating women: 1 with UC and 1 control. Both received daily 3.75 billion viable Lactobacillus bulgaricus for 28 days. SIgA was measured in breast milk and stools before and after the probiotic intake. The concentration of sIgA in breast milk before the probiotic intake in UC was 408.5 vs 137.4 μg/ml in contol. Fecal sIgA in UC was 420 vs 274 μg/ml in control. After 28 days of probiotic intake there was a decrease in breast milk sIgA in UC but an increase in control - 266.7 vs 914 μg/ml respectively. There was an increase in fecal sIgA both in UC and control - 674.4 vs 1033 μg/ml. It is tempting to speculate that the different sIgA secretion towards the probiotic may be a result of an altered mucosal immune response in UC.

Keywords: *Human breast milk • Secretory IgA • Probiotic • Ulcerative colitis*

1. Introduction

Secretory IgA (sIgA) is the main antibody in human breast milk. It plays a major role in the prevention of infection and the maturation and maintenance of the oral tolerance in infants – a suppressive phenomenon unquestionably involving numerous mechanisms. Identifiable experimental variables include: genetics; age, dose and timing of postnatal antigen feeding; antigenic structure and composition; epithelial barrier integrity, and the degree of concurrent local immune activation as reflected by micro-environmental cytokine profiles and the expression of co-stimulatory molecules on antigen presenting cells (APC) in the gut. The development of oral tolerance to food antigens and resident gut microbiota is of extreme importance for the development of the local and systemic immune response in infants [1-3].

In the colostrum, sIgA may appear in very high concentrations - up to around 5 g/L - initially. In the following days, the concentration reduces rapidly until the mature milk appears. At the same time, the milk volumes increase, providing similar total amounts of sIgA throughout lactation. The milk sIgA antibodies will be present on the mucosal membranes, primarily in the gastrointestinal tract and to some extent in the respiratory tract, preventing microbes from getting into host tissues [4].

Theoretically, the qualitative and quantitative composition of the gut flora can be modified by the oral intake of probiotics. So if it is possible to modify the gut flora it would theoretically be possible to alter the local and systemic immune response.

Only a handful of studies reveal immunological changes in breast milk after the intake of a probiotic and none of them focusses on sIgA. The aim of our study is

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to monitor the concentrations of sIgA in breast milk and stool samples in a patient with ulcerative colitis (UC) and a control before and after the oral intake of a probiotic.

2. Material and Methods

2.1. Sample

The study included 1 patient with UC and 1 control. The patient with UC was a 29-year old woman with proctosigmoiditis treated with Mesalasine (Salofalk™, Falk pharma GmbH, Freiburg, Germany) at a daily dose of 1000 mg taken orally, and local therapy with 2x250 mg Mesalasine (Salofalk™, Falk pharma GmbH, Freiburg, Germany) suppositories. She had been lactating for already 10 months. The patient had been off of probiotics, antibiotics or hormonal therapy for more than 1 year prior to her enrollment in the study. The rationale for the inclusion of a patient with UC was the assumption that in chronic intestinal inflammation there might be disturbances in the functional link between the mammary gland and the gut. The control was a 34-years old woman who had been lactating for 12 months. Both women had vaginal births, had been off probiotics, antibiotics or hormonal therapy for more than 1 year prior to their enrollment in the study and were at similar lactation stage. They breastfed 5-6 times daily.

2.2. Ethics

The study protocol was approved by the Ethics committees of Medical Science Council at Medical University (Sofia) and the University Hospital St. Ivan Rilsky (Sofia). The study was conducted in line with Declaration of Helsinki. All participants provided informed consent.

2.3. Probiotic

The probiotic used in this study was Normoflor™, Deodan Ltd, Sofia, Bulgaria. The probiotic contains *Lactobacillus bulgaricus - "I. Bogdanov patent strain tumoronecroticance B-51" - ATCC 21815*. The daily dose was 3x2 enterosolvent capsules taken orally (equal to 3.75 billion colony-forming units per gram). The probiotic intake lasted for 4 weeks (28 days).

2.4. SIgA collection and detection

2.4.1. Collection

Human breast milk and stools were collected on day 0 and day 28 of the study. All samples were stored in sterile conditions at -20°C prior to testing.

The breast milk was the first hindmilk excreted after midnight. It was collected by hand expression.

Table 1. Changes in the concentration of sIgA in human breast milk and stools in a control (CL) and a patient with UC before and after the intake of a probiotic.

		Studied persons	
		CL	UC
Body secretion	Day of the study	sIgA (μg/ml)	
Breast milk	Day 0	137.4	408.5
	Day 28	914	266.7
Stools	Day 0	274.2	420
	Day 28	1033	674.4

Stool samples were collected from the first defecation after midnight. All stool samples went through an extraction during which 100 mg of feces were diluted in 5 ml of physiological solution. The solution was mixed vigorously with a vortex for 30 seconds. The mixture was centrifuged at 2500 g for 5 minutes.

2.4.2. Detection

The detection method used in this study was indirect enzyme immunoassay (EIA) - Salimetrics Inc., USA.

The detection was conducted by the following steps. All reagents were brought to room temperature. Fifty μl of diluted goat antihuman sIgA conjugated to horseradish peroxidase was added to the tubes containing the diluted breast milk, stools or standards. After incubation and mixing, an equal solution from each tube was added in duplicate to wells of microtiter plate coated with human sIgA. After another incubation and washing, the substrate tetramethylbenzidine (TMB) was added to the wells and the microtitre plate was incubated again. The reaction was stopped and the optical density was read on a standard ELISA-reader Das srl, Rome, Italy at 450 nm.

The levels of sIgA were calculated using 4-parameter sigmoid minus curve fit.

3. Results

Before the intake of a probiotic, the concentration of sIgA in breast milk in UC was higher that the one in the control: 408.5 μg/ml vs 137.4 μg/ml respectively. At baseline the concentration of fecal sIgA in UC was higher than the one in the control: 420 μg/ml vs 274 μg/ml respectively. After 4 weeks of probiotic intake, the concentration of sIgA in breast milk in UC decreased but there was a 6-fold increase in the control - 266.7 (35% decrease) μg/ml vs 914 μg/ml (6-fold increase). After the probiotic intake there was an increase of fecal sIgA in both women - 674.4 μg/ml (62% increase) in UC vs 1033 μg/ml (4-fold increase) in control (Table 1):

4. Discussion

Secretory IgA together with serum IgA make up about 80% of all immunoglobulin. They are produced mainly in the intestinal mucosa where 2/3 of the immune system is found because of the heavy local microbial exposure there; between 3 and 5g are secreted daily only in the intestinal lumen [4-6]. More sIgA is produced in mucosal linings and excreted with exocrine secretions than all other types of antibody combined [7].

The bacterial and food antigens from the intestinal lumen are sampled by special epithelial cells, the so called M-cells covering the Peyer's patches (PP), multiple lymphocyte aggregations in the gut mucosa. In PP, the antigens are presented to lymphocytes by APC. The lymphocytes are activated by APC so they can start the production of IgA dimers with a joining chain (J-chain). Some of these activated lymphocytes migrate to various mucosal sites, including the intestine, but also to exocrine glands. During late pregnancy, such lymphocytes will migrate, or 'home' to the mammary glands, which is an effect of lactogenic hormones [4,5]. The 'homing' is conducted by the close interaction between the ligands of the homing receptors – the vascular addressins and the receptors themselves. By using the 'homing' mechanism, the gut-sensitized lymphocytes can reach not only the intestine, but also the mammary glands, the synovia, the nose associated lymphoid tissue, the uro-genital tract and any other mucosal surface in the human body [8].

After reaching a mucosal site, the activated lymphocytes initiate the production of IgA dimer antibodies with J-chain, which bind to a poly-Ig receptor. The receptor-IgA complex passes through the cellular compartments before being secreted on the luminal surface of the epithelial cells, still attached to the receptor. Proteolysis of the receptor occurs and the dimeric IgA molecule along with a part of the receptor known as the secretory component are free to diffuse throughout the lumen [9].

By using this mechanism sIgA is excreted by the mammary glands in the milk as the complete sIgA antibody. SIgA is more resistant to proteolysis than serum antibodies and can thus function in the gut. Due to this 'entero-mammaric link' of lymphocyte migration, the breast milk contains sIgA antibodies against the microbiota, food ingredients and other antigens, passing through the mother's gut [4,5]. Since memory lymphocytes are brought into the lactating mammary glands, previous antigenic experiences of the mother are also reflected among the broad set up of various specificities of her milk antibodies [10].

Breastfeeding has a strong protective effect against neonatal infection and sIgA is one of the main protective molecules in human breast milk [11].

The symbiotic relationship between the resident intestinal flora and the gut associated lymphoid tissue is of crucial importance for the maintenance of the intestinal homeostatis. The APC in the gut posseses specific pattern recognitioning receptors (PRRs), which bind to corresponding bacterial ligands, called pathogen associated molecular patterns (PAMPs). Specific PRRs are the toll-like receptors (TLR) of the dendritic and some enteroendocrine cells. The activation of the dendritic cells (DC) is due to the binding between the PRRs with specific bacterial PAMPs. By using this mechanism, the various types of PAMPs can selectively predetermine the activation of T-helper 1 (Th1), T-helper 2 (Th2) or T-regulatory (Treg) lymphocytes by the DC. Thus the Th1, Th2 and Treg can secrete numerous pro- and anti-inflammatory cytokines, which polarize the local and systemic immune response in different directions [1,12-14]. The oral intake of some Bifidobacteria and Lactobacilli have been proven to modify the inflammatory response because of their PAMPs [15,16] by stimulating the synthesis of the anti-inflammatory IL-10 and TGFβ [17,18].

Some authors report that probiotic intake in late pregnancy causes temporary infantile colonization [19]. Previous studies reveal an increase of the anti-inflammatory transforming growth factor β2 (TGF-β2) in breast milk after the intake of a probiotic [20]. We did not find any relevant articles concerning the relation between breast milk sIgA and probiotic intake. Our data demonstrate that at baseline, the concentration of sIgA in the breast milk of the patient with UC is about 3 times higher than that of the control. After the 4-week oral administration of probiotics there was a 35% decrease of breast milk sIgA in UC and 6-fold increase in sIgA in the control. At the same time there was an increase in fecal sIgA in UC – 674.4 (62%) μg/ml and an approximately 4-fold increase in the control (1033 μg/ml). These results eventually point the anatomic and functional link between the mucosal surfaces and raise the possibility of a re-distribution of activated B-lymphocytes from the gut to the mammary glands. The second question is to what extend the probiotic intake might confer the entero-mammaric axis.

It is tempting to speculate that the higher concentration of sIgA in breast milk in ulcerative colitis at baseline is a result of the intestinal inflammation and the increased lymphocyte activation in the gut, resulting in more intensive functional "communication" between the intestine and the mammary glands, thus aiming to protect the infant. This assumption, combined with the

increase of sIgA in the breastfeed and stools of healthy women after the intake of a probiotic and its decrease in breast milk but increase in stools in UC points to the question of a possible reverse re-distribution of activated lymphocytes from the mammary glands to the gut as possible reaction towards the preservation of the biological individuality of the mother against the "invasion" of the probiotic bacteria in UC.

Our results do not allow us to make any clinical recommendations regarding the use of probiotics in lactating patients with UC. The alterations of sIgA in breast milk after the intake of a probiotic and their role in clinical practice, combined with a detailed study of the entero-mammaric link demand for larger and integrative controlled trials with bigger study groups.

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Conflict-of-interest statement

There was no personal or financial relationship that could inappropriately influence the results of the study. There was no conflict of interest during or after the study.

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