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Fluctuation of Specific IgA Antibodies in Human Milk

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ABSTRACT. Cruz, J. R. and Arévalo, C. (Institute of Nutrition of Central America and Panama, Guatemala). Fluctuation of specific IgA antibodies in human milk. Acta Paediatr Scand, 74: 897, 1985.

The concentration of secretory IgA and the levels of IgA specific antibodies against *Escherichia coli* labile-toxin, *Shigella flexneri* 6, and rotaviruses were determined in milk samples obtained serially from women during the first 16 weeks postpartum. The mean concentration of secretory IgA followed the expected pattern; the levels of specific antibodies fluctuated in an unpredictable manner and independently of milk secretory IgA content, becoming undetectable in many instances. Under some circumstances, continued breast-feeding may not guarantee continued intake of antibodies against intestinal pathogens by the breast-fed infant.

Key words: Secretory IgA, specific antibodies, E. coli labile toxin, Shigella, rotaviruses.

Breast-feeding protects against intestinal, respiratory and allergic diseases (1–3). This protective effect is attributed to a variety of defence factors present in human milk such as lysozyme, lactoferrin, specific antibodies and cellular components of the immune-system (4–6). The concentrations of these components in milk vary as lactation progresses: that of lysozyme is lowest during the early postpartum period and increases gradually (7, 8); the concentrations of lactoferrin, cells and IgA follow a reverse pattern, being highest during the first few days after delivery (8, 9).

The major immunoglobulin in human milk is secretory IgA (SIgA) (10); SIgA antibodies against a wide variety of microorganisms and their products such as *E. coli* (11), *E. coli* heat-labile toxin (12), *V. cholera* toxin (12), *Shigella* (13), *Salmonella* (14), rotavirus (15, 16) and poliovirus (17) have been found in human milk. These antibodies are apparently produced in the mammary gland by IgA-committed lymphocytes primed in the intestine (18, 19). Thus, human milk contains antibodies against the pathogens present in the mother's environment; as suggested experimentally (20), these antibodies may prevent microbial adhesion to the intestinal mucosa. In fact, feeding human milk with anti-rotavirus IgA to immunodeficient children suffering from diarrhoea due to rotaviruses, stopped viral shedding and interrupted the symptoms (21).

The entero-mammaric traffic by lymphocytes may be modified by the presence of antigen in the intestine (22): In experimental animals, IgA producing cells migrate preferentially to areas of the intestine in which parasites lodge (23). In the human, the presence of parasites in the intestine induces an increase in the quantity of IgA excreted in faeces (24). Acute viral gastroenteritis results in a transient lymphopenia which occurs concomitantly with the infiltration of the jejunal mucosa by the mononuclear leukocytes (25). Additional information that supports the hypothesis that intestinal infection may sequester lymphocytes and prevent them from homing the lactating mammary gland is provided by polio vaccination of immune women (17): A single dose of live attenuated virus induced a marked decrease in the milk levels of anti-polio IgA antibodies, instead of the expected rise. The temporary absence in milk of specific IgA antibodies against intestinal pathogens may increase the likelihood of an infection by that given pathogen becoming symptomatic.

Recent reports (8, 26) provided evidence that antibody levels in milk fluctuate with time, independently of total SIgA concentration. Goldman et al. (8) reported that seven women

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from Texas, USA, showed at least five different patterns of anti-*E. coli* antibodies from the second to the twelfth week postpartum. These changes could be more dramatic in rural areas of developing countries where intestinal infections among adult women are very common (27).

The present study was undertaken with the purpose of determining the behaviour of milk antibodies against common enteropathogens during the first four months postpartum.

MATERIALS AND METHODS

Twenty lactating women from Santa María Cauqué, a rural community of Guatemala (27), were included in the study. Their age, weight and height are shown in Table 1. All the participants were asked to donate milk samples (≈10 ml) on days 5 through 9 and on day 11 after parturition, and weekly thereafter for 16 additional weeks. The specimens were kept frozen at −20°C and not thawed until immediately before their analysis, when they were freed of fat and cells. Total SIgA and IgA specific antibodies were determined by means of the enzyme-linked immunosorbent assay (ELISA) (28–30) using microplates (M-24, Dynatech Microtiter U plates). For anti *E.coli* labile toxin (ETLT), burro anti-cholera serum, and cholera toxin (kindly provided by Dr J. Robbins, USA FDA) were used to coat the plates. Goat hyperimmune serum against rotavirus and rotavirus type 2, strain D, obtained from the USA NIH through the Pan American Health Organization, were employed for anti-rotavirus (RTV) antibodies. *Shigella flexneri* 6 somatic antigens were used for the direct ELISA. Goat anti-human antiserum coupled with alkaline phosphatase (TAGO, Inc., Burlingame, California, USA) and p-nitro-phenyl phosphate (SIGMA, St. Louis, Missouri, USA) were used as the detection system. Serial dilutions (1 : 2–1 : 1 024) of the samples were tested; the reciprocal of the highest dilution having a positive reaction (optical density ≥0.2 above background) was considered the titer. Two-way analysis of variance and Spearman correlation tests (31, 32) were carried out for the statistical analysis.

Table 1. *Characteristics of the women included in the study*

Subject no.	Age (yrs)	Height (m)	Initial weight (kg)	Final weight (kg)
1	29	1.39	35.1	36.8
2	24	1.44	48.1	48.6
3	16	1.43	49.1	46.4
4	25	1.46	46.4	47.3
5	29	1.38	44.1	44.1
6	27	1.47	51.8	53.2
7	22	1.41	50.9	53.2
8	26	1.43	42.7	42.7
9	27	1.39	46.4	48.6
10	21	1.39	46.4	44.1
11	27	1.37	38.2	38.2
12	21	1.49	44.1	44.1
13	21	1.42	48.2	43.4
14	24	1.43	44.1	40.4
15	18	1.44	43.2	41.8
16	27	1.47	55.9	55.9
17	24	1.42	55.0	50.9
18	28	1.47	44.1	46.4
19	33	1.52	56.8	57.7
20	19	1.40	54.5	54.5
$\bar{x}$	24.4	1.431	47.26	46.92
SD	4.2	0.397	5.74	5.88

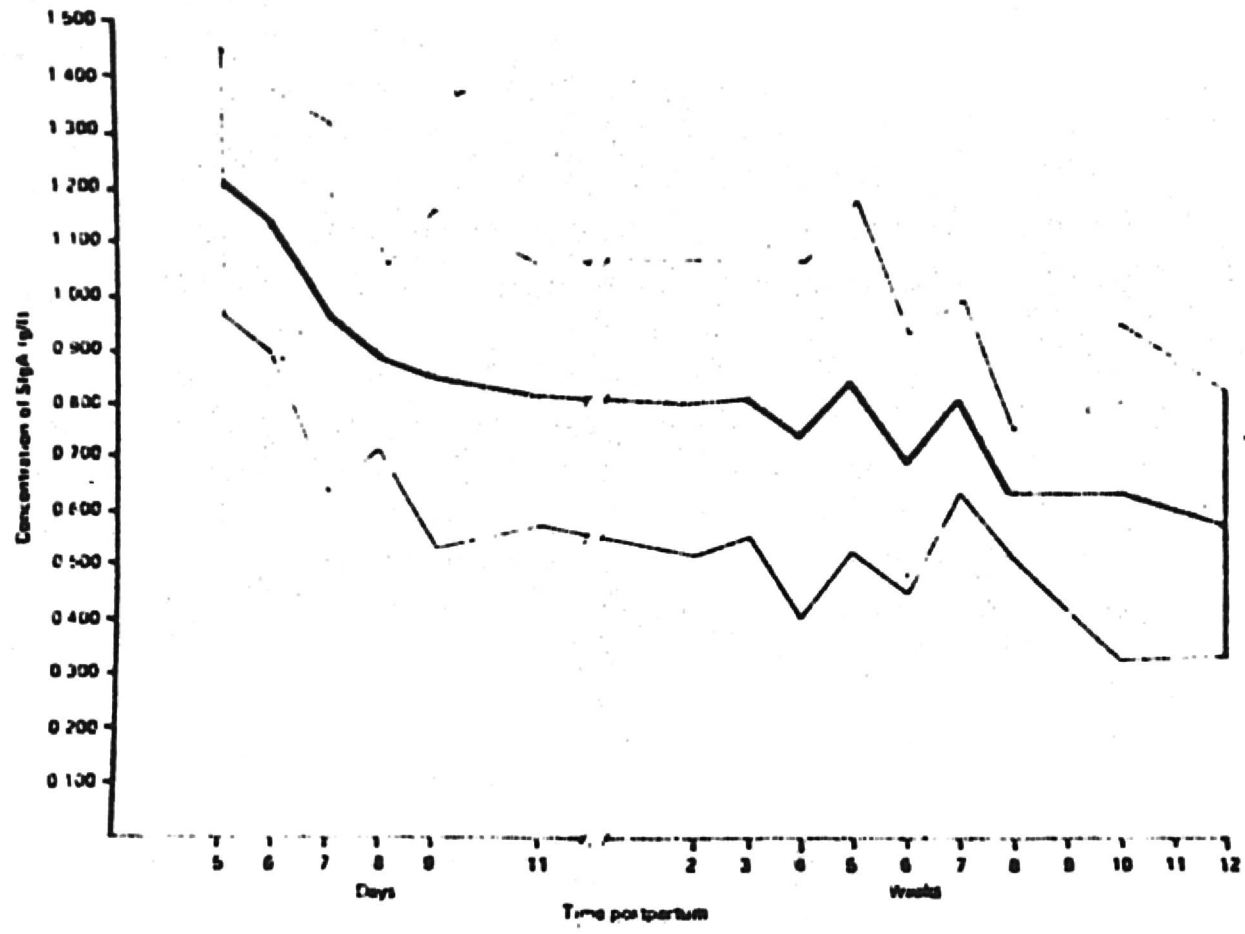

Fig. 1. Concentration (mean ± standard deviation) of secretory IgA in milk specimens collected 5 days – 12 weeks postpartum from 10 Guatemalan women.

RESULTS

The mean concentration of SIgA in colostrum and milk samples collected from 10 of the women followed the expected pattern (Fig. 1): It was at its highest peak in the early samples (1.215 g/l) with a rapid drop during the following six days (0.851 g/l), reaching the lowest value in the late specimens (0.585 g/l). The analysis of variance showed that these differences were significant ($F=1.859$, $p<0.05$). The titers of specific antibodies fluctuated over time with variable and unpredictable patterns in each of the individuals. Fig. 2 summarizes the findings in two of the subjects. In several cases, the levels of specific IgA antibodies became temporarily undetectable or reached very low titers. These fluctuations were independent of the total milk SIgA concentration (Table 2) and of the other specific antibodies tested (Table 3). In some instances, a rise in the milk content of one of the specific antibodies was accompanied by a decrease of another.

DISCUSSION

The results of the present investigation clearly demonstrate that the levels of specific antibodies in human milk of women from rural Guatemala fluctuate as lactation progresses. The changes observed did not follow a predictable, uniform pattern and were not associated with changes in the total SIgA concentration. These observations support the general idea that the findings are not influenced by differences in milk volume produced by the individuals at each period of study or by sampling techniques. If these factors were responsible for the observed fluctuations, we should have detected interdependency between total milk SIgA concentration and the titers of the various antibodies tested.

However, this was not the case, as shown by the results of the Spearman correlation test (Tables 2 and 3).

Goldman and coworkers (8) reported that the levels of antibodies against *E. coli* fluctuate in milk specimens of women living in the United States. We postulated that these changes, if associated with antigenic exposure at the intestinal level, could be more

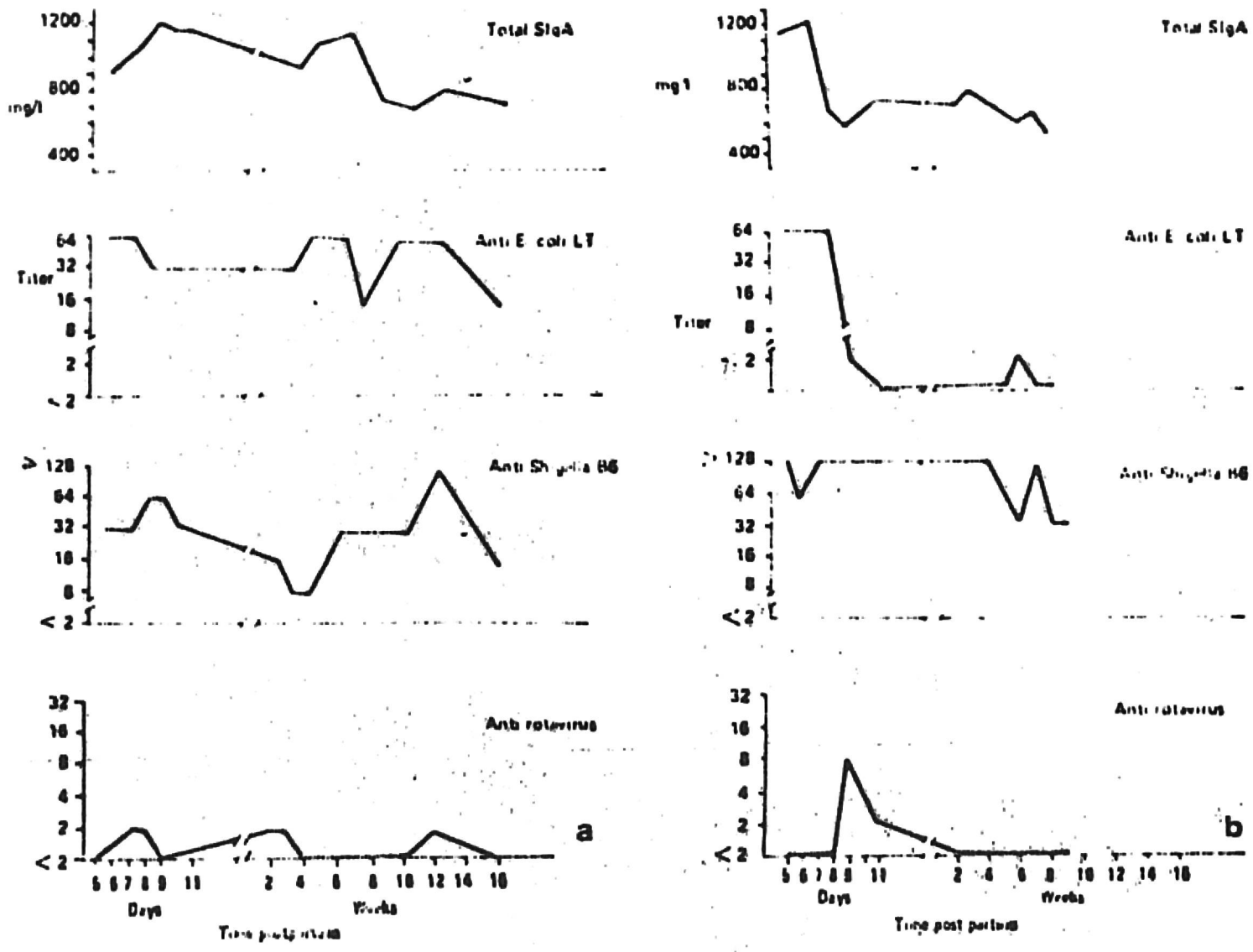

Fig. 2. Concentration of secretory IgA and levels of IgA specific antibodies against. *E. coli* LT, *Shigella* B6 and rotaviruses in milk specimens collected from two of the mothers.

Table 2. *Correlation between SIgA concentration and specific antibody levels in human milk*[a]

When observed $r_s \leq$ critical r_s value IgA antibody levels are independent of total SIgA. Dependent levels are italicized

Subject no.	Observed correlation (r_s) value			Critical r_s value ($p = 0.975$)
	E. coli LT	*Shigella*	Rotavirus	
1	0.500	0.308	0.410	0.50
2	0.530	0.406	0.151	0.58
3	0.033	0.424	0.270	0.52
4	0.494	0.510	0.470	0.52
5	*0.810*	0.000	0.220	0.55
6	0.387	*0.620*	0.230	0.52
7	0.004	0.415		0.55
			0.058	0.61[b]
8	0.056	0.055	*0.699*	0.55
9	0.399	*0.580*	*0.587*	0.50
10	0.145	0.160	0.080	0.52

[a] Spearman correlation test.
[b] For RTV only.

dramatic among women of areas with poor sanitation and high prevalence of enteric infections. Most of the mothers included in this study reflected drastic declines in at least one of the antibodies determined; even more important is the finding that the specific antibodies, especially those directed against rotaviruses, became temporarily undetectable in the majority of the subjects. The temporary absence of specific IgA antibodies against common enteropathogens may increase the likelihood of an enteric infection symptomatic in the breast-fed infant. Glass et al. (29) have shown that high levels of IgA antibodies in milk are associated with protection against cholera, even in the presence of *V. cholerae* in the intestine of the breast-fed infant. The implications for the breast-fed children of the observed fluctuations in specific IgA antibodies in milk should be determined in prospective studies, since continued breast-feeding may not guarantee a continuous ingestion of IgA antibodies against enteropathogens in sufficient quantities to be protective. Moreover, the factors responsible for the fluctuations of milk antibodies should be further investigated. In this regard, intestinal infections, either symptomatic or accompanied by disease, should be considered of high priority.

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Table 3. *Correlation among specific antibody levels in human milk*[a]

When observed r_s value≤ critical r_s value antibody levels are independent of each other. Interdependent titers are italicized

Subject no.	Observed correlation (r_2) value			Critical r_s value (p=0.950)
	EcLT/*Shigella*	EcLT/RTV	*Shigella*/RTV	
1	*0.775*	0.377	0.150	0.426
2	0.238	0.015	0.090	0.459
3	0.195	*0.467*	0.310	0.443
4	0.308	*0.787*	0.237	0.443
5	0.000	0.073	0.041	0.459
6	*0.500*	*0.689*	*0.658*	0.443
7	0.200	0.170	0.390	0.478
8	0.340	0.080	0.130	0.459
9	*0.450*	*0.600*	0.030	0.426
10	0.120	0.160	0.420	0.443
11	0.268	0.340	*0.770*	0.496
12	0.441	*0.743*	0.449	0.459
13	*0.677*	*0.527*	*0.621*	0.443
14	*0.559*	*0.468*	*0.865*	0.459
15	0.054	*0.695*	*0.901*	0.443
16	*0.513*	0.241	*0.747*	0.426
17	*0.638*	*0.701*	*0.676*	0.443
18	0.323	0.124	0.001	0.389
19	0.284	*0.859*	*0.504*	0.443
20	*0.709*	*0.720*	*0.969*	0.426

[a] Spearman correlation test.

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