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ORIGINAL ARTICLE

Exposures influencing total IgA level in colostrum

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Immunoglobulin A (IgA) is a predominant immunoglobulin present in human breast milk and is known to play an important role in infant gut immunity maturation. Breast milk composition varies between populations, but the environmental and maternal factors responsible for these variations are still unclear. We examined the relationship between different exposures and levels of IgA in colostrum. The objective of this study was to examine whether exposures analysed influence levels of IgA in colostrum. The present study used 294 colostrum samples from the MecMilk International cohort, collected from women residing in London, Moscow and Verona. Samples were analysed in automated Abbott Architect Analyser. We found an inverse correlation between time postpartum and colostrum total IgA level ($r = -0.49$, $P < 0.001$). Adjusting for maternal parity, smoking, fresh fruit and fish consumption and allergen sensitization, multiple regression model showed that IgA levels were influenced by colostrum collection time ($P < 0.0001$) and country of collection ($P < 0.01$). Mode of delivery influence did not appear to be significant in univariate comparisons, once adjusted for the above maternal characteristics it showed a significant influence on total IgA ($P = 0.01$). We conclude that the concentration of IgA in colostrum drops rapidly after birth and future studies should always consider this factor in analysis. IgA concentration varied significantly between countries, with the highest level detected in Moscow and lowest in Verona. Mode of delivery effect should be confirmed on larger cohorts. Further work is needed to determine ways to correct for IgA decline over time in colostrum, and to find the cause of variations in IgA levels between the countries.

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Introduction

Breastfeeding is known to be beneficial for maternal health as well as the developing infant, influencing short- and long-term health outcomes,[1] although it's protective effect on some diseases, such as allergy is still under debate due to conflicting evidence.[2] Breast milk composition is very diverse and contains a variety of cells, cytokines, signalling molecules, food antigens and microbes.[3–5] Conflicting data in regards to breastfeeding protective effects may be explained by individual variations of human milk composition, which varies greatly among mothers.

According to various sources, colostrum is a special milk that is secreted in the first 2–3 days[6] or 1–5 days[7] after delivery. It is known that the so-called colostrum contains higher levels of protein and lower lactose content in comparison with mature milk. At the same time colostrum is particularly rich in immunological components such as IgA, lactoferrin, a variety of growth factors and cells.[8,9]

Passive immunoglobulin (Ig) transfer from a mother to an infant is needed to compensate for immune 'immaturity' and this phenomenon is achieved by Ig transfer through breast milk during feeding. Human milk provides additional immune-active factors including IgA, anti-microbial peptides and cytokines, in addition to growth factors and essential nutrients, which promote development of the infant gut-associated lymphoid tissue.[3,10] Cytokines from colostrum and breast milk are not destroyed in the stomach; some are protected by being bound to other molecules such as soluble components of their receptors.[11] A number of protease inhibitors that are present in human milk have been proposed to limit the activity of pancreatic enzymes,[12,13] and in the critical first few weeks of life the gastric pH is high that considerably reduces peptic digestion.

A predominant type of immunoglobulin in the milk is very dependent on animal species, the same applies to the proportion of immunoglobulin in milk. While milk from a large variety of animals contains up to 75% of IgG, human milk has only about 2%. In human colostrum and mature milk, IgA is the predominant immunoglobulin, accounting for 88–90%.[14]

Various studies have demonstrated a number of maternal and environmental factors (e.g. parity, age, smoking, allergic status, country of residence, diet, etc.)[15–17] that influence human milk constituents but data is controversial and the degree of these effects is still unclear. Environmental and/or maternal factors

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influencing levels of immune active molecules have been reported previously but very limited knowledge on total IgA is available.

In this study we attempted to investigate the influence of exposures known to have potential to influence breast milk immune active molecules, such as parity, labour type, allergic sensitization, diet, smoking exposure, country of collection and time of colostrum collection postpartum, on the levels of total IgA in colostrum, analysing samples collected from different geographical regions.

Materials and methods

Study population

For the purpose of this study we used samples from the MecMilk cohort. Colostrum samples (day 0–6) collected from 294 mothers, recruited from hospital postnatal wards, from London, UK $n = 61$; Moscow, Russia $n = 161$ and Verona, Italy $n = 72$ were used for this study.

Women who consented to the study underwent an allergy skin prick test (SPT) and answered a 10-min questionnaire regarding their medical history, particularly for the presence of any allergic or other immunological disease or treatment. We used the following allergen solutions for SPT: histamine positive control, negative control, house dust mite (*Dermatophagoides pteronyssinus*), cat fur (*Felix domesticus*), five grasses mix, ryegrass, peanut, hazelnut, whole hen's egg (Stallergenes, Anthony, France) and cow's milk raw (ALK-Abello, Hørsholm, Denmark). Skin pricking was performed using lancets (ALK-Abello).

We considered a mother to be sensitized if she had a positive control wheal of ⩾3 mm and any of the other pricks wheal ⩾3 mm. Mothers were asked about their dietary preferences (probiotics, fish and fresh fruits intake frequency), antibiotic intake during pregnancy and smoking exposure. Medical files were reviewed to extract any relevant health information, and subjects were asked to provide samples of breast milk as described below.

Colostrum was collected once upto 6 days postpartum. Breast milk samples were collected by manual expression by collecting the drip from the contralateral breast since last feeding and stored at −80°C in a freezer until analysis.

Inclusion and exclusion criteria

In order to be eligible for inclusion the following criteria should be met:

Inclusion criteria: mothers willing to participate with healthy term infants.

Exclusion criteria: premature infants (<37 weeks), mothers taking immunosuppressive medications, any major birth defects, neonatal intensive care admission, intrauterine growth restriction or any other significant illness.

Ethics and research and development approval

Participant recruitment and sample collection were conducted following ethical approval by local research ethics committees in three countries involved into the project: West London REC 3, (ref. no. 10/H0706/3), the Ethical Committee of the Azienda Ospedaliera di Verona (approval no. 1288) (Italy) and the Moscow Institute of Paediatrics and Child Health of Ministry of Health of Russian Federation (approval no. 1-MS/11) (Russia). All participants provided a member of the research team with a written informed consent before enrolment in the study.

IgA analysis

Upon thawing, colostrum samples were centrifuged at 1500 ***g*** for 15 min at 4°C. Fat layers were trimmed using pipette tips. Levels of total IgA in colostrum were analysed with an automated Abbott Architect Analyser®, using a turbidimetric procedure; Beckman Array nephelometer. Some studies used nephelometer as an instrument for milk analysis.[18]

Statistical analysis

We tested differences between the groups using Mann–Whitney U and Kruskal–Wallis tests for continuous data. Pearson χ^2 test was used for categorical data. Multivariate linear regression using analysis of variance was used to provide mutual adjustment for confounding factors, such as: parity, smoking exposure, fresh fruit and fish consumption, colostrum collection time postpartum, maternal sensitization, country of residence and mode of delivery (caesarean section *v.* vaginal delivery). Results were considered significant when P-values were <0.05. Statistical analysis was carried out using SPSS Software Version 22.0 and GraphPad Prism Version 6.0.

Results

Among mothers who provided samples, women from Verona were older compared with both Moscow and London ($P < 0.01$), while the rate of caesarean sections and sensitization was significantly higher in London ($P < 0.01$). Mothers from Moscow were exposed to smoke much more often and had less fish and fresh fruits in their diet when compared with other countries. Time of colostrum collection postpartum varied among the countries with later collection in London and earliest in Moscow ($P < 0.01$). Detailed demographic data are presented in Table 1. Crude differences in the levels of total IgA depending on exposure are presented in Table 2.

We found a significant difference in the concentration of total IgA when compared between the countries (Fig. 1), with highest levels in the colostrum of women from Moscow [9.55 g/l (S.D. = 10.64)], followed by London [6.67 g/l (S.D. = 8.09)] and lowest levels in colostrum from Verona mothers [3.12 g/l (S.D. = 4.85)] ($P < 0.001$). We also detected a rapid decrease of total IgA concentration in colostrum over time ($r = -0.49$, $P < 0.001$) (Fig. 2).

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Table 1. *Study population demographic data*

	L	M	V	*P*-value (three countries)	*P*-value (two countries)
Self-reported allergy (maternal) [*n* (%)]	30 (18/61)	27 (44/161)	33 (24/72)	0.65[a]	L > M ($P < 0.01$)[a]
Positive maternal sensitization [*n* (%)]	30 (18/61)	9 (14/161)	13 (9/72)	<0.01*[a]	
Maternal age (years) [mean (S.D.)]	32.4 (4.62)	29.6 (4.55)	37.7 (5.35)	<0.01*[b]	V > L ($P < 0.01$)[c]
					V > M ($P < 0.01$)[c]
					L > M ($P < 0.01$)[c]
Mode of delivery [*n* (%)]					
Vaginal	67 (41/61)	87 (139/160)	82 (59/72)	<0.01*[a]	M > L ($P < 0.01$)[a]
Caesarean section	33 (20/61)	13 (21/160)	18 (13/72)		V > L ($P = 0.05$)[a]
Baby weight (g) [mean (S.D.)]	3465.4 (516.3)	3524.9 (444.5)	3329.1 (487.9)	0.01*[b]	M > V ($P < 0.01$)[c]
Previous deliveries [*n* (%)]					
Primiparous	54 (32/59)	42 (66/158)	38 (27/71)	0.15[a]	
Multiparous	46 (27/59)	58 (92/158)	62 (44/71)		
Exposed to smoke [*n* (%)]	26 (16/61)	59 (94/160)	32 (23/72)	<0.01*[a]	M > V ($P < 0.01$)[a]
					M > L ($P < 0.01$)[a]
Antenatal antibiotics [*n* (%)]	18 (11/61)	12 (18/151)	21 (15/72)	0.19[a]	
Daily fresh fruit intake [*n* (%)]	97 (58/60)	65 (104/161)	81 (58/72)	<0.01[a]	L > V ($P = 0.01$)[a]
					L > M ($P < 0.01$)[a]
					V > M ($P < 0.01$)[a]
Fish intake at least once a week [*n* (%)]	75 (45/60)	49 (79/161)	70 (49/70)	<0.01[a]	V > M ($P < 0.01$)[a]
					L > M ($P < 0.01$)[a]
Time of colostrum collection (hours postpartum) [mean (S.D.)]	67.7 (31.8)*	50.9 (14.2)*	57.7 (26.8)*	<0.01*[b]	L > M ($P < 0.01$)[c]
					V > M ($P = 0.02$)[c]

L, London; M, Moscow; V, Verona.
Following approaches were used for statistical analysis:
[a]Pearson χ^2 test has been used.
[b]Kruskal–Wallis test has been used.
[c]Mann–Whitney *U*-test has been used.

Table 2. *Crude differences in total IgA levels depending on exposure*

Exposure	Total IgA [mean (S.D.) concentration (g/l) in each group]			*P* value
Site of collection	L	M	V	Overall <0.01**
	6.67 (8.09)	9.55 (10.64)	3.12 (4.85)	M > L 0.05*
				M > V <0.01**
				L > V <0.01**
Parity	Primiparous	Multiparous		0.40
	8.77 (10.37)	7.05 (9.14)		
Smoking exposure	Exposed to cigarette smoke	Not exposed to cigarette smoke		<0.01**
	6.72 (8.59)	9.05 (10.64)		
Fresh fruit consumption	Every day	Less than daily		0.01**
	6.78 (8.99)	11.28 (10.99)		
Fish consumption	At least once a week	Less than once a week		0.25
	7.95 (10.30)	7.52 (8.49)		
Maternal sensitization	Sensitized	Not sensitized		0.39
	6.89 (8.25)	8.04 (9.99)		
Mode of delivery	Vaginal	Caesarean section		0.22
	8.55 (10.04)	4.10 (6.20)		

IgA, immunoglobulin A; L, London; M, Moscow; V, Verona.
Univariate analysis results (Mann–Whitney *U*-test have been used for comparison between each of two groups. Kruskal–Wallis test have been used for comparison between three sites of collection).

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Multiple linear regression highlighted time of sampling postpartum as having the most significant impact on the levels of total IgA ($\beta = -0.16$, $P < 0.001$). Country of collection ($\beta = -2.74$, $P < 0.01$) was another factor to influence levels of total IgA in maternal colostrum. Mode of delivery ($\beta = -4.67$, $P = 0.01$) was a third factor showing significance within the model (Table 3). However, this significance was not confirmed with univariate analysis [IgA mean concentration in colostrum of the mothers giving birth by vaginal delivery 7.68 g/l (S.D. = 9.35) and 6.07 g/l (S.D. = 9.58) in those having caesarean section; $P = 0.25$].

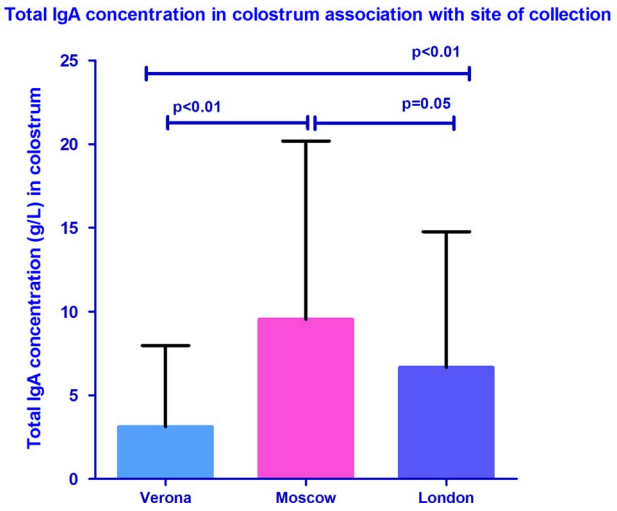

Fig. 1. Mean (S.D.) levels of total IgA (g/l) in colostrum of women from London, Moscow and Verona. IgA, immunoglobulin A.

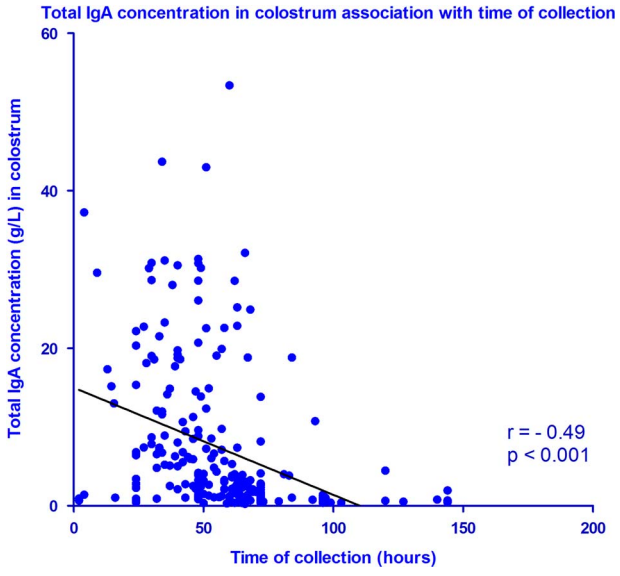

Fig. 2. Total IgA concentration (g/l) in colostrum decline over time. IgA, immunoglobulin A.

Discussion

The results of this study of 294 women from three countries, representing western, eastern and southern Europe, identified three significant determinants of total IgA levels in colostrum: method of delivery, colostrum collection time postpartum and geographical location. Colostrum IgA levels declined with time following birth. They were lowest following caesarean delivery and among women living in Verona. Levels of immune active factors such as IgA, in colostrum and breast milk, possess the potential to influence infant immune development. There is evidence that a number of maternal interventions and environmental exposures may influence colostrum and breast milk constituents and as such, can alter infant immune outcomes. We had an opportunity to examine some of these factors on breast milk IgA content in this multi-country study and they will be discussed in greater detail in the ensuing paragraphs.

Levels of immune active factors in colostrum and breast milk possess the potential to influence infant immune development and we believe that human breast milk composition may be modifiable. There is evidence that a number of maternal and environmental exposures may influence maternal colostrum and breast milk constituents, and even that such interventions can alter infant immune outcomes, although existing evidence is still conflicting.

Time of colostrum collection

Levels of total IgA in colostrum in our study were inversely correlated with time of collection postpartum and they declined rapidly after birth. Our results confirm earlier reports of IgA decline in breast milk over time after birth.[19,20] IgA is a predominant type of immunoglobulin in human colostrum and mature milk; it is an essential immune active component that provides protection against infection. During the 1st weeks of life, infant immunity is not fully developed and mucosal production of IgA in the gut is insufficient.[21] Colostrum provides the nursing infant with a bolus of IgA to prevent significant bacterial uptake by the gut and support the undeveloped intestinal barrier. With

Table 3. *Exposures influence on total IgA level in colostrum, according to final model of multiple linear regression analysis*

Exposure	β	S.E.	*T*	*P* value
Colostrum collection time (hours postpartum)	−0,16	0.03	−5.38	<0.001
Country of collection	−2,74	0.97	−2.82	<0.01
Mode of delivery	−4,67	1.82	−2.56	0.01
Exposures excluded in the process of analysis: maternal sensitization, smoking exposure, fish intake, fruits intake				Not significant

IgA, immunoglobulin A.

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increasing production of mucosal IgA by the infant and maturation of gut immunity, breast milk IgA requirements lessen and likely explain declining levels over time.[21]

Country of collection

Data from various trials suggests that colostrum and breast milk constituents may be influenced by the country of origin and a number of environmental conditions specific for a particular geographical region. In our study we found significant differences between populations, with the highest IgA levels present in colostrum of mothers from Moscow and the lowest levels in the colostrum of mothers from Verona. Recently, Orivuori *et al.*[16] studied breast milk from four countries of continental Europe and Finland and found lowest levels of secretory IgA (sIgA) in mothers from Germany. The reasons behind regional differences are not clear. One possible explanation in our study is a higher microbial load in Russia when compared with Italy and the United Kingdom. Tomicic *et al.*[17] detected higher levels of sIgA in the breast milk of Estonian mothers compared with Swedish mothers; endotoxin levels in house dust were found to be higher in Estonian homes. In Orivuori *et al.*'s[16] study, regional differences were no longer apparent following adjustment for maternal prenatal exposure to barns, cats and farm animals. Another reason behind this difference may be a much more homogenous population in Verona in comparison to two capital cities. Accumulated evidence suggests that future breast milk research should be more focused on international collaborations providing multicentre studies in order to assess epigenetic influence on breast milk composition.

Mode of delivery

Colostrum IgA levels were significantly lower following caesarean delivery, independent of time after birth, country of origin and other tested covariates. However, we failed to reveal a significant difference between total IgA concentration in colostrum of mothers that delivered vaginally compared with women having caesarean sections, when both groups were compared using univariate analysis. This inconsistency in results may be explained by a small sample size. In the populations studied, most of the mothers had vaginal delivery and only a small proportion had caesarean section. This limitation does not allow us to make proper conclusions on this matter.

While caesarean delivery can delay breastfeeding initiation,[22] little is known about its influence on breast milk composition. Dizdar *et al.*[23] reported lower levels of protein content in colostrum of mothers having caesarean section. In a small study of 19 women in 1981 by Kulski *et al.*,[24] colostrum IgA concentrations did not vary by type of delivery. However, Striker *et al.*[20] found that mode of delivery affected colostrum IgA concentrations in 82 women; 75% of mothers who had undergone labour before caesarean section exhibited IgA levels greater than the mean compared with one-third of mothers who delivered vaginally; 50% of elective caesarean-sectioned women had high colostrum IgA levels. Two studies have reported the microbiota of colostrum following caesarean section to contain fewer bifidobacteria than after vaginal delivery;[25,26] breast microbiota profiles following emergency caesarean were more similar to vaginal delivery. Further research is required to determine the impact of caesarean delivery on breast milk composition and subsequent interactions between breast milk microbiota and immunoactive factors.

Maternal sensitization

Our findings also do not provide a definitive answer on whether maternal sensitization or allergic status influences levels of IgA in human colostrum and breast milk, as it does for serum levels of immune markers. We failed to reveal any differences in colostral total IgA levels between sensitized and non-sensitized mothers. Our results are in agreement with a study done by Savilahti *et al.*[27] a decade ago, reporting no difference in total IgA concentration and cow's milk-specific IgA levels between allergic and non-allergic mothers with similar findings published by Orivuori *et al.*[16] recently.

Parity

We failed to find an association of maternal parity on levels of total IgA in colostrum. Groer *et al.*[28] observed large differences in breast milk immunoactive factors in relation to parity; cytokine IL-10 levels were highest in primiparous women. Similar associations have been reported for other breast milk cytokines, such as IL-7[29] and IL-8.[30] On the other hand, Kawano *et al.*[31] observed higher breast milk IgA levels on the 3rd day after birth in primiparous *v.* multiparous women. Positive correlations between mature milk IgA and parity have been reported in Bachour *et al.*'s and Orivuori *et al.*'s studies.[15,16] More research is needed to add more clarity on this subject.

Maternal diet

There is some evidence in the literature that dietary interventions may influence breast milk composition. Prescott *et al.*[32] showed that *Lactobacillus rhamnosus* HN001 or *Bifidobacterium lactis* HN019 supplementation resulted in a higher levels of IgA in breast milk compared with placebo; Kuitunen *et al.*[33] found that probiotic intervention was associated with decreased casein IgA levels in breast milk. Fish intake is also known to have a strong influence on breast milk composition as demonstrated in a Chinese study in which higher concentrations of sIgA in colostrum were observed among women residing in river and lake regions compared with those from coastal and inland areas.[19] While our study did not administer detailed diet history questionnaires, maternal dietary habits during pregnancy were assessed in regards to intake of fresh fruits and fish. We failed to reveal a significant influence of maternal prenatal diet on total IgA concentration in colostrum.

Smoking

Finally, we found higher levels of total IgA in the colostrum of mothers not exposed to tobacco smoke ($P<0.01$), although

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this significance disappeared in multivariate analysis. Assessing the influence of environmental tobacco smoke on breast milk immune composition Bachour *et al.*[15] found breast milk sIgA levels to be reduced by 27% in smoking mothers. Conflicting findings have been reported for cytokine levels in colostrum and breast milk according to maternal smoking status.[34,35]

Strength and limitations of the research

This study is one of the very few attempts to assess levels of total IgA in colostrum samples collected from three different geographical locations. All samples have been collected using similar standard operating procedures for collection and storage and were analysed in the same laboratory. The main limitation of our research is the cross-sectional design of our study since we collected only a single colostrum sample from each mother. In future, research breast milk samples should be collected longitudinally. A further limitation of this study was the significant differences in maternal age, allergic sensitization, parity, dietary patterns, mode of delivery, smoking exposure between the cohorts from the three countries where samples were collected. We attempted to adjust for all these factors, including them in a linear regression model. In our study dietary preferences were assessed, asking women questions on the frequency of fish and fresh fruits intake. However, no detailed dietary questionnaires were used. Methodological limitations may be related to IgA subclass detection since total IgA concentration was assessed but not individual IgA subclass. Owing to the small volume of colostrum samples we were not able to provide this analysis.

Summary

In this study we aimed to assess maternal and environmental factors influence on total IgA concentration in colostrum. We found that three factors influencing levels of total IgA in colostrum to a significant extent were time of colostrum collection postpartum, country of residence and mode of delivery. Our results support earlier reports that country of residence influences levels of total IgA highlighting a need for international prospective trials focused on assessment of genetics, dietary habits and environmental factors influence on breast milk composition. Our data illustrates the need for large prospective international studies on breast milk research.

Levels of total IgA declined over time and we suggest that it represents a high demand of a newborn baby for IgA in order to compensate for gut immunity 'immaturity'. With subsequent immunity maturation, the need for additional IgA decreases, accompanied by the decline of breast milk IgA concentration. Although type of delivery was shown to be one of the factors influencing colostral IgA levels, we failed to find a similar difference in a univariate analysis, due to a small sample size. Larger cohorts specifically focused on labour influence on breast milk composition are needed in order to make a proper evaluation.

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Conflicts of Interest

None.

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