Comparison of colostrum TGF-β2 levels between lactating women in Japan and Nepal

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Summary

Background: **Maternal milk-borne transforming growth factor (TGF)-β plays a potential role in the development of the mucosal immune system in infants. However, it remains unclear what factors determine TGF-β levels in breast milk. We hypothesized that microbial pressures during pregnancy might affect the expression levels of TGF-β in colostrum.**

Objectives: **This study compared TGF-β2 levels in colostrum of lactating women living in Japan and Nepal with contrasting hygiene statuses. Additionally, we identified environmental and intrinsic factors influencing TGF-β levels in colostrum.**

Methods: **Breast milk samples and structured questionnaires were collected from 80 women living in Japan and 208 women living in Nepal. A robust regression model was used to identify factors associated with colostral TGF-β levels.**

Results: **Analysis using the Mann-Whitney *U* test showed that TGF-β levels were significantly higher in Japanese women than in Nepalese women. Japanese women who consumed animal milk daily during pregnancy and had atopic dermatitis expressed lower levels of TGF-β in colostrum, as compared to Japanese women who did not. Among Nepalese women, large family size and higher birth order were associated with lower TGF-β levels and women who gave birth to infants with low birth weight had higher expression of TGF-β levels in milk than women who gave birth to infants with normal birth weight.**

Conclusion: **The results suggest that induction of TGF-β levels in colostrum depends on differences in the ethnicity of lactating women. Consumption of animal protein and parturition characteristics may affect TGF-β levels in breast milk, and may explain differences in these levels in breast milk between countries. *(Asian Pac J Allergy Immunol 2014;32:178-84)***

Key words: *colostrum, hygiene, Japan, Nepal, TGF-β2*

Introduction

A higher incidence of allergic diseases in developed countries may be explained by the "hygiene hypothesis", which was first described by Strachan.[1] This hypothesis suggests that improved public hygiene and sanitation facilities lead to an overall decrease in the exposure to microbial stimulation during infancy and early childhood. In addition, these improved conditions suppress the stimulation of Th1 immunity and increased Th2 responses to environmental stimuli, including allergens.[2] The incidence of allergic diseases during infancy may also be associated with maternal immune function transferred to the offspring through breast feeding. As breast milk contains a number of immunological substances, including IgA and cytokines, which play an important role in the protection of the offspring against the morbidity induced by infectious diseases.[3,4] Therefore, expression levels and compositions of immunological substances in breast milk should be properly regulated in the maternal body by various factors to promote survival of the offspring over microbial

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burden. However, the association between maternal microbial exposure and the expression levels of immunological substances in breast milk remains poorly understood.

Transforming growth factor (TGF)-β is a central cytokine that regulates mucosal immune responses such as IgA production and the induction of oral tolerance.[4,5] In human milk, TGF-β is abundantly present[3,4,6] and plays a potential role in the development of the mucosal immune system in infants.[7,8] Because the lactating mammary gland is an integral part of the common mucosal immune system which stands as a sentinel in combating pathogens that enter the body via the mucosal route, [9] we hypothesized that maternal microbial burdens in mucosal sites such as the intestine and airways might affect TGF-β levels in breast milk. However, a systematic review reports that the levels of TGF-β in human milk vary, and the association between the levels of TGF-β in breast milk and protection of childhood allergic diseases is ambiguous.[10] Only a few studies have reported the differences in thelevels of TGF-β in breast milk in lactating women living in difference regions or countries of origin.[11,12] To test the hypothesis, we explored differences in the levels of colostrum TGF-β2, a dominant isoform of breast milk TGF- β[13] between two Asian countries; Japan and Nepal, with contrasting hygiene statuses. Japan is a developed country with good hygiene and sanitation facilities. Diarrhea is a major cause of death of children under 5 years of age in Nepal; an estimated 15% of children under 5 years of age died due to diarrhea in 2008.[14]

Moreover, evidence suggests that not only bacterial exposures, but also nutrient supplementation and/or psychological health influence TGF-β2 levels in breast milk.[15-17] However, the factors associated with TGF-β2 levels in human milk are unknown. In addition, the majority of the studies have been conducted in developed countries. To our knowledge, no reports have been published comparing the factors associated with TGF-β2 in breast milk in developed and developing countries. Therefore, we additionally aimed to identify the various factors associated with TGF-β2 levels in colostrum according to country by epidemiological methods.

Methods

Subjects and colostrum samples

Data were collected from lactating women admitted to obstetric clinics/wards in the Yamanashi prefecture in Japan, as well as in the Kathmandu and Kabhrepalanchok districts in Nepal. The purpose of the study was explained to the subjects, and written consent was obtained from all the participants. All the women delivered singleton babies. Women were asked to collect 1−5 mL of breast milk into a sterile plastic container during their waking hours (i.e., from 6:00AM to 10:00PM). To avoid any misclassification due to changes in composition levels of immunological substances throughout the lactating period,[18] we analyzed milk samples collected within 5 days after delivery. In total, 80 samples from Japan and 208 samples from Nepal were analyzed.

Breast milk assay and Measurements

Colostrum samples were stored at −20°C and analyzed without centrifugation. Levels of TGF-β2 were measured using human-specific TGF-β2 ELISA kits (R&D Inc., MN, USA). The detection limit was set at 31.3 pg/mL.

Structured questionnaires and medical records were used to collect information on maternal characteristics and parturition characteristics of the subjects. Maternal characteristics included sociodemographic characteristics, dietary habits during pregnancy, and postpartum health conditions. Maternal dietary habits during pregnancy included the frequency of animal milk and yogurt consumption. Examination of the postpartum health condition included identification of the presence of puerperal fever. We also recorded self-reported doctor-diagnosed maternal allergies; however we were only able to collect detailed information of allergic diseases from women in Japan. Our preliminary study found that none of the women in Nepal (15 lactating Nepalese women) reported that they had allergies. Parturition characteristics included the type of delivery, previous births, and infant's weight at birth.

Statistical analysis

The Mann-Whitney *U* test was used to compare the TGF-β2 levels of milk between the Japanese and Nepalese women. The distribution of TGF-β2 levels was long-tailed and skewed. Therefore, we transformed the TGF-β2 levels by logarithmic transformation as an outcome variable. In order to identify the factors associated with the levels of TGF-β2 in colostrum, we applied a robust regression model, as the outcome variables included certain outliers, which were stratified by country. All the statistical analyses were performed using

STATA 12.1 (StataCorp LP, TX, USA), and the value for statistical significance was set at less than 5%.

Ethics

The Ethical Committee of the Faculty of Medicine at the University of Yamanashi and the Institutional Review Board of the Institute of Medicine at the Tribhuvan University approved this study protocol.

Results

Characteristics of lactating women in Japan and Nepal

Maternal and parturition characteristics were significantly different between Japan and Nepal. The women in Nepal (mean age: 24.6) were younger than women in Japan (mean age: 31.2, $p < 0.001$). More than half of women in Nepal had a caesarean section ($p < 0.001$). In addition, more infants had low birth weight in Nepal than in Japan ($p = 0.01$). Among Japanese women, 34 women (48.8%) had any type of allergy, and pollen allergy/allergic rhinitis/conjunctivitis was the most prevalent allergy among them (Table 1).

TGF-β2 levels in colostrum in Japan and Nepal

TGF-β2 levels in colostrum for women in Japan and Nepal were 1398.1 (713.0−2297.1) pg/mL and 968.3 (483.1−1770.9) pg/mL, (median [inter-quartile range]), respectively. Then TGF-β2 levels were significantly higher in colostrum from Japanese women than from Nepalese women ($p = 0.005$: Figure 1).

Factors associated with TGF-β2 levels in colostrum by country

Using a univariate robust linear regression model, we found Japanese women who ingested animal milk daily during pregnancy expressed lower levels of TGF-β2 in colostrum than Japanese women who did not. Moreover, women who had atopic dermatitis expressed lower TGF-β2 levels in colostrum than women who did not have atopic dermatitis. Among Nepalese women, large family size was significantly associated with lower TGF-β2 levels in colostrum. On the other hand, women who gave birth to infants with low birth weight had higher expression of TGF-β2 levels in milk than women who gave birth to infants with normal birth weight (Table 2).

Table 3 shows multivariate robust linear regression estimates. Among Japanese, daily intake of animal milk (B = −0.58, $p < 0.05$) and the presence of atopic dermatitis (B = −1.05, p = 0.02) were negatively associated with composition levels of TGF-β2 in colostrum. Among Nepalese women, TGF-β2 levels were lower in colostrum from women with large families (B = −0.07, $p < 0.01$) and multiparae (B = −0.38, $p = 0.04$) than in those from small families or primiparae.

Table 1. Characteristics of lactating women living in Japan and Nepal

Variables		Japan n = 80 (%)	Nepal n = 208 (%)	*p*-value*
Age	(mean ± SD)	31.3 ± 4.2	24.6 ± 4.8	< 0.001
Family size	(mean ± SD)	4.0 ± 1.1	6.3 ± 3.1	< 0.001
Delivery type	Vaginal	67 (83.8)	112 (53.9)	
	Caesarean section	13 (16.2)	96 (46.2)	< 0.001
Previous birth (multiparity)	Yes	47 (58.7)	65 (31.6)	< 0.001
	No	33 (41.3)	141 (68.5)	
Weight of baby at birth	< 2500 g	4 (5.0)	33 (16.5)	0.01
	≥ 2500 g	76 (95.0)	167 (83.5)	
Puerperal fever	Presence	10 (12.7)	12 (5.8)	0.05
	No	69 (87.3)	196 (94.2)	
Intake of animal milk	Every day	23 (29.1)	88 (43.1)	
	2−3 days/week	25 (31.7)	48 (23.5)	0.09
	None	31 (39.2)	68 (33.3)	
Intake of yogurt	Every day	18 (22.8)	11 (5.4)	
	2−3 days/week	52 (65.8)	51 (24.9)	< 0.001
	None	9 (11.4)	143 (69.8)	
Allergies	Asthma (+)	6 (7.6)	n/a	
	Atopic dermatitis (+)	6 (7.6)		
	Pollen allergy/ allergic rhinitis/ conjunctivitis (+)	37 (46.3)		
	Food allergy (+)	4 (5.1)		
	Other allergy (+)	5 (6.3)		

n/a: not applicable, SD: standard deviation.

*: Student t-test for numerical variables and a chi-square test for categorical variables were applied.

Discussion

This results of this study show that TGF-β2 levels are significantly higher in Japanese women than in Nepalese women. Additionally, we found that Japanese women who consumed animal milk daily during pregnancy or had atopic dermatitis had lower levels of TGF-β2 in colostrum than Japanese women who did not. Among Nepalese women, those who had a large family or higher parity had

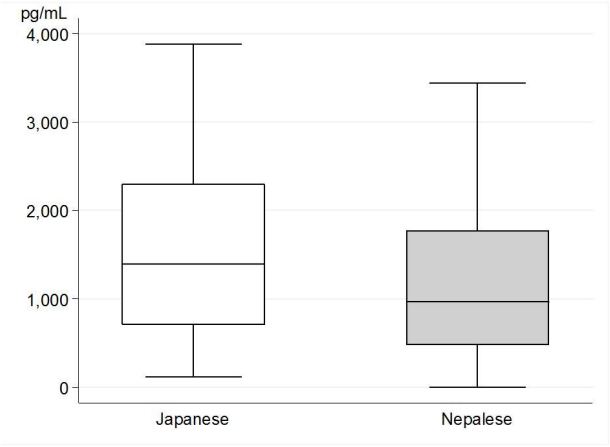

Figure 1. TGF-β2 levels in colostrum derived from Japanese and Nepalese women. The median levels (inter-quartile range) of TGF-β2 in colostrum obtained from Japanese (*white*) and Nepalese (*gray*) mothers. The colostral TGF-β2 levels were significantly higher in Japanese women than Nepalese women ($p = 0.005$).

lower TGF-β2 levels in colostrum. These results suggest that maternal microbial burdens and dietary habits affect TGF- β2 levels in colostrum, and these factors vary according to the country in which the subject lives.

We initially hypothesized that women living in developing countries exposed to greater levels of microbes due to poor hygiene and unsanitary conditions might have elevated TGF-β levels in colostrum compared to those in developed countries, because TGF-β is a key regulatory cytokine in the development of the mucosal immune response and, in particular, in the promotion of IgA production in the mucosa.[5,19] Contrary to our expectation, colostrum TGF-β2 levels were lower in women living in Nepal than in Japan, which warrants further investigation. It is possible that much higher parasite infection rates in Nepal than in Japan (although data on this are not available) may explain lower colostrum TGF- β levels in Nepal because parasite infection promotes Th2 responses, which could lead

Table 2. A univariate robust linear regression model for factors associated with TGF-β2 levels in colostrum among women in Japan and Nepal

		Japan		Nepal	
		B (S.E)	***p*-value**	**B (S.E.)**	***p*-value**
Age	years	−0.03 (0.03)	**0.27**	**0.01 (0.02)**	**0.76**
Family size	persons	0.03 (0.10)	0.77	−0.07 (0.02)	< 0.01
Delivery type	Caesarean section	−0.17 (0.30)	0.57	−0.15 (0.14)	0.27
	Vaginal	Reference		Reference	
Previous birth	Yes	−0.03 (0.22)	0.91	−0.21 (0.15)	0.17
	No	Reference		Reference	
Weight of baby at birth	< 2500 g	0.04 (0.51)	0.94	0.38 (0.19)	< 0.05
	≥ 2500 g	Reference		Reference	
Puerperal fever	Presence	0.33 (0.33)	0.32	0.05 (0.30)	0.86
	No	Reference		Reference	
Intake of animal milk	Every day	−0.57 (0.27)	< 0.05	−0.16 (0.16)	0.34
	2−3 days/week	−0.39 (0.26)	0.14	0.04 (0.19)	0.84
	None	Reference		Reference	
Intake of yogurt	Every day	−0.47 (0.40)	0.24	0.04 (0.32)	0.90
	2−3 days/week	−0.42 (0.42)	0.25	−0.14 (0.16)	0.41
	None	Reference		Reference	
Allergies	Have	0.25 (0.22)	0.26	n/a	
	None	Reference			
Types of allergy	Asthma (+)	−0.45 (0.42)	0.28	n/a	
	Atopic dermatitis (+)	−0.91 (0.40)	< 0.05		
	Pollen allergy/ allergic rhinitis/ conjunctivitis (+)	0.23 (0.22)	0.30		
	Food allergy (+)	0.32 (0.51)	0.53		
	Other allergy (+)	−0.52 (0.46)	0.26		

B: coefficient, S.E.: standard error, n/a: not applicable.

Table 3. A multivariate robust linear regression model for factors associated with TGF-β2 levels in colostrum among women in Japan and Nepal

		Japan		Nepal	
		B (S.E)	*p*-value	B (S.E.)	*p*-value
Age	years	−0.03 (0.03)	0.28	0.03 (0.02)	0.11
Family size	persons	0.01 (0.14)	0.96	−0.07 (0.03)	< 0.01
Delivery type	Caesarean section	−0.09 (0.32)	0.78	−0.27 (0.16)	0.09
	Vaginal	Reference		Reference	
Previous birth	Yes	−0.12 (0.30)	0.69	−0.38 (0.19)	< 0.05
	No	Reference		Reference	
Weight of baby at	< 2500 g	−0.12 (0.51)	0.82	0.37 (0.20)	0.06
birth	≥ 2500 g	Reference		Reference	
Puerperal fever	Presence	0.55 (0.34)	0.87	0.32 (0.33)	0.34
	No	Reference		Reference	
Intake of animal	Every day	−0.58 (0.29)	< 0.05	−0.27 (0.18)	0.14
milk	2−3 days/week	−0.26 (0.28)	0.36	−0.11 (0.21)	0.60
	None	Reference		Reference	
Intake of yogurt	Every day	0.02 (0.43)	0.96	0.10 (0.33)	0.75
	2−3 days/week	−0.26 (0.38)	0.49	0.01 (0.18)	0.95
	None	Reference		Reference	
Atopic dermatitis	Have	−1.05 (0.42)	< 0.05	n/a	
	None	Reference			

B: coefficient, S.E.: standard error, n/a: not applicable.

to low activity of TGF- β, a potent inhibitor of IgE class switching.[20] Alternatively, because TGF- β1, but not TGF- β2, is likely to be important in promoting IgA production in the mucosal immune system,[21] TGF- β2 may not actually reflect maternal microbial pressure and mucosal immune responses in mothers although TGF- β2 is abundant in breast milk.

High animal milk consumption was associated with lower TGF-β2 levels in colostrum in Japanese women. Animal milk contains a high load of enriched animal protein, which may lead to incomplete digestion and stimulation of Th2 responses.[22] Therefore, the lower TGF-β2 levels in colostrum might reflect Th2-polarized responses in Japanese women who ingested animal milk daily during pregnancy because TGF-β is a potent inhibitor of IgE class switching.[20]

Similar to a study conducted by Kuitunen et al.,[23] we found lower TGF-β2 levels in breast milk from women with atopic dermatitis than in that from women without allergies. Maternal allergic diseases may influence or impair regulatory T cells,[24] and reduction of T-reg cells consequently suppresses the expression of TGF-β in breast milk. However, we should note that the association between maternal allergic history and the levels of TGF-β in breast milk remains controversial.[10] Moreover, the results of studies of the association between breast feeding from mothers with a history of allergies and the onset of atopic dermatitis in offspring are inconclusive.[25]

Large family size and crowding in households may be related to poor hygiene and unsanitary conditions. People living with more family members are more likely to have an intestinal microbial infection than those living with fewer family members; in addition, crowding in households mainly occurs in developing countries.[26] Higher external endotoxin levels are associated with lower TGF-β levels in breast milk.[12] The "birth order effect" is noted in children with older siblings, and these children reportedly have a greater exposure to pathogens at a younger age, thus resulting in a high frequency of allergic diseases in children with lower birth order. This phenomenon also supports the "hygiene hypothesis".[1] Therefore, Nepalese women living with more family members who are multiparous may be exposed to more microbial burdens than Nepalese women who have smaller families or are primiparae and have lower TGF-β2 levels in colostrum.

Although only a univariate regression model showed the significant association between birth weight and TGF-β2 levels in colostrum, Nepalese women who gave birth to low birth weight infants expressed higher TGF-β2 levels in colostrum than Nepalese women who gave birth to babies with normal birth weight. IgA and TGF-β2 levels in colostrum are reported to be significantly higher among women giving birth to low birth weight or preterm infants,[27,28] which is consistent with the results of this study. This change of immunological composition in breast milk is considered to be a compensatory maternal response for premature neonates who have compromised immune systems and require additional protection from external microbes.

An advantage of this study is our large sample size that facilitates the examination of the levels of immunological substances in breast milk in different countries.[11,12] However, our study had several limitations; in particular, information on maternal allergies was obtained by self-report. In Nepal, the concept and knowledge of allergies is not well understood by the public. Although medical workers explained the definition of allergies to Nepalese participants in our preliminary study, none of the participants reported any allergies. Analysis would be improved if we were able to determine allergies using specific diagnostic tests, such as a skin prick tests.

Conclusion

In conclusion, TGF-β2 levels in colostrum may be influenced by maternal bacterial exposure and dietary habits during pregnancy. Nevertheless our findings suggest the presence of different TGF-β2 levels in developed and developing countries in Asia. Because colostrum feeding is considered to be a prominent method to protect neonates against infectious and allergic diseases, it will be important to investigate the relationship between maternal milk TGF-β2 levels and protection against infectious and allergic diseases in their offspring.

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