

Dear Editor

Residential Area, Birth Order, and Dietary Habit May Influence TSLP Levels in Colostrum: Comparative Study between Japan and Nepal

Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine that is produced mainly by epithelial cells.^{1,2} TSLP was originally identified as a factor derived from a thymic stromal cell line that could support the growth of a mouse B cell line. However, recent studies in mice and humans establish TSLP as a “master switch” for allergic inflammation.^{1,2} Interestingly, Macfarlane *et al.* reported that TSLP was present in human milk.³ However, it remains unclear what maternal environmental and intrinsic factors, as well as parturition characteristics, affect TSLP levels in human milk. This study therefore aimed to identify such factors influencing TSLP levels in human milk by epidemiological analysis. For this purpose, we obtained colostrum samples from lactating women living in Japan, a developed Asian country with good sanitation facilities, and Nepal, a developing Asian country with limited sanitation. Both countries also differ in cultural and economic factors as well as dietary habits.

Breast milk samples were collected from lactating women admitted in hospitals in Yamanashi prefecture, Japan, and in hospitals in Kathmandu district and Kavrepalanchok district, Nepal and were stored

at -20°C until the analysis. Women were asked to collect 1-5 mL breast milk into a sterile plastic container during 6:00AM and 10:00PM within 5 days after delivery. Concentrations of TSLP in 80 samples from Japanese women and 208 samples from Nepalese women were measured using human-specific TSLP ELISA kits (R&D Inc., MN, USA). The samples were analyzed without centrifugation, because our preliminary analysis demonstrated that results were not affected by centrifuging the samples. The detection limit was set at 31.3 pg/mL. Structured questionnaires and medical records were used to collect information on maternal characteristics. Maternal characteristics, which included sociodemographic characters (age and family size), parturition characteristics (delivery type, parity, and baby's weight at birth), postpartum health conditions (self-rated health and presence of puerperal fever), prenatal dietary habits, and presence of diagnosed allergies are described in Supplementary Table 1. Maternal dietary habits during pregnancy included frequency of animal milk and yogurt consumption (every day, 2-3 days a week, or never/seldom). Information on maternal allergic diseases was obtained only from women in Japan because, in our preliminary test, trained interviewers had asked 15 Nepalese lactating women about allergies, and none of them reported allergic diseases. Statistical analyses were performed using STATA11 (StataCorp LP, TX, USA), and we set the statistical significance level at <5%. This study protocol was approved by the Ethical Committee of the Faculty of

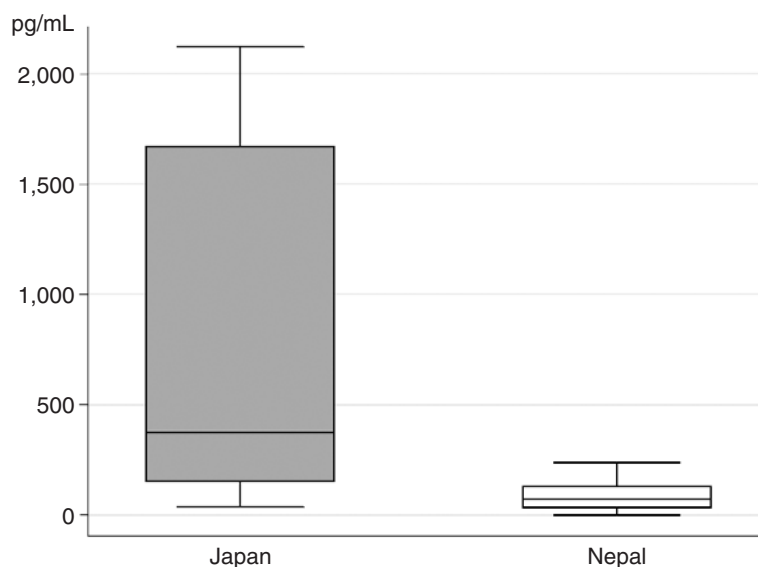


Fig. 1 The TSLP levels were significantly higher in the colostrum of Japanese women than in that of Nepalese women. The median (inter-quartile range) values of TSLP in colostrum of lactating women living in Japan (grey) and Nepal (white) were 412.2 (152.7-1614.9) pg/mL and 68.8 (not detectable-125.0) pg/mL, respectively ($p < 0.001$).

Table 1 Ordered logistic regression for associated factors of TSLP in colostrum by country

		Univariate ordered logistic regression				Multivariate ordered logistic regression			
		Japan		Nepal		Japan		Nepal	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age	Years	0.96	0.87-1.06	1.02	0.97-1.08	0.97	0.87-1.09	1.08	1.00-1.16
Family size	Persons	0.52	0.35-0.78	0.99	0.91-1.07	0.61	0.34-1.08	1.01	0.91-1.12
Delivery type	Vaginal	Ref		Ref		Ref		Ref	
	Caesarean	0.67	0.22-2.04	1.50	0.91-2.44	0.91	0.25-3.38	1.83	0.94-3.56
Birth order of baby	Primiparae	Ref		Ref		Ref		Ref	
	Multiparae	0.34	0.15-0.78	0.56	0.33-0.95	0.49	0.15-1.64	0.34	0.16-0.72
Weight of baby at birth	<2500 g	1.51	0.26-8.92	1.34	0.69-2.59	1.69	0.21-13.54	1.29	0.56-2.93
	≥2500 g	Ref		Ref		Ref		Ref	
Self-rated health	Very good/good	1.04	0.46-2.40	0.75	0.46-1.25	1.48	0.60-3.66	1.01	0.58-2.04
	Fair/bad/very bad	Ref		Ref		Ref		Ref	
Puerperal fever	Presence	0.73	0.23-2.33	1.68	0.53-5.27	0.80	0.21-3.02	1.37	0.31-6.12
	No	Ref		Ref		Ref		Ref	
Intake of animal-milk	Everyday	3.42	1.27-9.23	0.86	0.49-1.50	5.48	1.69-17.78	1.37	0.66-2.84
	2-3 days a week	0.99	0.38-2.57	1.46	0.75-2.87	0.87	0.29-2.67	2.21	0.89-5.46
	Never/seldom	Ref		Ref		Ref		Ref	
Intake of yogurt	Everyday	1.07	0.26-4.51	0.22	0.07-0.70	0.62	0.12-3.22	0.08	0.01-0.49
	2-3 days a week	1.37	0.39-4.81	0.91	0.52-1.62	2.39	0.50-11.41	0.88	0.44-1.78
	Never/seldom	Ref		Ref		Ref		Ref	
Diagnosed allergic diseases	Have	0.89	0.41-1.94			0.98	0.37-2.55		
	None	Ref		n/a		Ref		n/a	
Time of sampling	6:00AM-12:00PM	Ref		Ref		Ref		Ref	
	1:00PM-10:00PM	1.29	0.57-2.92	0.67	0.39-1.16	1.56	0.61-3.98	0.78	0.41-1.48

OR, odds ratio; CI, confidence interval; Ref, reference; n/a, not available.

Ordered logistic regression calculated OR for one step higher TSLP quartile by maternal characteristics.

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The Wilcoxon rank-sum test showed that TSLP levels were significantly higher in the colostrum of Japanese women than in those of Nepalese women (Fig. 1). The findings may be explained by the difference in hygiene status between the 2 countries. Good sanitation and hygiene status leads to low exposure to microbial and viral infections, which results in an insufficient stimulation of Th1 cells and expansion of Th2 cells.⁴ Importantly, TSLP induces Th2 responses, and in turn, TSLP is induced by the Th2 responses, thereby forming a vicious cycle in allergic diseases.⁵ Thus, greater Th2 responses in Japanese women than in Nepalese women might stimulate more TSLP production from the mammary gland.

An ordered logistic regression model showed that the TSLP levels were significantly lower in colostrum from multiparae than in colostrum from primiparae in women in both countries (Table 1). Children with older siblings are reported to have more exposure to pathogens at a younger age, which results in a low

frequency of allergic diseases in children with higher birth order compared with first-borns and this supports the hygiene hypothesis.⁶ Although the potential roles of TSLP in human milk in the development of allergy in the offspring remain to be determined, the present data suggest that a lower frequency of allergic diseases in children with higher birth order might be associated with lower expression levels of TSLP in colostrum.

Moreover, we found that the TSLP levels were higher in colostrum from Japanese women who consumed animal milk everyday than in that from Japanese women who seldom or never consumed animal milk during pregnancy (Table 1). A relationship between animal milk consumption and colostrum TSLP levels remains to be investigated. In contrast, TSLP levels were lower among Nepalese women who consumed yogurt everyday than in women who never consumed yogurt during their prenatal period (Table 1). Probiotics can influence the intestinal microbiota, inhibit Th2 responses, and increase anti-inflammatory cytokine⁷; thus, probiotics are being recognized as a useful tool to prevent or alleviate allergic diseases.⁸

High frequency of yogurt consumption during pregnancy might suppress Th2 responses via the probiotic activity and reduce colostrum TSLP levels in Nepalese women. The reasons why Japanese women did not show significant association of colostrum TSLP levels with yogurt consumption may underlie the different quality (e.g., different probiotics used in yogurt) of yogurt and quantity of yogurt consumption between the 2 countries. Moreover, dietary habits among Japanese and Nepalese mothers are significantly different, and the effect of milk protein and probiotics may depend on the combination of other nutrients in the diet.⁹ This might also explain why animal milk consumption was not associated with colostrum TSLP levels in Nepalese women.

In summary, this study suggests that residential area, birth order, and dietary habits during pregnancy may influence the expression levels of TSLP in colostrum. Importantly, we found much higher colostrum TSLP levels in Japanese women than those in Nepalese women, which might reflect different sanitation infrastructures between the 2 countries and support the hygiene hypothesis. The results will provide useful information for future studies that aim to investigate a role of human milk TSLP in the onset of allergic diseases in offspring.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1 is available online.

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