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Variation of bromine concentration as an essential trace element in human milk over lactation stages

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ABSTRACT

Introduction: Bromine has been newly discovered in human milk but its importance in the growth and development of infants is unclear. Only a few studies have reported the concentration of bromine in human milk and considered it as an essential element, whereas others highlighted its toxicity of bromism in humans. This study aimed to determine the concentration of bromine as an essential trace element in human milk using a validated acid digestion method and discuss its variation over lactation stages.

Method: Human milk samples were collected from three postpartum mothers and analysed using inductively coupled plasma mass-spectrometry (ICP-MS). The concentration of bromine was determined over a certain postpartum period, analysed using Microsoft Excel 2016, and reported descriptively.

Results: Method validation parameters for bromine showed good linearity ($R^2 > 0.999$), limit of detection (0.003 µg/L), limit of quantification (0.01 µg/L), accuracy (96%), inter-day (3.76%RSD) and intra-day (3.35%RSD) repeatability. The median concentration of bromine in human milk decreased over six months of lactation, in µg/L: 1210, 674, 722, 671, 511 and 538. At later lactation months which were 12th, 13th, 14th, 15th and 21st, the median bromine concentration was in µg/L: 780, 815, 645, 846, 910, respectively.

Conclusion: The acid digestion method by ICP-MS was robust and accurate in determining bromine concentration in human milk. The consistent variation of bromine in human milk over lactation stages may indicate its importance in supporting infant development in the first two years of age. Future research should explore the role of bromine in infants' development, its chronobiological importance, and the risk of deficiency or toxicity.

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Introduction

Human milk contains essential trace elements that can vary in concentration over the postpartum period to meet an infant's nutritional needs. These include zinc (Zn), copper (Cu), selenium (Se), iodine (I), molybdenum (Mo), manganese (Mn), iron (Fe), and bromine (Br) (Mohd-Taufek et al., 2016a). Unlike other well-reported elements, very few studies have discussed about bromine in human milk. It was the first time reported in 2016 that median bromine concentration (1066 μg/L) was relatively higher than other elements such as selenium, iron, and iodine, but comparable to those of zinc (1639 μg/L) in an Australian population (Mohd-Taufek et al., 2016b). Bromine has been discovered as an essential trace element in human for its role as a key cofactor in tissue development in the type IV collagen scaffold presented in basement membranes, which support epithelial cells (McCall et al., 2014). However, data and information about bromine concentration and its role in humans remain scarce and require further research.

The essentiality of trace element bromine could have been overlooked due to its prominent toxicity profile that has been widely highlighted in the literature (Frances et al., 2003; Lugassy & Nelson, 2009; Rho & White, 2018). Although rare, the occurrence of bromide intoxication or bromism in adults characterised with neuropsychiatric symptoms have been reported from medicinal exposure (James et al., 1997; Frances et al., 2003). Moreover, a case of a 22-day-old girl with bromism who presented with excessive sleepiness and decreased oral intake due to ingestion of elixir containing potassium bromide was also reported (Lugassy & Nelson, 2009). Despite being the first drug as anti-epilepsy, potassium bromide has a narrow therapeutic index, of which toxicity characteristics include severe skin reactions, lethargy, cachexia, delirium/psychosis, and exacerbation of seizure activity (Rho & White, 2018). The neuropsychiatric symptoms were reported with a serum bromide concentration of 1717 mg/L (Frances et al., 2003) whereas severe bromism was found in a patient with serum bromide of 3180 mg/L (Horowitz, 1997). Therefore, its clinical use has declined for decades but bromide can still be found in prescription and over-the-counter (OTC) preparations owing to its sedative effect (Lugassy & Nelson, 2009). For example, a case of chronic bromide toxicity has been reported from the recurrent abuse of OTC dextromethorphan hydrobromide (Monks, Yen & Myers, 2020). Additionally, severe bromism could also happen from consumption of cola containing brominated vegetable oil (Horowitz, 1997).

In recent years, bromine has been recognised as part of more than 20 elements that are essential for life. It is unclear about the role of bromine in infants other than tissue development. Nevertheless, infants are generally at high risk of trace element deficiency due to higher metabolism and need for their rapid growth and development (Angelova et al., 2014). Unlike bromine toxicity, its deficiency has been rarely reported, and thus reference ranges in human biological samples have not been established. A review has summarised that decreased bromine concentration was found in insomnia and long-term dialysis patients and suggested that evaluation of human bromine status might be useful in correcting bromine-dependent health issues (Canavese et al., 2006). It was reported that bromine was significantly lower in the scalp hair samples of dialysis patients compared to healthy subjects (Ochi et al., 2011). The analysis of dried blood spot also has been reported for bromine and iodine (He et al., 2020). However, different biological matrices contain different concentrations of trace elements, which require specific reference range.

The presence of bromine in human milk at a relatively similar concentration to zinc poses questions about whether infant's need of bromine is sufficiently sourced from breast milk. In infants, high metabolism may deplete bromine reserve in the body and signs and symptoms of its deficiency have not been identified. For example, zinc deficiency in exclusively breastfed infants have been frequently misdiagnosed as eczema or impetigo before the correct diagnosis of nutritional zinc deficiency attributable to the decreased zinc content in human milk at the later stage of lactation (Kienast et al., 2007). The composition of human milk nutrients has been reported to vary over lactation stages with some trace elements such as zinc and copper had been observed to decrease throughout the postpartum period (Terrin et al., 2015). Some nutrients may decrease or maintain their concentration due to various intrinsic and external factors. However, there are limited data on the variation of bromine concentration in human milk. It is important to document more data about bromine in human milk to evaluate the longitudinal changes relative to the development of infants. This study aimed to determine bromine concentration in human milk of postpartum mothers using a validated method and discuss its essentiality in infants.

Methodology

This study received ethics approval from the International Islamic University Malaysia (IIUM) Research Ethics Committee (IREC) (ID No.: IREC 2021-053).

Participant recruitment, data collection, sample collection, and analyses have been conducted following the protocol as described in the previously published article (Mohd Taufek et al., 2023). The developed and validated method has been used to analyse bromine concentration in human milk. The certified reference material for bromine in human milk is currently unavailable, thus spiked samples were used to measure accuracy using 1000 mg/L of Br single-element standard solution (Merck Certipur).

The 0.1 ml of Br single-element standard solution 1000 mg/L was diluted with 1% (v/v) nitric acid up to 50 ml for the purpose of standard stock solution. Then, 1 mL of each of 5 samples of milk was spiked with 0.005 g/mL bromine and made up to 10 mL by adding 1% (v/v) nitric acid. The 1% (v/v) nitric acid was made by diluting 15.4 mL of 65% (v/v) nitric acid (Merck Suprapur) with 1 L of water. A total of 105 milk samples were analysed using the method. All the results were analysed for descriptive analysis using Microsoft Excel version 2016.

Results

A total of three participants consented to the study and were included as case studies. Participant X was a 30-year-old mother who did not take any supplements and donated her milk at her time convenience in the first six months postpartum. Participant Y was a 27-year-old mother who took supplements for selenium, manganese, zinc and copper. Participant Z was a 27-year-old mother who took supplement for zinc and iron. Both participants Y and Z donated their milk at the later stage of lactation which were 12th month until 21st month at their convenience. All participants were Malay, had normal body mass index at the time of milk collection, and delivered male infants of the same birth weight (3 kg) at full term (37≤ weeks of gestation). Only participant Z had a history of gestational diabetes and asthma. Other details regarding the participants have been published in a previous study (Mohd Taufek et al., 2023)

The calibration data for bromine is presented in Table 1, with $R^2 > 0.999$ is considered good linearity. The inter-day and intra-day repeatability of spiked bromine samples are shown in Table 2 which indicate good percentage recovery (80-120%) and %RSD (Mohd Taufek et al., 2023).

Table 3 showed that median bromine concentration was the highest in the first month postpartum in comparison to the later months for Participant X. The minimum and maximum range provided more information on the variation of bromine across six months of the lactation period. Although the median bromine concentration dropped to half in the second month, it seemed to remain stable until the sixth month.

Table 4 showed that bromine concentration observed in two different postpartum mothers at the 12th, 13th, 14th, 15th and 21st month postpartum were stable with little fluctuations.

Table 1: LOD and LOQ, slope and correlation coefficient.

Trace element	LOD (µg/L)	LOQ (µg/L)	Slope	Correlation coefficient (R^2)
Br	0.003	0.01	y= 420x + 539	0.9994

Table 2: Percentage recovery, inter-day (n=3), and intra-day repeatability

Trace element	Percentage recovery (%)	Inter-day [%RSD]	Intra-day [%RSD]
Br	96	3.76	3.35

Table 3: Concentration of bromine of participant X over six months postpartum.

Trace element	Mean/Median	Month postpartum					
		1	2	3	4	5	6
		n = 15	n = 8	n = 10	n = 15	n = 6	n = 10
Br (µg/l)	Mean ± SD	1340 ± 503	678 ± 101	718 ± 123	739 ± 259	540 ± 95	574 ± 163
	Median (range)	1210 (886-3010)	674 (517-847)	722 (528-902)	671 (494-1500)	511 (431-678)	538 (431-1000)

n: number of milk samples available

Table 4: Concentration of bromine of participants Y (12^{th} – 15^{th}) and Z (21^{st}) month postpartum.

Trace element	Mean/Median	Y				Z
		Month postpartum				
		12	13	14	15	21
		n = 16	n = 4	n = 6	n = 4	n = 11
Br (µg/l)	Mean ± SD	788±144	863±252	641±79	879±210	888±224
	Median (range)	780 (573-1190)	815 (614-1210)	645 (545-746)	846 (696-1130)	910 (354-1180)

n: number of milk samples available

Discussion

We report a validated acid digestion method to determine bromine concentration in human milk that obtained good values and acceptable validation parameters. These are comparable to a previous study that used an alkaline dissolution method (Mohd-Taufek et al., 2016a). Our method is applicable to monitor bromine concentration in human milk in the future to establish the reference range and assist in the identification of bromine deficiencies or toxicities. For the current study, the method successfully measured bromine in the human milk of three postpartum mothers.

Despite different individuals providing milk samples at different periods of postpartum at their convenience, our findings provided important information about bromine concentration in human milk up to 21 months postpartum. We propose that the consistently high bromine concentration in different individuals at different postpartum periods may indicate that infants require abundant of bromine for growth and development. The nutritional aspect of bromine across two years of infants age needs to be explored, and its concentration should be maintained within a certain reference value to ensure sufficient intake. Exclusively breastfed infants aged six months and above are generally recommended to be fed with complementary food to prevent nutritional deficiencies based on developmental readiness (Pérez-Escamilla et al., 2019). Considering that bromine concentration in human milk was comparable to zinc which presented at a higher concentration than other elements (Mohd-Taufek et al., 2016b; Mohd Taufek et al., 2023), we speculate that deficiency in infants is possible, and its identification requires further investigation. For example, inadequate levels of zinc were reported to increase the risk of infections, impairment of growth, neurological function, and several complications particularly affecting rapidly growing preterm infants such as necrotising enterocolitis (Terrin et al., 2015). Additionally, higher risk of pneumonia and stunting were seen in children with zinc deficiency (Hamed et al., 2019; Khairun et al., 2019). Since bromine has not been routinely monitored in human milk as well as in the blood of mother-infant dyads, its deficiency or toxicity are currently unknown. Future studies evaluating this aspect are recommended.

In participant X, the median bromine concentration dropped to half in the second month (674 µg/L) compared to the first month (1210 µg/L) then were relatively stable throughout the six months postpartum (Table 3). Limited data are available addressing bromine concentration in human milk. A study in Australia has reported that bromine concentration was not significantly different before and after pasteurisation, ranging from 834 to 1443 µg/L in 16 different mothers who donated their milk (Mohd-Taufek et al., 2016b). These values were similar to our findings, when referring to the concentration range. The difference between the minimum and maximum values of bromine concentration in each month suggests that chronobiological changes in bromine contribute to huge variation longitudinally. This aspect can be explored like other nutrients in human milk, since dysregulated circadian biology may affect the concentration of bromine and thus infant health outcomes (Hahn-Holbrook et al., 2019; Italianer et al., 2020). On the other hand, organobromine pollutants have been reported to contaminate the breast milk of Swedish population due to environmental contamination (Norén & Meironyté, 2000). Bromine exposure from the environmental pollutants have been readily reported in the literature from agricultural products (Shtangeeva, 2022) and important highlight was the genotoxicity risk owing to increased bromide body burden (Nusair et al., 2019). More data are needed to clarify the essentiality and toxicity of bromine in human and specifically in human milk.

Table 4 showed the bromine concentration of different participants who donated their breast milk after 12 months postpartum. Interestingly, the concentration of bromine was relatively consistent with median level of between 600-900 µg/l between 12 to 21 months postpartum. This study observed that bromine concentration in human milk varied over the postpartum period. The factors for this variation could be similar to those of the other nutrients including lactation stages, dietary intakes or supplements (Samuel et al., 2020). We were not able to confirm any significant changes in the pattern of bromine variation due to insufficient study population size. Future research is needed to confirm the changing pattern of bromine concentration in human milk at the population level, and its association with infants' health outcomes.

This study has the limitation of the small number of study participants. However, this study validated a simple and robust method that is applicable to monitor bromine concentration in human milk. Studies with large sample sizes are required to determine any association between maternal factors and bromine concentration. However, the findings from this study open more opportunities to explore bromine from nutritional aspects of the growth and development of infants.

Conclusion

The relatively high concentration of bromine in human milk indicates its essential role in infant growth and development. Bromine exhibited longitudinal variation in concentration in human milk like other essential trace elements such as zinc. Future studies may determine the reference range, and explore potential deficiency and toxicity.

Author Contribution

NHMT, ASMS & JB designed the study, collected samples and data. NBA, USMJ and ARFN analysed the samples and data. All authors wrote and reviewed the manuscript.

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Ethical Approval Statement

This study received ethics approval from the International Islamic University Malaysia (IIUM) Research Ethics Committee (IREC) (ID No.: IREC 2021-053).

Informed Consent Statement

Informed consent was obtained from all individual participants included in the study.

Conflict of Interest

All the authors declare that there is no conflict of interest.

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