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Biological monitoring of iodine content in human breast milk over six months postpartum: A case study

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Abstract

Introduction: Iodine deficiency was commonly reported in infants and partly attributable to low breast milk iodine content. The role of iodine is crucial in preventing brain damage and hypothyroidism in infants. It is important to monitor the concentration of iodine in breast milk of postpartum mothers. This study aimed to validate an analytical method to determine iodine concentration in human breast milk for biomonitoring purposes. **Materials and method:** Expressed breast milk samples were collected several times a day throughout six months postpartum from a healthy lactating mother. Samples were prepared with nitric acid digestion and analysed by inductively coupled plasma mass-spectrometry. Data analysis was conducted using Microsoft Excel 2016 for assessment of validation parameters and longitudinal concentration of iodine. **Results:** The method validation parameters showed that linearity of calibration graph was 0.9987, limit of detection and limit of quantification were 0.218 μg/L and 0.661 μg/L, respectively. A recovery of 100.3% showed good accuracy, whereas inter-day and intra-day repeatability were 5.91% and 3.60%, respectively. The median iodine concentration was the highest in the first month (160.0 μg/L), then dropped to lower than recommended level (110 μg/L) from the second until six months postpartum (range: 31.9 - 98.7 μg/L). Fluctuation in median iodine concentration occurred over six months postpartum but circadian rhythm was observed to be consistent with "V" shaped curve pattern indicating higher concentration was exhibited in the morning and at night compared to evening. **Conclusion:** The analytical method was robust, accurate and reliable for measuring iodine concentration in human milk and applicable for biomonitoring. Deficiency in breast milk iodine content was observed in the second until six months postpartum. Iodine concentration in breast milk exhibited consistent circadian variation over six months postpartum.

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Introduction

In early life, infants primarily rely on breast milk as the main source of nutrients including iodine (Mosca & Giannì, 2017). Iodine is obtained from the dietary sources to produce thyroid hormone, thyroxine and triiodothyronine. Lack of iodine will consequently lead to poor neurodevelopment, cognitive impairment and mental retardation in children, and other medical conditions collectively termed as Iodine Deficiency Disease (IDD). IDD has been reported extensively worldwide and substantial reduction of infants' morbidity and mortality has been observed following iodine supplementation in various forms, for example in countries including Zaire, Papua New Guinea, Indonesia, China and Europe (Eastman & Zimmermann, 2018). Infants are particularly susceptible to IDD due to high iodine requirement for growth and development since they are born with very small amounts of iodine body reserve (Dror & Allen, 2018).

The monitoring of breast milk iodine concentration (BMIC) is important in infants who exclusively receive mothers' milk or donors' milk in the first six months of age. Iodine status in infants has been reported to be more accurately reflected by the maternal breast milk iodine status (Nazeri et al., 2018). It has also been demonstrated that higher BMIC at the second week postpartum was associated with a larger increase in infant growth and development compared to later stage (Ellsworth et al., 2020). BMIC is mainly affected by foods, supplements, or medications as it is not synthesised in the human body. Supplementation intervention has been demonstrated to slightly increase iodine status in postpartum women although not able to resolve deficiency status (Mulrine et al., 2001). Additionally, BMIC is also varied according to each breastfeeding session, either colostrum or later milk stages, between days postpartum and individuals (Ballard & Morrow, 2013).

Biological monitoring of iodine concentration in human milk is important for maintaining adequate amount of iodine requirement in breastfed infants. The standard monitoring of urinary iodine concentration does not usually reflect the BMIC (Huang et al., 2023). Literature has been conflicting addressing correlation of urinary iodine and BMIC. It was noted that limited monitoring was available of nutrition-sensitive data across the food system in Southeast Asia and Western Pacific Regions (Peters et al., 2023). A nationwide survey in 2010 indicated that Malaysia was at borderline adequacy despite absence of goitre endemic but urinary iodine level of most of the population was <100 μg/L (Selamat et al., 2010).

The lack of available data on the BMIC in Malaysian population could stem from limited access to analytical instruments and ethical considerations regarding the collection of human milk samples. ICP-MS is a highly sensitive instrument as it can detect metal and nonmetal elements in a sample at very low concentrations with extremely low detection limits up to ppt (parts per trillion) (Catenza & Donkor, 2022). The aim of this study was to develop and validate a method to quantify the amount of iodine present in human breast milk and ascertain its application in biomonitoring.

Materials and methods

Study participant

A postpartum woman was recruited to supply breast milk samples throughout the first six months postpartum. The participant was enrolled as a case study and was provided with a participant information sheet. She was informed about the study, and data collection sheet that included participant's relevant health information. Expressed breast milk (EBM) samples were provided in plastic containers labelled with date and time of which the milk samples were collected. The samples were collected throughout six months of lactation from June until November 2023. Samples were kept in a freezer at -21ºC until analysis at the Institute of Oceanography and Environment, University Malaysia Terengganu. The samples were analysed using ICP-MS with the conditions reported in the previously published study (Mohd-Taufek et al., 2023).

Sample preparation, blank preparation and wash solution

The milk samples, blank samples, and wash solution were prepared following the protocol that has been published previously (Mohd-Taufek et al., 2023). Sample preparation was done by mixing 1 mL of thawed milk with 9 mL nitric acid 1% (v/v) to form homogenous solution. The nitric acid 1% (v/v) was prepared by diluting 15.4 mL of nitric acid 65% (v/v) (Merck Suprapur) with deionised water up to 1 L. The blank samples were prepared for 10 sets using 10 mL nitric acid 1% (v/v) into a conical tube. The wash solutions between samples analysis were the Milli-Q water and acid washout was done for every 10 samples using the 1% (v/v) nitric acid.

Standards preparation

For standards preparation, a 1000 mg/L of iodide standard solution (Tracecert®) was used. Then, 0.1 mL of iodide standard solution was added into 50 mL of 1% (v/v) nitric acid in a polypropylene conical tube to prepare the standard stock solution. Six calibration standard solutions were then prepared by adding 1 mL of milk sample into 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL of iodide stock solution. Each standard solution was diluted to 10 mL with 1% (v/v) nitric acid, resulting in six solutions with final concentrations ranged from 0.02 μg/mL to 0.12 μg/mL.

Quality control

The certified reference material for human milk iodine is currently unavailable, and thus spiked samples were used to measure accuracy by using 1000 mg/L of iodide standard solution (Tracecert®). Then, 0.1 mL of single-element standard solution 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 five milk samples were spiked with 0.1 mL of 0.002 μg/mL iodine and made up to 10 mL by adding 1% (v/v) nitric acid. The validated method was then used to analyse all milk samples donated by a postpartum mother over six months postpartum. Spiked samples where known quantities of a substance added to a sample were freshly prepared daily. Analysis of study samples were run concurrently with the blanks, iodide standard solution (Tracecert®), and iodine-spiked milk samples. Samples were analysed using ICP-MS (Perkin-Elmer SCIEX model ELAN 9000) connected with DELL PC equipped with ELAN Instrument Control Session software (PerkinElmer Inc., Massachusetts, USA).

Sample analysis and analytical method validation

Sample analysis was conducted using the instrument and protocol that has been previously reported (Mohd-Taufek et al., 2023). Validation parameters assessments were reported based on linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), accuracy and repeatability. A calibration graph of concentration *vs*. response was generated for final concentrations of 0.02 μg/mL, 0.04 μg/mL, 0.06 μg/mL, 0.08 μg/mL, 0.1 μg/mL, and 0.12 μg/mL.

Accuracy was assessed by comparing the result obtained from the analysis of spiked milk samples using 1000 mg/L of iodide standard solution (Tracecert®) calculated in the form of percent recovery.

Repeatability of the method was assessed on the same day (intra-day) using five spiked milk samples, and on three different days (inter-day), expressed as relative standard deviations (%RSD) and pooled relative standard deviation (RSDpooled) respectively.

Data analysis

Data on method validation parameters as well as breast milk iodine concentration were analysed using Microsoft Excel 2016. The collected samples were sorted into days, weeks, and months postpartum. Data were divided into three-time intervals which were 0400–1159, 1200–1959 and 2000–0359 to measure the variation over 24 hours according to the data and time stated for the milk samples. Data were analysed and reported descriptively.

Results

The calibration graph (Figure 1) for iodine over six concentration levels ranging from 0.02 μg/mL to 0.12 μg/mL was linear with correlation coefficient (r^2) value of 0.9987. The LOD and the LOQ were 0.218 μg/L and 0.661 μg/L respectively. The accuracy value that was calculated in the form of percent recovery

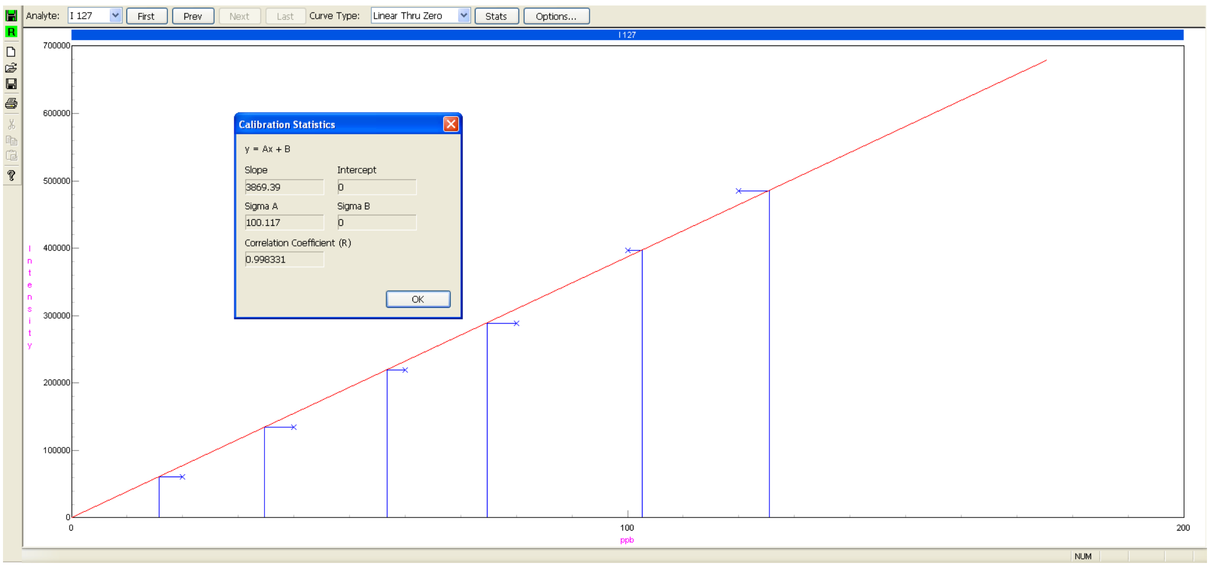

Figure 1: Calibration graph of concentration *vs.* response

Table 1: Recovery, inter-day (n=3), and intra-day repeatability of ICP-MS

Trace element	Unspiked concentration (µg/L)	Spiked concentration (µg/L)	Expected concentration (µg/L)	Recovery [%]	Repeatability% RSD (n=5)	
					Inter-day	Intra-day
I	11.5 ± 0.67	192.0 ± 4.53	180.5 ± 0.67	100.3 ± 2.70	5.91	3.60

Table 2: Concentration of iodine over six months postpartum

Trace element	mean/ median	Month postpartum					
		1	2	3	4	5	6
		n = 17	n =55	n = 44	n = 26	n =31	n = 18
I (μg/L)	mean ± SD	167.2 ± 69.0	45.8 ± 21.1	104.6 ± 41.3	39.6 ± 22.3	34.5 ± 16.2	39.8 ± 23.8
	median (range)	160.0 (72.9-276.0)	41.6 (11.3-96.8)	98.7 (30.8-215.0)	38.5 (7.2-115.0)	31.9 (9.4-70.2)	35.3 (10.4-85.7)

n: number of milk samples available

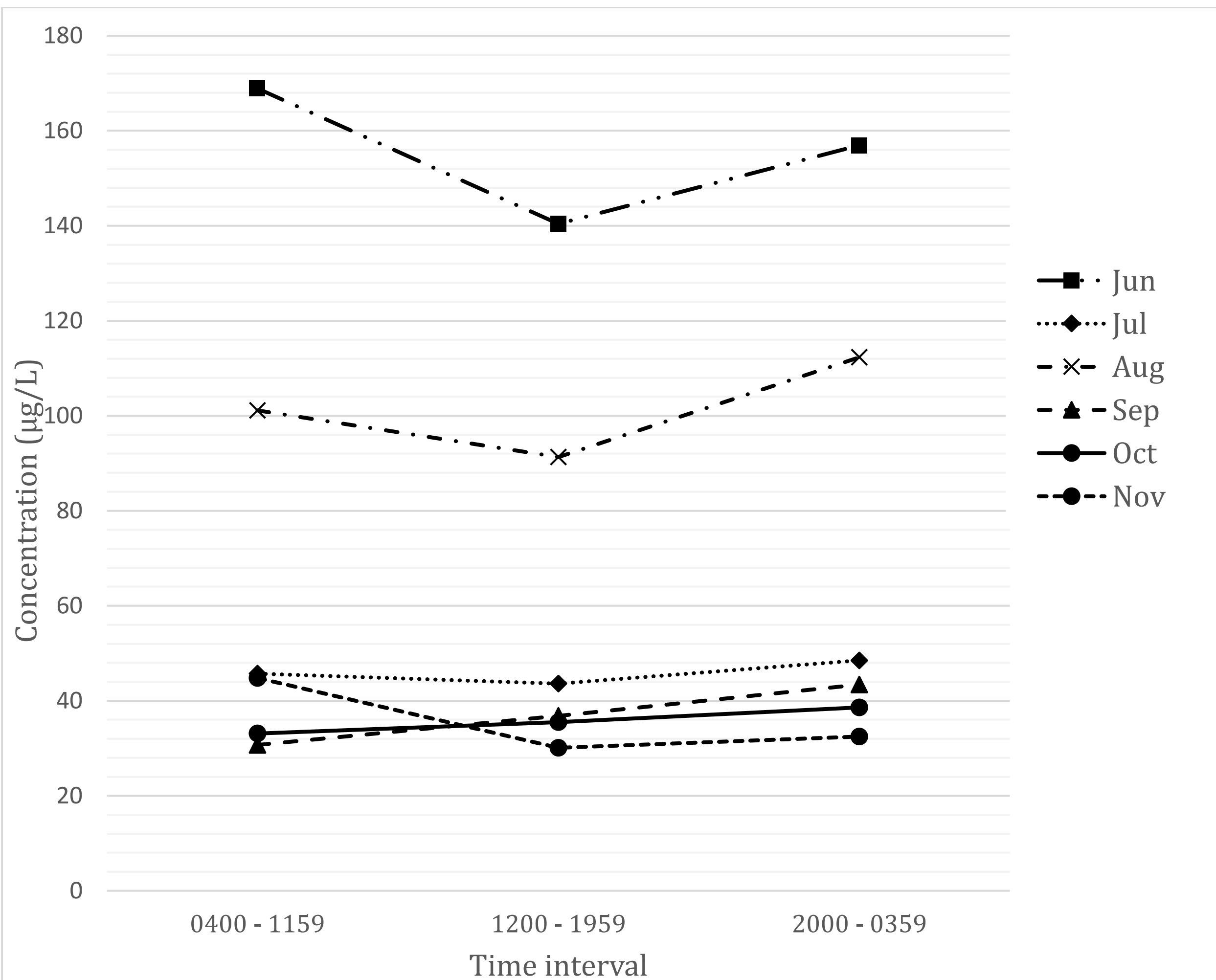

Figure 2: Median concentration of iodine (μg/L) in three-time intervals during 24-hour period over six months postpartum.

by using iodine-spiked milk samples was 100.3%. This finding indicated high recovery that eliminates the presence of matrix effect during sample analysis. The intra-day repeatability measured on the same day was 3.60%, and the inter-day repeatability measured on three different days was 5.91% (Table 1).

Application in biological monitoring of human milk iodine

The method was used to measure iodine concentration in 191 milk samples collected from a lactating mother over a period of six months. The participant was a 39-year-old Malay woman who had her first child, a male infant at 38 weeks of gestation by caesarean section, weighing 3.41 kg. The infant exhibited typical growth and development without any additional health issues apart from jaundice shortly after birth. Exclusive breastfeeding was maintained for the initial six months, followed by a combination of breastfeeding and introduction of solid foods. The mother claimed to include rice, fish, chicken, vegetables, fruits, and conventional supplements with zinc and iron in her dietary intake.

Table 2 showed the concentration of iodine over the period of six months. The highest median concentration of iodine was found in the first month postpartum (160.0 μg/L) then dropped to about a quarter (41.6 μg/L) in the second month. The subsequent months showed the fluctuations in the median concentration of iodine, but low concentration of less than 40 μg/L were observed in the fourth month onwards. Additionally, the widespread concentration range such as 7.2 – 115 μg/L in the fourth month showed similar trend in the fifth and sixth month.

Figure 2 demonstrated the circadian pattern of iodine concentration over six months postpartum. The median concentration of iodine throughout the day followed a 'V' shaped curve for all months except for the fourth (Sep) and fifth (Oct) month. The BMIC in the third month observed an improved median concentration but declined again in later stages, with the lowest BMIC being in the sixth month (Nov). Despite the circadian pattern being slightly different in the fourth, fifth and sixth month postpartum, the BMIC exhibited generally higher concentrations in the morning and at night, compared to afternoon/evening.

Discussions

The present study reports a validated analytical method measuring iodine concentration in human breast milk. The method exhibited strong linearity ($r^2 > 0.99$) with the recovery value within the acceptable range of 80-120%. The LOD and LOQ values indicated that the method was sensitive, whereas the inter-day and intra-day repeatability were considered precise as it falls below the limit of 20% based on the guideline of Codex Alimentarius Commission Joint FAO/WHO 2017. The method was robust, accurate and reliable for measuring iodine concentration in human breast milk and is applicable for biomonitoring of BMIC in population of lactating women.

Our study found that the median concentration of iodine in the first month postpartum was the highest at 160 μg/L, which met the requirement of Recommended Dietary Allowances (RDA) of 110 μg/day for infants up to six months of age. However, the median iodine concentration in the second month had declined below the adequate level, which was at 41.6 μg/L. The BMIC in the third month showed an increase in the median concentration of 98.7 μg/L, yet, still below the recommended level. Then, it further decreased to below 40 μg/L in later months postpartum. It could be noted that the lower and upper range for the fifth and sixth month fell below 100 μg/L. The low BMIC from the second month of lactation onwards could be due to insufficient dietary intake and supplementation of iodine in the postpartum mother. In Taiwan, it has been demonstrated that the estimated average BMIC was 111.6 μg/day (IQR: 78.3–172.1), which was sufficient for infants aged up to six months (Huang et al., 2023). However, global data highlighted that despite variation across populations, BMIC should be reaching a higher level of 150 μg/L in the first six months to ensure infants would not be at risk of iodine deficiency (Dror & Allen, 2018). Our findings suggest that postpartum women and infants in Malaysia could be at risk of IDD if surveillance, prevention, and intervention strategies are not taken.

To address this issue, daily supplementation during lactation was proven effective in increasing BMIC following dose-response relationship. A study of donor human milk iodine concentration in Spain found that supplementation with iodine was associated with high BMIC of median (IQR) concentration of 148.5 (97.6 - 206.1) μg/L in 70% donors and thus considered as iodine-sufficient population (Ureta-Velasco et al., 2022). Although Malaysia has experienced a large decrease in incidence rate of IDD, it is still regarded as an area of iodine insufficiency (Wei et al., 2023). Therefore, our findings suggest that monitoring of BMIC in lactating women population is crucial to establish iodine sufficiency status in vulnerable populations of postpartum mothers and their infants. Data on BMIC will be able to indicate iodine supplementation in addition to iodine enriched food products to improve clinical outcomes in infants.

The circadian rhythm of BMIC in our study was reported following three different time intervals. There was a consistent pattern on iodine concentration throughout the day where lower concentrations were found in the afternoon/evening compared to the morning concentration and at night. This finding was consistent with other studies conducted in Northen China where the circadian rhythm exhibited a distinct "V" pattern over 24 hours (Zhang et al., 2023). The same study also reported that iodine concentration in breast milk significantly correlated with dietary iodine intake of the postpartum woman. We speculate that the utilisation and metabolism of iodine in the body may follow specific diurnal pattern based on the need to regulate the circadian rhythm of the infants.

The limitation of our study includes the lack data on the iodine intake of the postpartum woman and findings from one participant does not reflect a true representation of iodine status in Malaysia population. However, our findings found a case of poor iodine status of a postpartum woman in the first six months of postpartum that indicate the needs of BMIC monitoring in Malaysia population. Women living in regions impacted by iodine deficiency, who exclusively breastfeed their babies, should consider incorporating a supplement containing at least 200 μg of iodide per day, following the RDA.

Conclusion

The analytical method was robust, accurate and reliable for measuring concentration of iodine in human milk and applicable for monitoring BMIC in lactating women to prevent IDD in infants. The BMIC in the first month postpartum met the RDA requirement needed by infants but iodine insufficiency occurred and BMIC decreased over lactation stages. The circadian variation of “V” shaped curve of BMIC was observed. Future studies with larger sample size are required to assess BMIC in Malaysia.

Authors contributions

N.H.M.T., A.S.M.S., and J. B. conceptualised and planned the experiment, oversaw sample and data collection, conducted data analysis, while A.Z.A. analysed the samples and data. All authors contributed to the writing of the manuscript.

Ethical Approval Statement

Ethics approval was obtained from IIUM Research Ethics Committee with the project ID IREC 2021-053.

Informed Consent Statement

Informed consent was received from the study participant.

Conflict of interest

There is no conflict of interest for all authors.

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