Maternal iron intake, haemoglobin, and breast milk iron concentration in exclusive breastfeeders

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Abstract. Maternal iron status (intake and haemoglobin levels) and the iron concentration in breast milk are critical determinants of infant health and development. This research aimed to analyse the associations among maternal iron intake, maternal haemoglobin levels, and the iron content of breast milk. This cross-sectional study was conducted in Malang City, Indonesia, from July to September 2025, involving 31 mothers providing exclusive breastfeeding for their infants. Maternal dietary iron intake was assessed using a semi-quantitative food frequency questionnaire (SQ-FFQ), breast milk iron (Fe) content was analyzed using spectrophotometry, and haemoglobin levels were measured by flow cytometry. The mean iron content of breast milk was 0.06+0.10mg/dL, while the mean of maternal haemoglobin concentration was 12.3+1.1g/dL. The average dietary iron intake among mothers was 22.5+12.3 mg/day. The Spearman correlation analysis showed no significant correlation between breast milk iron content and maternal haemoglobin level ($r=0.0068$, $p>0.05$). Maternal dietary iron intake showed no significant correlation with breast milk iron content ($r=0.063$, $p>0.05$) and with maternal haemoglobin concentration ($r=0.344$, $p>0.05$). These findings imply that variations in maternal dietary iron and haemoglobin levels have minimal influence on breast milk iron concentration, which is likely maintained through physiological regulation independent of maternal intake.

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1 Introduction

Iron is a critical micronutrient, essential for numerous physiological processes, including oxygen transport, energy metabolism, and, most crucially, infant neurodevelopment. The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months of life, recognizing breast milk as the indispensable source of all required nutrients, bioactive components, and immunological agents for the infant [1]. Consequently, the nutritional adequacy of breast milk is intrinsically linked to early-life health outcomes and the prevention of nutritional deficiencies [2].

Maternal iron deficiency, frequently leading to Iron Deficiency Anaemia (IDA), represents a significant global public health challenge, affecting millions of women worldwide, particularly during the peripartum and lactation periods [3-5]. Low maternal iron stores, often reflected by reduced haemoglobin and ferritin levels, are associated with adverse outcomes for the mother, including fatigue and impaired immune function [3]. Maternal iron status is often variable and suboptimal in vulnerable populations. However, a paradox in lactation physiology is that maternal iron deficiency does not necessarily reduce breast milk iron concentration. The mammary gland appears to maintain a stable iron supply to the infant through tightly regulated homeostatic mechanisms, prioritizing infant nutritional needs despite compromised maternal iron stores. Consistent with this concept, no correlation was found between maternal Hb levels and breast milk iron status among anaemic lactating mothers. This suggests that the iron concentration in human milk is highly resilient, maintained through robust homeostatic mechanisms even when maternal reserves are depleted. Furthermore, iron resilience indicates the absence of a significant association between maternal haemoglobin concentration and breast milk iron concentration, indicating that breast milk iron levels remain relatively stable despite maternal anaemia. This resilience serves as a primary protective factor for the exclusively breastfed infant's initial iron status.

Despite the established stability of milk iron, the precise extent and underlying factors governing this resilience remain the subject of active investigation. Most existing literature focuses on the relationship between clinical biomarkers (such as maternal haemoglobin and serum ferritin) and milk composition. There is a distinct gap that directly correlates quantified dietary iron intake with clinical status and, subsequently, the trace element content of the resulting breast milk, particularly within populations where habitual dietary intake is low [6, 7]. Understanding the interplay among these three variables (dietary intake, maternal status, and milk content) is paramount to determining the true physiological burden that poor maternal nutrition places on the quality of early infant feeding.

This study aims to critically analyse the relationships among maternal dietary iron intake, maternal haemoglobin status, and the resultant iron concentration in the breast milk of exclusively breastfeeding mothers. By rigorously quantifying these three factors within a cohort of mothers, we seek to determine the extent of breast milk's homeostatic mechanism and identify the clinical and nutritional thresholds, if any, at which severe maternal iron deficits begin to compromise the elemental composition of milk.

2 Methods

This quantitative study employed an observational design and a cross-sectional approach. The research population consisted of all breastfeeding mothers in Kedungkandang, Malang City. The study was conducted from August to September 2025 in the area of Puskesmas Kedungkandang. Sampling was performed using purposive sampling technique, with inclusion criteria encompassing breastfeeding mothers with infants aged 0–6 months, in good health, without a history of chronic diseases, non-smokers, non-alcohol consumers, and willing to sign informed consent. The sample in this study comprised 31 breastfeeding mothers. Data on iron intake were obtained through direct interviews with mothers using the SQ-FFQ (Semi-Quantitative Food Frequency Questionnaire), which had previously been tested for validity and reliability in a similar population. Breast milk samples were collected in a standardized manner from all participants using the following procedure: samples were taken from one breast using the Spectra 9 Plus Electric Double Pump with a pumping duration of 20 minutes. Sample collection was performed in the morning between 07:00–09:00 WIB. Prior to collection, mothers were asked to refrain from breastfeeding or pumping for at least two hours to ensure the obtained sample represented a mixture of foremilk and hindmilk. Mothers were positioned in a comfortable upright sitting position during the pumping process. This study included mature breast milk samples, i.e., breast milk produced after day 14 postpartum, thus excluding colostrum and early transitional breast milk (0–14 days postpartum) to ensure homogeneity of lactation stage among participants.

The collected breast milk samples were stored in sterile, metal-free 15 mL polypropylene (PP) containers. The minimum sample volume established was 5 mL per participant. Immediately after collection, samples were stored in a cool box at 2–8°C and subsequently transferred to a −20°C freezer within no more than two hours from collection. Analysis of Fe content was performed in a single batch for all samples to minimize inter-run variation, with the interval between the last sample collection and laboratory analysis not exceeding four weeks. Fe content was analysed using the UV-Vis spectrophotometry method with nitric acid (HNO_3) reagent at a wavelength of 510 nm. Normality testing of data was conducted using the Shapiro-Wilk test. Data were analysed using Spearman's correlation test with a significance level of $p<0.05$. This study has obtained ethical approval with number DP.04.03/F.XXI.30/00847/2025.

3 Results and Discussion

The characteristics of mothers who exclusively breastfed, along with the results of this study, are presented in Table 1.

Table 1. Characteristics of mothers who exclusively breastfed and results of this study.

Variable	n (%)	Mean±SD
Age		
18-24	8 (25.8)	
25-31	11 (35.9)	
32-39	12 (38.7)	
Education		
Elementary	4 (12.9)	
Junior High School	5 (16.1)	
Senior High School	20 (64.5)	
Diploma/Bachelor	2 (6.5)	
Occupation		
Full-time mom	26 (83.9)	
Staff	2 (6.4)	
Others	3 (9.7)	
Iron content of breast milk		0.06±0.10 mg/L
Maternal haemoglobin concentration		12.3±1.1 g/dL
Dietary iron intake among mothers		15.01±4.12 mg/day

Iron concentration remains within the typical physiological range observed in human milk, although positioned toward the lower end of reported values [8]. Another study also documented the average levels of 0.33 mg/L (range 0.26–0.46 mg/L) in healthy lactating women [9]. The differences in iron concentrations observed in this study compared with previous reports may be attributed to variations in human milk sampling procedures, sample handling, and analytical methods, which are known to affect the measured composition of breast milk [10]. These data suggest that Fe concentrations below 0.5 mg/L may be considered relatively low, particularly when compared with other study populations.

Maternal Fe intake and Hb levels may be influenced by several factors, including Fe absorption. Absorption of non-heme iron is affected by the presence of iron enhancers, competitors, and inhibitors. The dominance of non-heme iron intake among subjects (68%) with low bioavailability (1-6%) indicates that although intake appears quantitatively adequate, the absorbed amount may be limited. Table 2 presents the correlations between breast milk Fe content, maternal Hb concentration, and maternal dietary Fe intake.

Table 2. Correlation analysis of breastmilk iron, maternal Hb, maternal dietary iron intake.

	Breast milk iron content	Maternal haemoglobin (Hb) concentration	Maternal dietary iron intake
Breast milk iron content	—		
Maternal haemoglobin concentration	0.063	—	
Maternal dietary iron intake	0.175	0.344	—

Notes. Values are Spearman's correlation coefficients (r). $p<0.05$*

This study showed no significant correlation between maternal haemoglobin (Hb) and breast milk iron (Fe) concentrations. This finding contrasts with a literature review reporting that anaemic mothers tend to have lower iron concentrations in their breast milk, and that iron supplementation in women with low baseline iron status may increase human milk iron levels. However, among healthy lactating women with adequate iron status, studies consistently indicate no significant relationship between maternal dietary iron intake and breast milk iron concentration [11]. Another study also confirmed that maternal anaemia had no significant impact on the iron concentration of breast milk in the early postpartum period [12]. This indicates that iron levels in human milk are tightly regulated and remain relatively stable unless the mother is iron-deficient [11]. Iron metabolism during breastfeeding prioritizes maternal physiological needs and infant protection over altering breast milk iron concentrations [13]. This involves the upregulation of transferrin receptor (TfR1) on mammary epithelial cells when maternal iron stores decline, enabling the mammary gland to maintain iron extraction from maternal circulation even under iron depletion conditions.

The mean dietary iron intake among breastfeeding mothers in this study was adequate, according to the international recommendations of the Institute of Medicine (9–10 mg/day) and the World Health Organization (10–30 mg/day) [14-15]. Although this intake appears adequate, correlation analysis showed a weak positive association between dietary iron intake and breast milk iron concentration ($r=0.175$, $p>0.05$) and maternal haemoglobin levels ($r=-0.310$, $p>0.05$). Several recent studies support the biological plausibility of these findings. The iron homeostasis system is dynamic; however, several studies have demonstrated the presence of iron transporters, such as divalent metal transporter 1 (DMT1) and ferroportin, during early neonatal growth. DMT1 and ferroportin are distributed on the mammary epithelial cell membrane and play a role in the coordinated regulation of iron influx and efflux. Most of the iron in breast milk is bound to lactoferrin, which not only serves as an iron-carrier protein for the infant via specific receptors in the gut but also protects iron from binding to antinutritional agents. This multilayered regulation makes breast milk iron concentration relatively stable within its physiological range.

4 Conclusion

This study showed that there was no relationship among maternal dietary iron intake, maternal haemoglobin status, and iron concentration in breast milk of exclusively breastfed mothers. This may be due to the low number of respondents or the homeostatic mechanism of iron in breast milk. These findings suggest that nutrition policies should be extended from pregnant women to breastfeeding mothers, including routine haemoglobin monitoring and mandatory iron supplementation for six months of lactation, as a complementary strategy to prevent long-term iron deficiency and support maternal and infant health.

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