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Human Milk's Polyamine: A Potential Advancement as Biomarker

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ABSTRACT

Polyamine composition in human milk comprises putrescine, spermidine, and spermine, each playing distinct roles in infant development and digestive health. Variation in composition occurs across lactation stages and influenced by factors like maternal dietary intake. Imbalanced diets may hinder infant growth, weaken immunity, and heighten infection risks. This cross-sectional observational study analyzed 30 human milk samples from Melaka, Seremban, Selangor, and Kuala Lumpur using High-Performance Liquid Chromatography (HPLC) to quantify polyamine content. Maternal diets were recorded in Nutritionist Pro software, with macronutrient intake calculated automatically. Statistical analysis using Statistical Product and Service Solutions (SPSS) assessed the association between maternal diet and polyamine composition. Carbohydrates were the most consumed macronutrient (59.1%, *M* = 214.42 g), followed by fat (22.8%, *M* = 37.36 g) and protein (18.1%, *M* = 63.40 g). Among polyamines, spermine showed the highest concentration (70.2%, *M* = 5.4 nmol/L, SD = 5.99), followed by putrescine (22.3%, *M* = 1.74 nmol/L, SD = 3.38) and spermidine (7.4%, *M* = 0.58 nmol/L, SD = 0.88). No significant difference was found between maternal dietary intake and polyamine composition. However, a negative correlation existed between polyamine levels and certain macronutrients such as lipids, suggesting involvement in other metabolic processes. This study indicates that while dietary intake may not directly influence polyamine levels in human milk, polyamines may interact with specific nutrients in maternal diets, impacting their metabolism.

Keywords: polyamine; human milk; maternal dietary intake.

1. INTRODUCTION

Human milk is the only source of critical nutrients with a complete nutritional profile for the first few months of a newborn life. Polyamines are naturally occurring bioactive compounds found in various foods, including human milk. These compounds, derived from amino acids, are associated with numerous benefits for infants. Their synthesis within the mammary gland, particularly the primary polyamines putrescine, spermidine, and spermine, is crucial for infant growth and development (Muñoz-Esparza et al., 2021). Each mother has a different level of polyamine in human milk depending on lactation stage and maternal dietary intake by the mothers. Therefore, this study aims to investigate the polyamine composition in human milk using High-Performance Liquid Chromatography (HPLC) and explore the relationship between maternal dietary intake and polyamine levels.

2. METHODOLOGY

The method by Kamaruzzaman et al. (2018) was used in this study aligned to achieve the objective of this study. The primary research question focused on investigating the level of polyamine in human milk and determining whether variations in maternal dietary intake influence the levels of polyamine in human milk. A collection of sample lactating mothers from diverse locations, ensuring representation of different dietary habits and demographic factors. Eligible participants were included healthy mothers who exclusively breastfeed their infants and willing to provide human milk samples.

2.1. Polyamine Quantification by HPLC

Polyamine analyses were conducted using the Agilent 1200 Quaternary DAD High-Performance Liquid Chromatography (HPLC) System, a highly sensitive and accurate analytical technique commonly used for separating, identifying, and quantifying compounds in complex mixtures (Kamaruzaman *et al.*, 2018). Each sample and standard were run twice to identify any potential inconsistencies, and the measurement was consistent, accurate and reliable. The excitation wavelength and emission wavelength are set to 254 nm and 360 nm, respectively. These specific wavelengths were chosen because they corresponded to the optimal excitation and emission wavelengths for detecting polyamines.

2.2. Statistical Analysis

According to Kamaruzaman et al. (2018), the statistical analysis was performed using Statistical Product and Service Solutions (SPSS) statistic 28 version. The data are expressed as numbers and percentages for categorical variables. The total macronutrient intake by each of the mothers was analyzed in the SPSS after analysis in Nutritionist Pro. The correlation between the polyamine composition and macronutrient intake was assessed using Pearson's correlation to determine the strength and significance of the relationship between the variables. A p-value of less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1. Maternal Diet Composition

The study's results in **Table 1** reveal that lactating mothers in the sample had a macronutrient intake consisting primarily of carbohydrates 59.1% (M = 214.42 g), followed by protein 18.1% (M = 63.40 g) protein and 22.8% (M = 37.36 g) fat, in accordance with Malaysia's Recommended Nutrient Intakes (RNI) guidelines. Research by Mohamed *et al*. (2022) suggests that the Malaysian population should consume a diverse range of foods as per the Malaysia Food Pyramid, yet many rely heavily on rice rather than others, impacting their macronutrient balance. Therefore, rice consumption can impact a person's macronutrient intake balance. Wubetie & Mekonen *et al*. (2023) supported that the nutritional status of lactating mothers is an important public health issue since their nutritional status can affect both the quantity and quality of nutrient concentration in human milk, and maintaining the nutrients in human milk further depletes their body stores. However, high carbohydrate consumption could support milk production and increase the energy required during lactation. Infants ingest carbohydrates in the form of lactose due to the lack of development of the gastrointestinal tract. The production

of glucose from broken-down lactose can be used to synthesize arginine which is one of the precursors of polyamines. Therefore, a high intake of carbohydrates may lead to higher levels of polyamines in the body.

Table 1. Percentage consumes and Descriptive Statistics Macronutrients (g)

Macronutrients	Percentage (%) (n=30)	Mean	Std. Deviation
Protein	18.10	63.40	49.77
Carbohydrate	59.10	214.42	219.63
Fat	22.80	37.36	35.97

3.2. Correlation between Maternal dietary intake with Polyamine Composition

Polyamines were detected in all human milk samples collected from mothers. **Table 2** depicts the percentage and descriptive statistics for polyamines. Spermine had the maximum concentration at 70.2 % (*M* = 5.4 nmol/L, *SD* = 5.99) compared to putrescine at 22.3 % (*M* = 1.74 nmol/L, *SD* = 3.38) and spermidine at 7.4 % (*M* = 0.58 nmol/L, *SD* = 0.88). According to Munoz Esparza *et al*. (2021) found that spermine levels in human milk were significantly higher than in infant formulas owing to the fat content of human milk. A huge metabolic difference could occur since spermine is dependent on other polyamines due to spermine as a protective measure during the perinatal period, while lower putrescine and spermidine in breast milk were influenced by hormonal factors (Ali *et al*., 2013). Previous research discovered that polyamine content in human milk is significantly correlated with maternal diet. However, in this study no correlation was found between macronutrient and polyamine levels in human milk. All results are not significant (*p*-value > 0.05). It shows that polyamine composition was not only associated with the maternal diet but could also be correlated with other anthropometry factors such as BMI, socio-economic, and health issues (Almeida *et al.*, 2021). Even though the association was not statistically significant, **Figure 1** displays a negative correlation between the level of polyamines and lipid content. Sagar *et al.* (2021) postulate that polyamines regulate adipose metabolism. It can aid in fat breakdown and prevent fat storage in the body. Therefore, the finding of a negative correlation between polyamines and fat suggests that the body produces fewer polyamines in response to a high-fat diet.

Table 2. Percentage and Descriptive Statistics for Polyamines

Polyamines	Percentage (%) (n=30)	*M*	*SD*
Putrescine	22.3	1.74	3.38
Spermidine	7.4	0.58	0.88
Spermine	70.2	5.49	5.99

Polyamines	R-value (*p*- value)		
	Protein	Carbohydrate	Fat
Putrescine	-0.054 (0.778)	-0.024 (0.899)	-0.125 (0.509)
Spermidine	0.101 (0.594)	0.122 (0.519)	-0.068 (0.721)
Spermine	-0.215 (0.253)	-0.174 (0.358)	-0.209 (0.269)

Figure 1. Pearson's correlation (R) between macronutrients and polyamines

4. CONCLUSION

This study successfully achieved its objectives by examining the polyamine composition in human milk. Most mothers had a high carbohydrate intake, followed by fat and protein. Using High-Performance Liquid Chromatography (HPLC), we determined the proportion of polyamines in each mother's milk. Spermine emerged as the predominant polyamine, with the highest concentration (70.2%), followed by putrescine (22.3%) and spermidine (7.4%). This finding is marginally significant in comparison to previous research. However, this study found no significance between dietary intake and the levels of polyamines in human milk. As for the recommendation, further research is warranted in Malaysia to understand variations in breast milk composition among different populations, and the sample should be more than 30 to get more accurate data.

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