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Macro Nutrient and Micro Nutrient Profiling of Breast Milk: A Nutritional Quality Assessment

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Abstract
Human breast milk plays a vital role in supplementation for infants and is influenced by maternal health, regular diet and regional conditions. Here we hypothesized that the nutritional composition of breast milk was altered based on maternal age, regular diet and regional locations that lead to new-born infant growth and development in early months. In this work, we collected nearly 60 human (22-33 age) breast milk samples within a 150 km radius in Hyderabad. All biochemical (protein, pH, total fat, lactose, iron, calcium and nitrogen) analysis was carried forward into a UV-visible spectrophotometer at different wavelengths. Obtained results were showed there alterations in milk compositions are influenced by maternal especially dietary factors. Urban and rural mothers showed variations in all these biochemical analyses based on age, veg and non-veg food intake, area of living and several comorbidities. Overall these findings reveal that emphasizing the role of maternal supplementation and lifestyle in maintaining breast milk quality and effectiveness on infant growth and development provides a clear idea for targeted micro and macro nutritional interferences.

Keywords: Breast milk, Biochemical analysis, Nutrition and New born-infant, Infant growth.

1. Introduction:

Breastfeeding is universally acknowledged as the ideal method of infant nutrition, offering vital nourishment and fostering a unique bond between mother and child. Human milk is not only a complete source of nutrition for infants under six months but also contains a variety of bioactive components such as hormones, stem cells, complex sugars like human milk oligosaccharides (HMOs), exosomes, and microRNAs, whose roles in infant health and development are still being explored (Casavale et al., 2019). According to few well known organizations exclusive breastfeeding for the first six months of life due to its numerous health benefits for both infants and mothers. Human milk plays a pivotal role in infant immune function, gut development, and appetite regulation, while also protecting against infections, allergies, and chronic diseases. It reduces the risk of gastrointestinal infections, respiratory tract illnesses, necrotizing enterocolitis, and sudden infant death syndrome (SIDS) (Victora et al., 2016; Le Doare et al., 2018). Furthermore, breastfeeding has been associated with enhanced neurodevelopment and cognitive abilities that persist into childhood and adulthood (Spatz et al., 2018; Graf et al., 2021). For preterm and low-birth-weight infants, human milk serves as a critical medical intervention, reducing risks of severe complications such as intraventricular hemorrhage, sepsis and retinopathy of prematurity (Hair et al., 2016).

In addition to its macronutrients carbohydrates, proteins and fats human milk contains bioactive compounds that aid infant development. Lactose, the primary carbohydrate, facilitates calcium absorption and supports gut microbiota composition (Hester et al., 2012). Proteins, including whey and casein, contribute to growth and immune defense, while essential fats like docosahexaenoic acid (DHA) support neural and retinal development (Guo, 2014). However, factors such as maternal nutrition, body weight, and regional dietary habits influence milk composition, potentially impacting its nutritional quality (Andreas et al., 2015).

Despite its known benefits, challenges such as insufficient milk supply, poor maternal nutrition, and lack of support hinder optimal breastfeeding practices. Additionally, concerns over iron bioavailability and the risk of deficiency in exclusively breastfed preterm or low-birth-weight infants highlight the need for further research into micronutrient supplementation during lactation (WHO, 2020). This study aims to examine the biochemical properties of human milk and its impact on infant growth during lactation. It also explores the rheological behavior of human milk proteins and their role in stabilizing emulsions, contributing to our understanding of their nutritional and functional properties.

2. Sample Collection:

All the breast milk samples were collected from 60 lactating women aged 20-33 years within a 150 km radius of Hyderabad, following strict inclusion criteria to ensure reliability. Mothers with chronic illnesses, metabolic disorders, or substance abuse were excluded. Detailed surveys captured maternal health, dietary habits, anthropometric data and lactation history. This approach enabled a comprehensive analysis of biochemical variations in breast milk influenced by maternal age, diet and regional (Urban and rural) areas.

3. Materials and Methodology:

3.1 Sample Collection:

Milk samples were collected from collected from 60 lactating women aged 20-33 years within a 150 km radius of Hyderabad, ensuring a broad representation of urban and rural areas. The diversity of geographic locations allowed the study to account for environmental and demographic factors that may influence the biochemical composition of the milk. The lactating women were grouped into different categories based on their diet, employment status and comorbidity conditions. The vegetarian and non-vegetarian dietary groups were included to assess the impact of diet on milk composition, while employment status (employed vs. unemployed) was also considered as a potential factor in milk composition. Additionally, a comorbidity group was introduced to investigate the potential effects of underlying health conditions on breast milk quality.

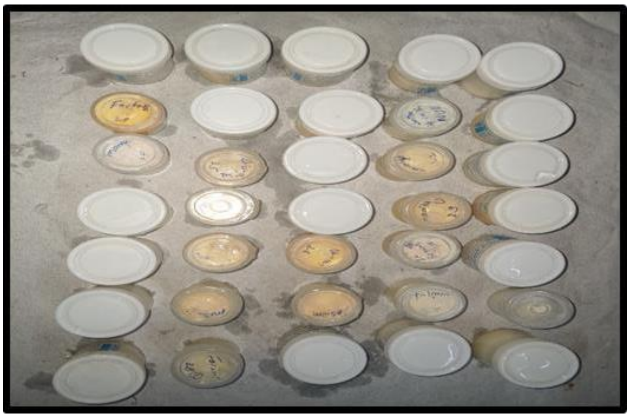

Figure 1: Collected breast milk samples from different regions.

In total, 60 mothers were divided into 12 groups based on their region (urban or rural), dietary preference (vegetarian, non-vegetarian and a mix), employment status, and comorbidity condition. Each group contained 5 mothers, making the total sample size for the study 60 (Figure 1). The milk samples were collected through careful, hygienic procedures and stored in sterile containers. The mothers provided detailed information regarding their age, diet, and employment status, allowing for a comprehensive analysis of how these factors influenced the milk's biochemical composition.

Determination of pH in Breast Milk:

The pH of breast milk samples was determined using an electro-pH meter to ensure precise measurements. Aliquots of 3 mL of milk were thawed overnight at 4°C to maintain structural integrity, followed by centrifugation at 15,000 × g to isolate the skimmed milk supernatant. The fat layer was carefully removed using sterile swabs to prevent contamination of the electrode, ensuring accurate readings. The supernatant was transferred to sterile microcentrifuge tubes, warmed to 37°C in a water bath, and measured using a digital pH meter with a temperature-compensated glass electrode. Triplicate readings were taken for accuracy and consistency (Bradford M. M. 1976).

Estimation of Proteins in Breast Milk:

For protein quantification, 2 g of lyophilized milk samples were mixed with 4 mL of biuret reagent in 15 mL Eppendorf tubes. The reagent was prepared using copper sulfate, potassium tartrate, and sodium hydroxide solutions, adjusted to 3% NaOH. The samples were thoroughly mixed, filtered to remove insoluble particles, and analyzed at 524 nm using a UV-visible spectrophotometer, where the biuret complex shows peak absorbance. This process ensured accurate protein measurement (Spector 1978).

Estimation of Fat in Breast Milk:

The total fat content in breast milk was estimated using Forcato's method (2005), which involves treating the milk with sulfuric acid and amyl alcohol to dissolve protein and release fat globules. The procedure starts by mixing 10 mL of milk with 10 mL of sulfuric acid, followed by the addition of 1 mL of amyl alcohol after 5 minutes. The mixture is then centrifuged at 1200 rpm for 5 minutes, allowing the fat to separate and float to the top. The sample is then heated at 65°C for 5 minutes to ensure proper phase separation, and absorbance readings are taken at 208 nm using a spectrophotometer. All samples were measured in triplicate to ensure accuracy, and were preserved with azidiol at 4°C to prevent degradation. This method allows for precise and reliable estimation of fat content in breast milk (Forcato 2005).

Estimation of Lactose in Breast Milk:

The total lactose content in breast milk was determined using a method adapted from Zhang et al., (2010), which involves the hydrolysis of lactose with sulfuric acid and the subsequent reaction with a cysteine hydrochloride-tryptophan reagent, followed by spectrophotometric analysis. First, 2.8 mL of 75% sulfuric acid was added to 5 mL of milk to hydrolyze lactose into its monosaccharides. The mixture was blended and incubated at 46°C for 5 minutes to ensure complete hydrolysis. Then, 0.2 mL of the cysteine hydrochloride-tryptophan reagent was added to form a coloured complex with the hydrolyzed lactose. The reaction was incubated at 46°C for 30 minutes, followed by cooling for 5 minutes using tap water. Absorbance was measured at 518 nm using a UV-visible spectrophotometer, with calibration performed using a blank reference (Zhang et al., 2010).

Estimation of Total Iron in Breast Milk:

The estimation of total iron in breast milk was performed using a method involving 1-(2-Pyridylazo)-2-naphthol (P-PAN) and a series of reagents for accurate measurement. The process began with lyophilizing 25 mL of milk samples overnight at -196°C to remove moisture, then dissolving the resulting powder in dilute nitric acid to a final volume of 25 mL. To analyze iron content, a 1.0×10^{-3} M PBN solution was prepared, and iron (II) ions ranging from 7.5 to 25 mg were added to the solution along with thiosemicarbazide and a phosphate buffer (pH 6.0). The final volume was adjusted to 25 mL, maintaining 50% ethanol. The absorbance of 1 mL of the processed sample was measured at 550 nm using a UV-visible spectrophotometer, with a reagent blank used for calibration (Sharma et al., 2009).

Estimation of Calcium in Breast Milk:

To estimate calcium in breast milk, 10 mL of milk samples were thawed and mixed with 10 mL of 65% nitric acid (HNO_3), followed by a 24-hour incubation at a low temperature for complete digestion of the milk matrix. After incubation, the samples were heated on a hot plate for one hour to further digest and react. The digested solutions were filtered to remove any un dissolved residues and analyzed for absorbance at 530 nm using a UV-Vis spectrophotometer. Measurements were performed in triplicate to ensure accuracy, with the average absorbance values used for quantification (Jannah et al., 2022).

Estimation of Nitrogen in Breast Milk:

The estimation of nitrogen content in breast milk samples is carried out using a standard method that involves converting nitrogen into ammonium sulfate and subsequently measuring ammonia levels. Initially, a 1 mL aliquot of the milk sample is mixed with 4 mL of 40% sodium hydroxide (NaOH) solution. The mixture is distilled for about 10 minutes, during which ammonia gas is liberated. The ammonia is then absorbed in 5 mL of 2% boric acid (H_3BO_3) solution, which also acts as an indicator for the titration process. The ammonia-boric acid solution is titrated with an N/70 sulfuric acid (H_2SO_4) solution. The endpoint of the titration, indicated by a colour change, reveals the amount of ammonia, which directly correlates with the nitrogen content in the milk sample. The amount of sulfuric acid required for neutralization and we used the following equation mentioned below to calculate the nitrogen concentration in the sample. This method provides an accurate and reliable means of quantifying nitrogen in the milk (Goyal et al., 2022).

Total volume

(A-B) of sample
100 *Strength* of
Percentage of Nitrogen = ------------- x ----------------------x----------- N/70 FI,S01
Volume of Quantity of
Digested sample taken
Sample for digestion
A = *Titer* value of the sample B Blank

4.Results:

- **P^H Levels Analysis:**

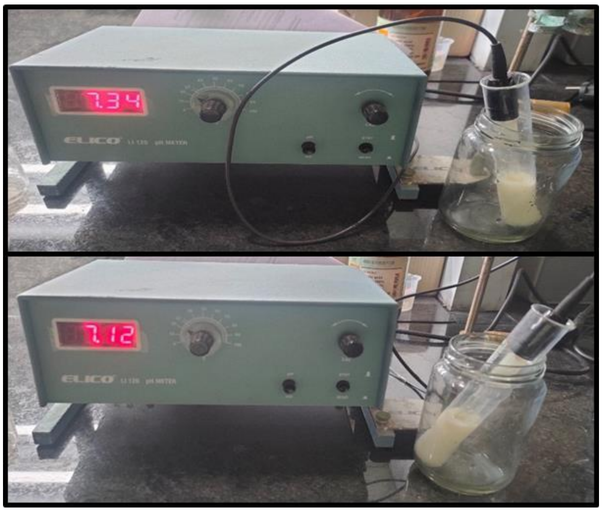

Figure 2: pH values were identified in different breast milk.

These pH values exhibited clear regional variations, with urban areas showing consistently higher pH values than rural areas. Whereas, individuals particularly, those with a veg-intake-women delivered persons and employed status showed lower pH levels around 7.09 compared to urban counterparts, where pH values were higher (7.32-in urban unemployed individuals with comorbidities) showed in (Figure 2). Additionally, unemployed individuals and those with all these comorbidities will show slightly higher pH values in both regions, suggesting that employment status and health condition might influence the pH in all delivered women.

- **Protein Content Analysis:**

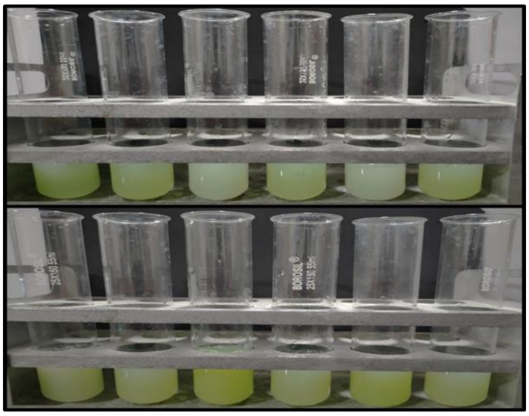

Figure 4: Total protein content was estimated in different regional breast milk samples.

The overall protein content from this data demonstrated that higher differentiation between rural and urban populations. In rural areas, non-veg-intake had the highest protein content nearly (3384 µg /ml in employed individuals), while vegetarians showed significantly lower values (1154 µg /ml). Whereas, urban areas showed the same result though the difference was less pronounced. For instance, urban vegetarians had 2309 µg /ml, which was higher than the protein content of rural vegetarians but lower than that of rural non-veg-intake women. Interestingly, the comorbidity groups in both rural and urban peoples had a notably higher protein level, with rural unemployed comorbidity individuals reaching up to 3650 µg /ml (Figure 4). This suggests that health conditions might be elevated protein content regardless of employment or personal diet after delivery.

- **Fat Content Analysis:**

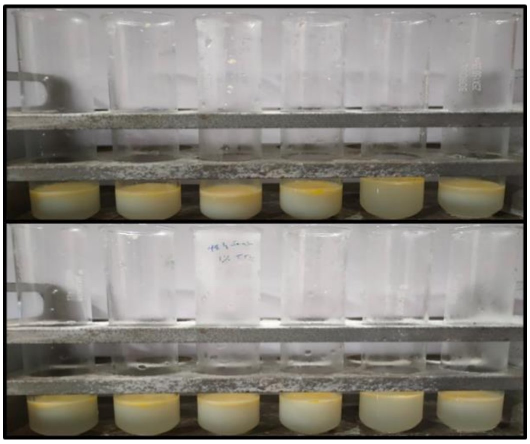

Figure3: Total fat content were estimated in different breast milk samples.

Overall, this fat content was relatively, higher in rural areas across both dietary groups, with non-veg-intake persons showing a little higher fat content than their urban counterparts. Rural un-employed non-veg intake persons had the highest fat content (1.492 µg/ml), while urban vegetarians recorded the lowest (1.395 µg/ml). Urban non-veg-intake persons showed an increase in fat content (1.5 µg/ml) compared to rural vegetarians (Figure 3). This indicates that dietary habits (vegetarians and non-vegetarian peoples) significantly impact fat content with rural non-veg-intake persons having a slight advantage.

- **Lactose Content Analysis:**

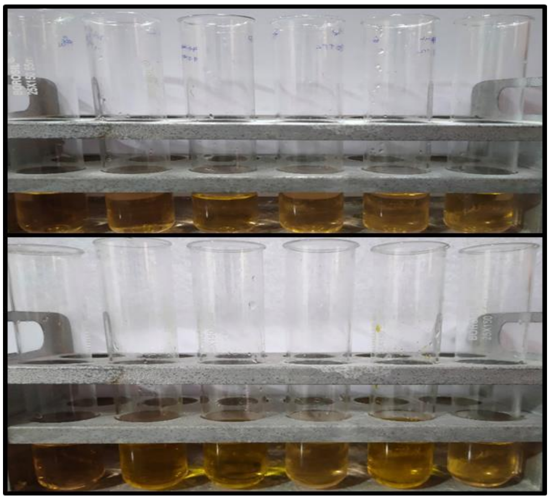

Figure 5: Lactose content was identified from all breast milk samples.

In this work, lactose levels were somewhat higher in urban individuals than rural ones. Urban vegetarians exhibited the highest lactose content (378 µg/ml), while rural vegetarians had 339 µg/ml. non-veg-intake persons in both rural and urban areas showed similar trends, with urban non-veg intake having slightly lower lactose content (359 µg/ml) than rural non-veg-intake persons (398 µg/ml). In both regions, comorbidity groups exhibited moderate lactose content, suggesting that health status does not show any significant impact on lactose levels (Figure 5).

- **Iron and Calcium Content Analysis:**

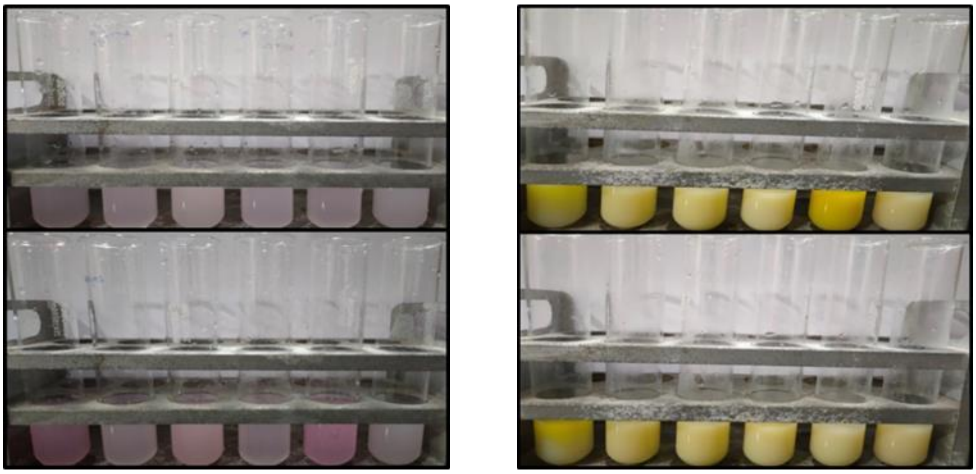

Figure 6 A & B: Iron and calcium content were estimated in different breast milk samples.

Iron and calcium content also exhibited regional and dietary differences. In rural areas, non-vegetarians consistently had higher iron content (190.75µg/ml) compared to vegetarians (178.98µg/ml) (Figure 6 A). This trend was even more pronounced in urban areas, where non-vegetarians had the highest iron content (224.8µg/ml). Urban vegetarians had a slightly higher calcium content (256.80µg/ml) than their rural counterparts (243.81µg/ml), indicating a possible regional advantage in calcium levels for urban populations (Figure 6 B). Rural comorbidity groups exhibited the highest calcium levels (255.81µg/ml), suggesting that comorbidities might be linked to higher mineral content.

S.No	Region	Personal diet	pH	Protein µg /ml	Nitrogen µg/ml	Calcium µg/ml	Iron ug/ ml	Lactose µg /ml	Fat µg /ml
1	Rural	Vegetari	7.0	1154	0.1941	243	178	339	1.48

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2	Rural	Non-Vegetarian	7.11	3384	0.1778	249	190	398	1.492
3	Rural	Vegetarian	7.13	1984	0.1637	241	194	334	1.487
4	Rural	Non-Vegetarian	7.15	2623	0.1647	238	203	372	1.531
5	Rural	Veg/Non-veg	7.17	2091	0.1745	255	199	365	1.467
6	Rural	Veg/Non-veg	7.19	3650	0.1904	225	212	345	1.526
7	Urban	Vegetarian	7.21	2309	0.1785	256	203	378	1.395
8	Urban	Non-Vegetarian	7.23	1577	0.1884	242	224	358	1.554
9	Urban	Vegetarian	7.25	3558	0.2115	221	184	373	1.452
10	Urban	Non-Vegetarian	7.27	1510	0.2007	247	202	357	1.467
11	Urban	Veg/Non-veg	7.30	2787	0.1910	251	221	387	1.435
12	Urban	Veg/Non-veg	7.32	2324	0.1967	246	198	375	1.485

Table 1: All Biochemical Analysis in Breast Milk Samples from Different Regions.

- **Nitrogen Content Analysis:**

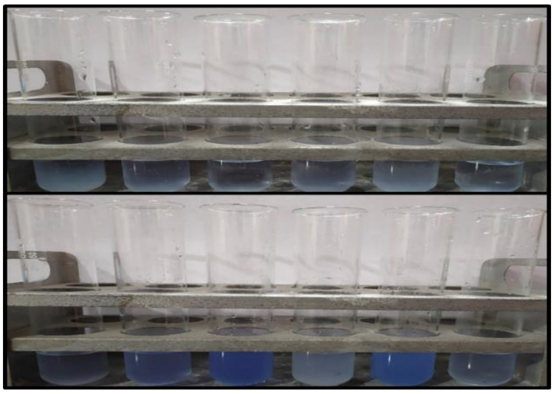

Figure 7: Total nitrogen content estimation in breast milk all samples.

However, nitrogen content was varied in both regions, in urban unemployed vegetarians showing the highest levels of (0.2155µg/ml). Contrastingly, rural-unemployed vegetarians had the lowest nitrogen content (0.1637µg/ml). This shows a general tendency for urban populations to have higher nitrogen content, especially among unemployed individuals, compared to rural delivered women. The nitrogen content in comorbidity groups in both regions was also higher than in the general unemployed groups, further indicating the potential impact of health status on nitrogen content.

5. Discussion:

Maternal Diet and its Effect on Breast Milk pH:

In this work, our results was showed that pH of breast milk was 7.09 to 7.19 along with comorbidity and unemployed peoples showed higher pH values than rest of others. These findings suggest that these comorbidities might influence the pH regulation, potentially due to the metabolic deviations or immune system responses that could affect the composition in breast milk. Similarly, previous studies also suggested that maternal health conditions alter the composition of breast milk, including pH also (Smith et al., 2018)

Conversely, urban mothers exhibited higher pH values in their breast milk, ranging from 7.21 to 7.32, with the highest pH observed in the especially in comorbidity-unemployed group. This data also confirmed with previous studies have noted that urban living populations tend to have better access to diverse and nutrient-rich foods, which could contribute to the higher pH values observed in their breast milk (Johnson et al., 2020).

Maternal Diet and its Effect on Protein Variation in Breast Milk:

Alterations in total protein content in mothers' breast milk among women based on their age (elder and younger), personal diet and financial status are influenced by multiple biological functions, socio-economic status and environmental factors. In our study, we observed that protein content in breast milk differed significantly across age groups, dietary patterns, and financial statuses. This data corroborated with previous statements that have explored the way maternal characteristics, age, nutritional intake and socioeconomic status, impact breast milk composition. Firstly, maternal age is important for the determination of breast milk composition, including total protein content. Several studies have shown that younger mothers below particularly those under the age of 25, tend to have lower protein content in their breast milk compared to older mothers aged above 25 years (Davis et al., 2019). Conversely, older mothers who are more nutritionally stable and have a lot of awareness will have reason to produce breast milk with higher protein content (strict-diet and protein intake), as they may have better access to nutrients or improved absorption and utilization capabilities (Lott et al., 2013).

Maternal Diet and its effect on Total Fat Content:

In our study, we observed notable differences in human breast milk total fat content across the different regions in both urban and rural areas, dietary habits and employment statuses, pointing to the complex interplay of all these factors (Table 1). These findings align with previous reports, suggesting that regional and socio-economic factors, including personal dietary habits and lifestyle, play a pivotal role in determining the fat composition of breast milk (Lee et al., 2017; Smith et al., 2018). In rural living areas, breast milk's fat content was slightly increased higher than in urban regional peoples, with the rural non-veg-intake grouped mothers (1.492 µg/ml) showing marginally higher fat content than the rural vegetarian group mothers (1.484 µg/ml). This will be consistent with previous results that have reported higher total fat content in the breast milk of mothers with diets rich in animal products (Green et al., 2021). Additionally, employment status in rural areas appeared to influence total fat contents, with un-employed individuals showing higher fat content (1.531 µg/ml) compared to their employed counterparts (1.484 µg/ml in veg-intake mothers and 1.492 µg/ml in non-veg-intake mothers). These results align with the notion that a non-veg-intake diet and consumption of over-processed food items (chizzes and refined oils) typically rich in animal overall fat content, contribute to higher fat levels in breast milk at the time of delivery (Lee et al., 2019).

Maternal Diet and its effect on Lactose Content in Breast Milk:

Next in this work we focused on carbohydrates. Whereas, in this work we observed alterations occurred in total lactose content across different maternal groups in this study we highlighted the importance and influence of dietary patterns along with health conditions that connection to socio-economic status on the nutritional composition mother's breast milk. Lactose is a primary carbohydrate in mother's breast milk that play crucial role for providing energy to infants and plays a role in gut health and development (Rasmussen et al., 2014). Our results suggest that both regional and dietary factors play an important role in shaping the total lactose content in breast milk, with noticeable differences observed between rural and urban mothers, as well as between veg and non-veg followed diets.

In rural areas, non-veg intake mothers exhibited overall higher lactose content (398.18µg/ml) compared to their veg-intake mothers (339.94µg/ml). Whereas, this data aligns with existing literature indicating that non-veg followed diets, which are typically richer in several nutrients like proteins and saturated fatty acids, support higher production of lactose. This could be happened with to the availability of essential amino acids and fatty acids, those are required for optimal lactose synthesis at the time of delivery. This much significance differences obtained between non-veg and veg intake mothers suggested that regular dietary factors, particularly the consumption of animal-based products, may directly influence the lactose production process in the mammary glands at the time of pregnancy. Additionally, this higher lactose content in unemployed non-veg intake mothers (372.06 µg/ml) compared to unemployed veg-intake-mothers (334.61 µg/ml) further emphasizes the role of diet in determining the milk carbohydrate composition, particularly in rural regions where accessible to diversified food number might be limited for all selected groups (Dufour et al., 2018).

Maternal Diet and its effect on Iron Variation in breast Milk:

These alterations of Fe^{+2} content in mother breast milk among the women based on age, hemoglobin, green-leafy vegetable intake and regular diet has been widely studied. These factors will play a vital role in determining Fe^{+2} content in mothers breast milk. However, our findings suggest that this Fe^{+2} content in breast milk that can influenced by a combination of mother's nutritional status, age and economic factors. Age is an important determinant of maternal Fe^{+2} statuses. Younger mothers, particularly those in the early stages of lactation, tend to have lower levels Fe^{+2} in breast milk. Several studies have shown that iron content in breast milk is lower during the first few months post-partum, with an increase in concentration during later stages early up to 6 months (Pike et al., 2021).

Like that, younger mothers may have lower Fe^{+2} storage due to lower dietary Fe^{+2} intake process or higher Fe^{+2} demands during pregnancy time, that could be affect the total Fe^{+2} content in their breast milk. Overall, these observations aligns with our data, where younger women, especially those in rural areas or remote areas, showed lower Fe^{+2} levels in their breast milk compared to older mothers. As age increases, especially among mothers with improved nutrition or better access to healthcare, Fe^{+2} levels in breast milk tend to stabilize or increase, showing an overall improvement in maternal-health and nutrition.

Overall Calcium Content in Breast Milk:

Alterations in total Ca2+ content in human breast milk were influenced by maternal age, Ca2+ supplementation, dietary habits and financial support. Whereas, observed results showed that this maternal age can impact Ca2+ levels, as older mothers may experience reduced Ca2+ absorption efficiency and bone mineral density loss, potentially resulting in lower Ca2+ concentration in breast milk (Bertino et al., 2019). Whereas, younger mothers, typically have better Ca2+ absorption, which could be contribute to higher Ca2+ content in their milk, although dietary and health factors can influence this. Ca2+ supplementation during pregnancy and lactation is known to increase calcium levels in breast milk helping to meet the increased calcium demands during lactation (Kuhn et al., 2020). In addition, the consumption of dairy products and other Ca2+-rich foods, which are easily absorbed by the body, plays a significant role in determining Ca2+ levels in breast milk (Gartner et al., 2016). Women who consume a diet rich in milk at the time of delivery, leafy greens and all types of fortified foods tend to have higher Ca2+ concentrations in their milk after delivery, underscoring the importance of an adequate diet during pre and post-lactation periods.

Differentiation in Nitrogen Content in Breast milk:

These alterations overall nitrogen content in human breast milk based in across different groups can be attributed to several factors, including maternal age (young and elder) external nitrogen supplementation, dietary intake and financial support. Whereas, nitrogen a vital element in protein metabolism, plays an important role in the nutritional composition in breast milk and its contents are often influenced by dietary habitats and overall nutritional status. For instance, older mothers might show different nutrient absorption capabilities and metabolic rates compared to younger mothers, potentially impacting nitrogen levels in their breast milk. Studies have shown that maternal age can affect the breast milk composition, with older mothers often having altered metabolic processes in their kidney that could influence the nitrogen content of their milk (Reynolds et al., 2016). Additionally, age-related changes in kidney function for production and filtration and protein turnover rate can lead to variations in nitrogen excretion and retention, potentially influencing breast milk for maintains nitrogen levels (Ishii et al., 2018).

Overall, this biochemical analysis revealed that regional and dietary factors along with financial and health status, significantly influence the composition of various nutrients. Urban populations showed higher pH, lactose and nitrogen content levels, while rural populations, particularly those consuming a non-veg-intake personal diet, showed higher protein, fat and mineral content (iron & calcium). Additionally, comorbidity groups in both regions exhibit higher protein and mineral content levels suggesting that health status could play a vital role in those influences on the biochemical profile. These findings underline the complex interactions between diet, employment, health conditions and regional factors in shaping the nutritional composition of individuals.

6. Conclusion:

Overall, this study investigates the effects of several maternal factors such as regular diet, financial status, and age of mothers, BMI and comorbidities on other biochemical compositions of breast milk in mothers and its implications for newborn infant growth and maternal health conditions. Urban living mothers, with better access to diversified, nutrient-rich diets, showed higher fat & protein content in their milk, while rural mothers exhibited increased iron levels, potentially influenced by regional dietary habits. Ca+2 and N levels in breast milk were strongly linked to maternal diet and supplementation, with older mothers experiencing reduced calcium absorption. Non-veg diets supported higher fat, protein and iron content levels, whereas veg diets, particularly in rural areas, were associated with lower milk quality due to limited food diversity in the time of pre and post-delivery. Comorbidities such as especially gestational diabetes and preeclampsia caused adaptive increases in Fe+2 and Ca+2 levels, especially in rural populations, to support infant growth and development. Breast milk pH showed altered variations, with urban mothers having higher pH values due to fortified diets. Employment status showed limited impact, although rural unemployed mothers had marginally higher fat content, reflecting reduced stress and better dietary focus and regulations.

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