Original Article

Effect of malnutrition on biochemical parameters of breast milk in lactating mothers

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Abstract

Context: There is growing recognition of the role of diet and other environmental factors in modulating the composition of breast milk. The present study was designed to demonstrate the effect of mother's socioeconomic status and mother's nutritional status on phospholipids and other biochemical parameters on breast milk composition of Indian women. **Methods:** Forty moderately malnourished full term and 40 severely malnourished mothers were compared with 40 well-nourished mothers. Breast milk samples were expressed from lactating mothers 15-20 days post-partum after lunch. Breast milk total phospholipids levels, individual phospholipids (separated by thin layer chromatography (TLC), electrolytes, lactose and protein levels were compared among the groups.Quantitative data were analyzed using unpaired t test with SPSS software, version 17. **Results:** Total phospholipid level was significantly lower in severely malnourished women (31.64 ± 2.04) as compared to the well-nourished group (34.00 ± 1.88). Among individual phospholipids there was a trend of decrease in sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol levels form well-nourished to severely mal nourished group. Triglyceride (well nourished-6.04 ± 0.34, moderately malnourished-5.94 ± 0.22 and severely malnourished-4.48 ± 0.43 gm/100ml) and protein content (well nourished-0.97 ± 0.07, moderately malnourished-0.94 ± 0.08 and severely malnourished-0.91 ± 0.08 gm%) was found to be decreased while no significant change could be observed in cholesterol, lactose, sodium and potassium content of milk among 3 groups. **Conclusion:** Malnutrition plays significant role in altering some biochemical parameters which in turn can affect infant's health and may exert severe complication. Maternal nutrition and diet should be given more attention in our country where child health and nutrition are major issues of national concern in paediatric public health. Awareness should be raised for nutritional requirements of mothers and child further the economic level of the country should be improved.
Keywords: Malnutrition, Lactating mothers, breast milk.

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INTRODUCTION

Malnutritioncontinues to be a major health burden in developing countriesparticularly in southern Asia and sub-Saharan Africa. Deficiency of macronutrients (protein, carbohydrates and fat) leads to protein–energy malnutrition and deficiency of micronutrients (electrolytes, minerals and vitamins leads to specific micronutrient deficiencies (Millward and Jackson, 2004). It is globally the most important risk factor for illness and death, with hundreds of millions of pregnant women and young children particularly affected due to the nutrients insufficiency in breast milk (Müller and Krawinkel, 2005).Human milk plays a vital role in survival and healthy development of infants not only due to the presence of nutrients butnon-nutritive bioactive factors that promote protection of infant against infection and inflammation and contributes to immune maturation, organ development, and healthy microbial colonization (Ballard and Morrow, 2013). Maternal diet and nutritional status have been found to influence both the quantity and the quality of milk as the energy, protein, and other nutrients in breast milk come from the mother's diet or from her own body stores. When women do not get enough energy and nutrients in their diets, repeated,

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closely-spaced cycles of pregnancy and lactation can reduce their energy and nutrient reserves, a process known as maternal depletion http://pdf.usaid.gov/pdf_docs/Pnacw588.pdf. Further, population of each geographical zone has particular eating habits which may influence lipid and fatty acid and other nutrient profile of human milk. There is growing recognition of the role of diet and other environmental factors in modulating the composition of breast milk. Studies have documented the biochemical composition of human breast milk from different parts of the world India (Frigerio, 1991 and Brüssow et al, 1996) and demonstrated the effect of maternal nutrition on the nutritional quality of human milk but there is paucity of data form. So the present study was conducted to evaluate the effect of malnutrition on the biochemical composition of human breast milk in Indian women.

MATERIAL AND METHODS

The present case control study was conducted in collaboration of department of Neonatal ICU & department of Biochemistry, Seth G. S. Medical College, Mumbai, India during the year of 2010 to 2011. The study protocol was approval by institutional Ethics Committee prior of starting the study.

Study Population

Forty moderately malnourished full term mothers (BMI 18-20 Kg/m^2, Serum total protein-> 6.3 gm% serum albumin- > 3.7%, Skin fold thickness- > 9.0 and haemoglobin- 10.0-12.0 gm/dl) and 40 severely malnourished full term mothers (BMI <18 Kg/m^2, Serum total protein- < 6.3 gm% serum albumin- < 3.7%, Skin fold thickness- < 9.0 and haemoglobin- <10.0 gm/dl) were recruited as a case groups. Forty well-nourished full term mothers (BMI >20 Kg/m^2, Serum total protein-> 6.3 gm% serum albumin- > 3.7%, Skin fold thickness- > 9.0 and haemoglobin- >12.0 gm/dl) with good general health were included as a control group. Mothers with multiple births, vaginal delivery, and baby separated from mothers also HIV positive, diabetic mothers and mothers having cardiac and hepatic disorders were excluded from the study. All the participants were recruited form the Maternity ward of the institute after obtaining well informed consent. The age range of all study participants was 18-35 years.

Sampling of milk

Breast milk samples were expressed by 120 lactating mothers 15-20 days post-partum after lunch by complete emptying of both the breasts at ward 38 Neonatal ICU. On the day of sample collection, after the lunch, the en tire contents of both the breast were expressed with a Lloyd-B hand pump. Only 2-3ml of sample was taken and dispensed into a plain sterile tube for the test after mixing the sample properly and the remainder was fed to the infant. The samples were Breast milk samples were preserved at -20^{o}C until analyzed. Human milk was defatted by 15 min of centrifugation at 1,000 3 *g*.

Biochemical paramters in milk

1. Total phospholipids levels were measured by the method of Fiske and Subbrao, 1925.Briefly, total phospholipids from the milk are extracted with ethanol ether mixture. The extract of phospholipids was then digested with the digestion mixture of sulphuric acid -perchloric acid to convert organic phosphorus into inorganic phosphorus. The inorganic phosphorus then formed was made to react with ammonium molybdate in acid solution to form phosphomolybdate. The phosphomolyb date thus formed was reduced by metol into molybdenum blue, a blue colored complex. The intensity of blue colored complex was estimated colourimetrically, which was proportional to the amount of inorganic phosphorus present in the milk sample. The absorbance of which was measured colorimetrically at 700nm.
2. Individual phospholipids were separated by thin layer chromatography **(**Tuckley and Storrey, 1974**).** Briefly, 0.5ml milk sample and 0.5ml of ethanol: ether mixture was centrifuged for 20 minutes at 300xg to get a tightly packed deposit. Supernatant was separated with the help of Pasteur pipette and 2ml of it was taken in the test tube. The test tube was heated in water bath for 15-20 minutes till the contents of the test tube were reduced to more than half. Then the tubes were kept in oven at 100^{0}C and the aliquots were evaporated to dryness. Few drops chloroform: methanol mixture (2: 1) was added and the sample was applied on the plate. The plate was then kept in a tank saturated with solvent system chloroform: methanol: distilled water (65: 25: 4) till the solvent reached the solvent front. Then plate was taken out and the solvent was allowed to evaporate at room temperature. For detection of spots the plate was kept in the iodine chamber. The separated components were visualized as dark brown spots which were then scrapped and analyzed for phospholipids.
3. Total cholesterol and triglycerides were estimated using enzymatic methods of Allian and Lucy, 1974;Fossati, and Prencipe, 1982 respectively.

For the extraction of cholesterol: 0.2 ml **m**ilk sample was taken in a test tube and 0.75 ml of 1: 2 (v/v) $CHCl_3$: MeOH was added and the contents were mixed well on the vortex rotor. It was followed by the addition of 0.25 ml of $CHCl_3$ and vortexed well. Tubes were centrifuged at 1000 rpm for 5 minutes at room temperature to give a two phase system (an aqueous top and organic bottom). The bottom phase was recovered carefully with the help of the Pasteur pipette. The organic bottom phase was used for cholesterol estimation by kit method.

For the extraction of triglycerides: 0.1 ml of milk sample was taken in a test tube and then 0.25 ml of alkaline ethanol was added to it. The mixture was kept at 70^0C in hot water bath. Then 0.5 ml of magnesium sulphate solution was added to it and mixture was centrifuged at 3000 rpm. The clear supernatant was separated with the help of a Pasteur pipette and was used for estimation of triglyceride by kit method. Breast milk lactose was determined using the method of Folin and Wu, 1920. O Sodium and potassium levels were determined by flame photometry.

Statistical Analysis

Data were analyzed using SPSS version 17. All the quantitative parameters were expressed as mean and Standard Deviation (SD) and qualitative parameters were presented as frequency (%). To compare a continuous variable between groups, the Student's unpaired t-test was performed. P value of <0.05 was considered statistically significant.

RESULTS

The mean age of lactating mothers of different groups were- 1) well-nourished mothers: 24.33 ± 4.4 years, 2) Moderately mal nourished mothers: 26 ± 3.39 years and 3) Severely mal nourished mothers- 26.2 ± 4.46 years. The mean BMI of lactating mothers of different groups were- 1) well-nourished mothers: 23.6 ± 2.07 Kg/m^2, 2) Moderately mal nourished mothers: 18.84 ± 0.66 Kg/m^2 and 3) Severely mal nourished mothers- 16.34 ± 0.60 Kg/m^2.

Phospholipid composition of breast milk in different groups of mothers:

Analysis of total phospholipids showed that total phospholipid levels were significantly lower in severely mal-nourished group as compared to the control while no significant difference could be observed between healthy and moderately malnourished women (p = 0.82). Major individual phospholipids class sphingomylin percentage was significantly decreased (p = 0.0001) in severely malnourished women than in the well-nourished and moderately mal nourished women. Similarly phosphatidyl choline (p = 0.002) and phosphatidyl ethanolamine (p = 0.002) content was significantly lower in the severely malnourished group than healthy and moderately malnourished women. Difference in mean prcentages of phosphatidyl choline, phosphatidyl ethanolamine and sphingomylin were found to insignificant in well-nourished and moderately mal nourished groups. Difference in mean percentage of phosphatidyl inositol and phosphatidyl serine was not significant in all the three groups. Thus it is observed that values of total phospholipids and individual phospholipids sphingomylin, phosphatidyl choline and phosphatidyl ethanolamine are decreased drastically in the milk because of severe malnourishment of mother while their content remains unchanged even if the mothers are moderately malnourished at an early stage of lactation. But there is no effect of moderate or severe malnourishment of mother on the phosphatidyl serine and phosphatidyl inositol content of breast milk at an early stage of lactation.

Table 1: Total and individual phospholipid content in the breast milk samples of mothers

Parameter	Well-nourished mothers (group-1) (N=40)	Moderately nourished mothers (group-2) (N=40)	Severely malnourished mothers (group-3) (N=40)
Total Phospholipids (mg %)	34.00 ± 1.88	33.95 ± 1.34	31.64 ± 2.04 *
Sphingomyelin (mg %)	13.26± 0.76	13.20 ± 0.74	12.11 ± 0.82 *
Phosphatidyl Choline (mg %)	9.35 ± 0.71	9.33 ± 0.72	8.80 ± 0.85**
Phosphatidyl Ethanolamine (mg%)	6.76 ± 0.51	6.73 ± 0.43	6.36 ± 0.61 **
Phosphatidyl Serine (mg %)	2.82 ± 0.41	2.88 ± 0.31	2.68 ± 0.4
Phosphatidyl Inositol (mg %)	1.80 ± 0.21	1.81 ± 0.19	1.75 ± 0.22

*p = 0.0001, **p = 0.002group 2 was compared with group 1 and 3 while group 3 was compared with group 1 and 2.

Neutral lipid, Lactose, Protein and Mineral content in the breast milk samples of mothers

Triglyceride is the major neutral lipid of breast milk and it was significantly decreased (p = 0.0001) in the severely mal nourished group. Triacylglycerol content of well-nourished and moderately mal nourished group was almost constant as there was no significant difference (p = 0.1224). No significant difference could be observed in cholesterol (p = 0.2780) among the three groups. (**Table-2**) Protein content in breast milk was significantly decreased (p= 0.0005) in group-3 as compared to group-1. Results indicating that there was a profound effect of severe malnutrition of mother on her breast milk protein content while there is no effect of moderate malnourishment of mother on its milk protein content at an early stage of lactation. No significant difference could be observed in Lactose content, sodium* and potassium levels among all three group ($p=0.072$, $p \geq 0.05$ and $p > 0.22$ respectively). (**Table-2**)

Table 2: Neutral lipid, Lactose, Protein and Mineral content in the breast milk samples of mothers

Parameter	Well-nourished mothers (group-1) (N=40)	Moderately nourished mothers (group-2)(N=40)	Severely malnourished mothers (group-3) (N=40)
Triacylglycerol (g / 100ml)	6.04 ± 0.34	5.94 ± 0.22	4.48 ± 0.43**
Cholesterol (mg / 100ml)	22.04 ± 2.13	21.54 ± 1.96	21.68 ± 1.88
Lactose (gm %)	7.19 ± 0.22	7.11 ± 0.17	7.03 ± 0.51
Proteins (gm %)	0.97 ± 0.07	0.94 ± 0.08	0.91 ± 0.08 ***
Sodium (mEq/L)	11.17 ± 0.42	11.03 ± 0.16	11.2 ± 0.23
Potassium (mEq/L)	16.03 ± 0.72	15.9 ± 0.48	15.88 ± 0.29

***p = 0.0005, ** p = 0.0001.

DISCUSSION

Human milk is a dynamic system where composition is influenced by several factors. After reviewing the studies related to malnutrition and human milk it was obvious that there is need for further investigation in this area. Present study was conducted on Indian lactating women to demonstrate the impact of malnutrition on the biochemical composition of breast milk. Phospholipids are important components of human breast milk. Process of brain maturation of infants continues up to few months even after the birth in which phospholipids play a crucial role. According to the previous studieshuman milk contains about 0.5g/L phospholipids which are about 0.5% of total milk lipids. In the present study the range of total phospholipid content was found to be 31.64 to 34 mg%. These results are quite in agreement with the similar study performed in Nigeria by Glew et al, 1995. They studied phospholipids from the milk of 16 mothers (13 moderately malnourished and 3 severely malnourished) at lactation stage ranging from 1 to 4 months. They found average total phospholipid to be 0.4 mole % in moderately malnourished mothers (BMI-20.2 Kg/m^2). Bracco et al, 1972reported the quantity of phospholipids in pooled milk (from Swiss milk bank) to be 0.26% of total lipids which is little less than the percentage of phospholipids found in our study. The results for total phospholipid content in the present study were comparable with the earlier studies which were based on the milk of well-nourished mothers, Low total phospholipids content in severely malnourished mothers group can be explained by the fact that the membrane surrounding fat globule becomes thinner in malnourished and synthesis of phospholipids in the mammary gland of malnourished mothers diminishes more as they have less reserves of subcutaneous fat. In the present study the percentage of sphingomyelin was found to be 12.11-13.26 mg % and phophatidyl choline was found to be 8.80-9.35 mg % in the group of well-nourished and moderately malnourished mothers. This is quite in agreement with the previous studies (Mc Nary et al, 1996; Bitman et al, 1984). There was no major difference in the content of these two phospholipid classes inthe group of well-nourished and moderately malnourished mothers as there was no major difference in their socio economic class as well as their diet pattern. But the trend of decrease was observed in the group of severely malnourished mothers. As recalled from their diet pattern their total energy intake was significantly less than the other two groups and also their diet was lacking in the variety of lipids and fatty acids especially PUFAs due to lower socioeconomic status. Lesser sphingomyelin and phosphatidyl choline means there is less choline available to infant in the form of phospholipids the decrease in sphingomyelin and phosphatidyl choline content in mature milk as compared to colostrums is described by various authors. But the present study is the first study in which it is reported that it is more reduced in severely malnourished mothers. Nervonic acid an important component of PUFA is esterified in sphingomyelin in phospholipids of human milk because nervonic acid-sphingomyelin is the most suitable form of absorption for human infant (Bettger et al, 2003). In present study, the amount of phosphatidylethanolamine in the group of moderately malnourished mothers and well-nourished mothers was higher than that present in the severely malnourished group. Esterification of PUFAs is carried out mainly by phosphatidyl ethanolamine and therefore PUFAs are preferentially accumulated in phosphatidyl ethanolamine. The ratio of PUFA: saturated FA also increases as lactation progresses. Low content of PUFAs in the diet or lesser capacity of mammary glands to synthesize PUFAs may lead to decrease in the content of phosphatidyl ethanolamine in the severely malnourished mothers. Similar type of study was conducted by Glew et al, (1995) in Nigeria but they reported that there was no significant effect of mother's nutritional status on milk phospholipids content. The sample size in their study of severely malnourished mothers was three which was very low. The values for phosphatidyl serine and phosphatidylinositol reported in table 1 were also in good general agreement with the earlier studies (Shoji et al, 2006; Bitman et al, 1984). Size of milk lipid globule goes on increasing as lactation proceeds as more lipids are contained in the core of lipid globule and the membrane becomes thinner. Core of lipid globule mainly contains triglycerides. In the present study it was observed that percentage of triglycerides in severely malnourished mothers was lower than the other groups this suggests that milk lipid globule size is smaller in these mothers. This is in accordance with our earlier conclusions that mammary glands may have lesser capacity to synthesize the lipids in severely malnourished mothers. Cholesterol the second neutral lipid class is a component of milk lipid globule membrane. The cholesterol in human milk supplies an infant with close to six times the amount of cholesterol most adults consume from their food. Content of cholesterol remained same in all the three groups of mothers in the present study. When the values of lactose from well-nourished group were compared with moderately malnourished group and severely malnourished group there was no significant difference observed at this early stage of lactation. In the recent past very little attention is drawn towards the lactose and carbohydrate content of breast milk in malnourished condition in early lactation. Emmett and Rogers (1997) reported that lactose is generally the most constant constituent of human milk, least affected by external or internal factors. There is a positive association between milk volume and lactose concentration and thus lactose is the major contributor to the osmolality of human milk. Synthesis of lactose determines the volume of milk secreted by the mammary gland. Secretion of the lactose can be affected by the secretion rates of electrolytes. Results of this study, confirming the point that there is no change in the lactose content of human milk because of malnourished condition of mother are consistent with the fact that there was no change in the electrolytes- sodium and potassium composition of breast milk in well-nourished and malnourished mothers. Our results were comparable to the earlier studies by Morrison's (1968) and Gebre-Medhin et al (1976). Edozien et al (1976) reported total production of lactose was reduced in the milk of malnourished mothers. But the reason behind this may be that lactose percentage was calculated on the basis of total production after considering both quality and quantity of milk.

In the present study the protein concentration in the group of well-nourished and moderately malnourished mothers was found to be 0.94- 0.97g %. The protein content of milk in the present study was estimated by Folin-Lowry method and the results obtained are comparable to the earlier reports (Hanafy, et al, 1972 and Casey, C.E. and Hambidge, 1983).Protein concentration in the group of severely malnourished mothers in our study was found to be significantly low. The reason for low protein content found in the group of severely malnourished mothers may be this study was carried out for the early lactation period. At early lactation stage mothers are not completely recovered from the stress of pregnancy and delivery, especially malnourished mothers definitely take more time for recovery. Along with lipids, lactose and proteins, minerals are also important constituent of human milk. There were two reasons behind considering the milk mineral content of milk while studying biochemical contents of breast milk in malnutrition. Primarily these parameters were not considered by any author in earlier studies in malnutrition. Secondly imbalance in milk mineral content may cause serious effects. The trapping of lactose within the Golgi spaces creates an electrochemical difference that is responsible for the transport of sodium and potassium into the milk. It is known that monovalent ions like sodium potassium and chloride cross both Golgi and apical plasma membrane of mammary alveolar cell. The mechanism by which the concentration of these milk constituents is regulated is poorly understood. Allen et al (1991) after correlating secretion rates established that ionic composition of human milk as far as sodium, potassium and free phosphate is conis concerned tends to be characteristic of a given women. Many authors have published data for sodium and potassium during the course of lactation for normal (well nourished) mothers. Sodium and potassium content in the milk samples studied was 11.03-11.17mEq/L and 15.88-16.03 mEq/L respectively. Further there was no difference in the values of sodium and potassium in the milk of all the three groups. In the opinion of Barness et al. (1981) no relationship has been demonstrated between maternal salt intake and human milk sodium content. So from the present study, it can be concluded that malnutrition plays significant role in altering some biochemical parameters which in turn can affect infant's health and may exert severe complication. Awareness should be raised for nutritional requirements of mothers and child further the economic level of the country should be improved so that and the money become available for low income families to buy foods and thus the mothers-child health can be boosted.

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