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## Original Research Article

## Comparative study of phospholipid content in breast milk of mothers at preterm and term babies

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## ABSTRACT

**Introduction:** Phospholipids play important roles in cell membrane integrity, neural and brain development, and inflammatory responses. Phospholipids are also important for the growth of infants and the neurological development and the neurological disorders.

**Aim:** The aim of the present study was to determine and compare phospholipid content in breast milk of mothers at preterm and term babies.

**Materials and Methods:** The present study was a hospital- based comparative and analytical study. The study was conducted over a period of 1 year on 60 samples. The study group comprised a total 60 individuals in which breast milk of mothers of preterm babies comprised of 30 individuals and breast milk of mothers of term babies comprised of 30 individuals. The phospholipid content were qualitatively analyzed by the Thin Layer Chromatography and quantitatively analyzed by the method of Connerty.

**Results:** The results of the present study show that, the Mean±SD of the total phospholipid content (mg/dl) in breast milk of mothers of preterm babies was 84.44±10.45 mg/dl and the Mean±SD of the total phospholipid content (mg/dl) in breast milk of mothers of term babies was 45.1±7.15 mg/dl. The results of the present study indicate that the difference between the preterm and term babies was found to be significant statistically ( $p < 0.0001$ ).

**Conclusion:** The present study concluded that phospholipids content was significantly higher in breast milk of mothers in preterm babies as compared to term babies. Difference in composition of individual phospholipid may contribute to difference in rf values of individual phospholipids in breast milk of mothers of term and preterm babies.

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## 1. Introduction

Phospholipids (PLs) are the lipid molecules containing phosphorus, having a polar portion and non-polar portion in their structures. They are the main component of cell membrane, arranged in bi-layer having excellent biocompatibility.<sup>1</sup> They include primarily phosphatidylcholine (PC), phosphatidylethanolamine (PE),

Phosphatidylinositol (PI), and phosphatidylserine (PS), while sphingomyelin (SM), is the dominant species of sphingolipids.<sup>2</sup> Glycerophospholipids and sphingolipids are quantitatively the most important PLs in milk. They represent about 0.5%–1% of milk fat and about 60%–70% of the PL in milk are present in the milk fat globule membrane (MFGM), placed mainly in the external bilayer membrane of phospholipids.<sup>3</sup>

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The composition of milk from mothers who delivered before completion of gestation period and of mothers who delivered after completion of gestation period suggested that preterm milk might not meet the nutritional requirements of the preterm infant, potentially increasing the risk of impaired growth and development. Metabolic studies on preterm and term milk have furthermore revealed significant differences in metabolites that are important for infant development, including phosphocholine, citrate and lactose, cholesterol, saturated fatty acid and monounsaturated fatty acid as well as glutamine and lysine.<sup>4</sup>

Breast milk of completely matured human contains 3%-5% fats, 0.8%-0.9% proteins, 6.9%-7.2% carbohydrates in the form of lactose, and 0.2% mineral constituents. In human milk, lipids are secreted in the form of fat globules. Composition of total phospholipid content comes out to be 75mg/100ml.<sup>5</sup> Milk fat is another form of intracellular fat that is found in the mammary glands of female mammals. In this form, fat globules or droplets are formed through the synthesis of phosphatidylecholine to regulate the formation of fat globule. These fat globules are surrounded by a structural membrane composed of phospholipids, cholesterol, enzymes, proteins and glycoproteins.<sup>6</sup>

Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis. The principle of chromatography is based on where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. Thin-layer chromatography is a planar chromatographic technique extensively used as a rapid and straight forward tool for analysis. In TLC prepared samples are dissolved in an appropriate solvent and applied in the form of spots or narrow bands to the plates. Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. Normally silica gel is used as the stationary phase it can be considered polar. Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places.<sup>7</sup>

## 2. Aim and Objectives

The aim of the present study was to determine and compare phospholipid content in breast milk of mothers at preterm (n=30) and term (n=30) babies of the different age group and were statistically analyzed.

## 3. Materials and Methods

The present hospital-based comparative and analytical study was conducted over a period of one year on 60 patients. The samples of breast milk of mothers of preterm babies

in the age group of 18-36 years and samples of breast milk of mothers of term babies in the age group of 21-32 years were included in the study. The phospholipid content of all the participants were quantitatively analyzed by the method of Connerty and qualitatively analyzed by the Thin layer Chromatography.

## 4. Results

Observed that total phospholipid content (mg/dl) is more in breast milk of mothers of preterm babies as compared to the breast milk of mothers of term babies were statistically significant ( $p < 0.0001$ ).

Table 2 shows a Mean $\pm$ SD of the rf values of all the phospholipids and were statistically significant. If we compared the rf values of phospholipids in breast milk of mothers of term and preterm than the preterm breast milk phospholipids shows the higher rf value in comparison to the breast milk of term babies.

## 5. Discussion

The present study showed a comparison of phospholipid content (mg/dl) in breast milk of mothers of preterm and term babies. It was observed that the statistically significant difference of phospholipid content between breast milk of preterm and term babies mothers milk. The results of the study are consistent with the results of other studies conducted by Giuffrida F et al (2013),<sup>8</sup> Bitman J et al. (2019).<sup>9</sup> In the present study the Mean $\pm$ SD of concentration of phospholipids by method of connerty which was for term samples 45.1 $\pm$ 7.15mg/dl and the preterm was 84.44 $\pm$ 10.45mg/dl which was higher in the breast milk of mothers of preterm babies as compared to the term babies is also consistent with this explanation since the mammary gland at term may be more mature and less permeable than the preterm mammary gland which has been less prepared physiologically and hormonally for the secretory function. The concentration of most of these phospholipid containing particulate components falls very rapidly and thus, this alternative explanation appears to be most valid only for the initial stages of lactation (colostrums and transitional milk)<sup>9</sup> and this difference was highly statistically significant with  $p < 0.0001$ . Qualitatively we are separates the individual phospholipids on the basis of rf values. The rf value of individual phospholipids is more in the breast milk of mothers of preterm babies as compared with the term milk phospholipids. Among the both groups (preterm and term) the rf value of the individual phospholipids are in the order of phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and sphingomyelin (SM) were visualized from top to bottom. The results of Rf values of our study are similar with the other studies conducted by Huang Z et al. (2020).<sup>10</sup>

**Table 1:** Mean±SD of the total phospholipid content in breast milk of mothers at preterm and term babies (mg/dl)

Method(Method of Connerty)	Number	Mean±SD of TPL	P value	Significance
Term	30	45.1±7.15	<0.0001	Highly significant
Preterm	30	84.44±10.45		

**Table 2:** Observed that total phospholipid content (mg/dl) is more in breast milk of mothers of preterm babies as compared to the breast milk of mothers of term babies were statistically significant (p<0.0001)

Phospholipids	Participants	Number	Mean±SD (rf value)	p value
Phosphatidylecholine	Term	30	0.40±0.04	< 0.0001
	Preterm	30	0.50±0.03	
Phosphatidylethanolamine	Term	30	0.66±0.04	< 0.0001
	Preterm	30	0.77±0.04	
Sphingomyelin	Term	30	0.08±0.03	< 0.0001
	Preterm	30	0.19±0.02	
Phosphatidylinositol	Term	30	0.70±0.03	< 0.0001
	Preterm	30	0.84±0.03	
Phosphatidylserine	Term	30	0.30±0.03	< 0.0001
	Preterm	30	0.34±0.03	

## 6. Conclusion

The present study shows the difference between the phospholipid content in breast milk of mothers of preterm and term babies and it was concluded that phospholipids content was significantly higher in breast milk of mothers in preterm babies as compared to term babies. Difference in composition of individual phospholipid may contribute to difference in rf values of individual phospholipids in breast milk of mothers of term and preterm babies.

## 7. Source of Funding

None.

## 8. Conflict of Interest

None.

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