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Study of Probable Effects of a New Pregnancy on Some Milk Constituents in Lactating Women

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Abstract: The probable effects of a new pregnancy on changes in some components of breast milk were studied. Milk samples were collected from 90 healthy Egyptian women; 45 lactating non-pregnant (control group) and 45 lactating pregnant (study group) with different gestational ages, of which, 9 women were followed up to the third trimester (follow up subgroup). The gross chemical composition of milk was determined and compared between the two groups. Samples from the follow-up subgroup were further analyzed for protein fractions and immunologic components. The major findings showed that total solids, fat, lactose and ash contents were lower, while protein and non protein nitrogen were higher in breast milk of the study group than those of the control group. Fat was widely variable component both in study and control group. The level of sodium was too much higher and the levels of calcium and potassium were lower, while magnesium was undetectable. The composition of breast milk of the study group showed significant changes over the 40 weeks of gestation. With advancing gestation age, there was a decline in total solids, fat and lactose contents while protein and non-protein nitrogen increased. As gestational age advanced, sodium was highly increased while, calcium and potassium were decreased but magnesium was undetectable over time. Protein fractions showed different behaviors throughout the gestation age. IgA, lactoferrin and lysozyme concentrations increased significantly with the gestational age. According to our findings, the milk composition with developing pregnancy tended to pass toward the picture of colostrum rather than mature milk.

Key words: Breastfeeding, Pregnancy, Breast milk composition, Immunological components.

INTRODUCTION

Human milk is considered to be the optimal form of nutrition for infants. It provides both nutritive and nonnutritive benefits to infants. The composition of breast milk is widely variable both within and between breast-feeding women[3].

For many years many discussions about breastfeeding during pregnancy have been mentioned. With the renewed interest in this issue, there is still more controversy regarding safety of breast milk as a nutritional source for infants.

Although breastfeeding during pregnancy is a common practice in many cultures, most of mothers who have become pregnant while still breastfeeding have concerns of safety, nutrition and comfort when it comes to breastfeeding during pregnancy. There continue to be many unanswered questions as: is the pregnancy harmful to the breastfeeding baby? And when a mother can take the decision of weaning?

To date, researchers have assessed the effects of pregnancy on lactating women with respect to the quantity of the expressed milk. However, there is a paucity of information in the literature, on the effect of pregnancy on the composition of breast milk. Marquis *et al.*,[22] reported that the influence of some maternal factors, such as the practice of continuing to breastfeed during a new pregnancy on human milk composition is not well understood.

Therefore, the present study was carried out to assess the probable effects of pregnancy on the gross chemical composition, protein fractions and immunological components of breast milk.

MATERIAL AND METHODS

Subjects: Ninety healthy lactating women were invited to participate in the current study and classified into 2 groups; a control group included (45) lactating non-pregnant women, and a study group included (45) lactating pregnant women with different gestational ages (8-11 weeks, 12-15 weeks, 16-19 weeks and 20-40 weeks-delivery). Of the 45 lactating pregnant women, only 9 women continued to follow up until the third trimester.

Inclusion Criteria: The inclusion criteria for participants were: 1) age: between 18-36 years; 2) no lactational amenorrhea; 3) socioeconomic level: is moderate; 4) healthy status (Physically and mentally) is

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good; 5) no recent medication (i.e. drugs or hormonal contraceptives use); and 6) duration of lactation (single baby): between 6-14 months.

Milk Sampling: Breast milk samples (30-50 ml) were collected from participants by expressing both breasts in sterile dark glass bottles using a manual breast pump (at 8.00 to 11.00 a.m) after 2 hours of the last breastfeeding. The collected samples were immediately frozen at - 18°C and kept at this temperature until analysis.

Chemical Analysis: Total solids were determined by a forced air drying oven at 100°C. Total nitrogen was determined by the semi micro-Kjeldahl method. The factor of 6.25 was used for conversion of nitrogen to protein. Fat was determined by the Gerber butyrometer method; and lactose content was determined at 490 nm using Jenway 6405 UV/Vis Spectrophotometer. Ash content was determined by incineration at 600°C in a muffle furnace. Sodium and potassium were determined using a Flame Photometer M7 D. Calcium and magnesium were determined by titration method as described by Schwarzenbach and Biedermann[29]. Electrophoretic patterns of protein fractions were evaluated by polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli[18]. Lactoferrin, lysozyme and IgA assays were determined using ELISA kit according to Kearney *et al.*,[15].

Statistical Analysis: All analyses were carried out with SYSTAT version 10 (SPSS, Chicago, IL). Data are presented as the means ± SD and significance for all two-tailed probability tests was set at $P < 0.05$ for major components and at $P < 0.01$ for minor components.

RESULTS AND DISCUSSION

The present study was carried out as a cross-sectional study comparing the effects of pregnancy occurs on the composition of pregnant mother's milk. Totally 108 breast milk samples were analyzed; 45 samples as control group, which have been collected from lactating non-pregnant women and 45 as study group, which have been collected from lactating pregnant women with duration of pregnancy ranged from 8-11 weeks (16 samples) of which only 9 women of them could be followed up by 9 milk samples in the second trimester and 9 samples in the third trimester for the same pregnant lactating woman, 12-15 weeks (12 samples), 16-19 weeks (10 samples) and from 20-40 weeks (7 samples).

Gross Chemical Composition: A comparison of gross chemical composition in breast milk of the study group versus that of the control group is shown in Table (1). The mean composition of lactating pregnant mother's milk was significantly different from that of lactating non pregnant mother's milk. Total solids, fat, lactose and ash contents were lower, while protein and non protein nitrogen were higher in breast milk of the study group than those of the control group. The level of sodium was too much higher and the levels of calcium and potassium were lower, while magnesium was undetected.

Lower solids content in breast milk of study group means that the moisture content was higher in lactating pregnant mother's milk, which become more watery and subsequently less nutrient content. Similar observations were reported by Ahmed[1]. Fat was the widely variable in breast milk both within and between groups, however, the fat content in study group was generally lower than that in control group. Higher total protein and non protein nitrogen in study group may be due to the high level of progesterone as in earlier study conducted by Toddywalla[31]. There were significant variations as regard to the four key mineral elements studied. In comparison with those in the control group, sodium was extremely higher while, calcium and potassium were significantly lower and magnesium was below a detectable level. The high level of sodium in breast milk is of particular physiologic importance in the feeding of neonates, because clinical problems may arise if there is an excessive newborn intake of sodium. Hence, the kidney of the neonate has a limited capacity to deal with a heavy load of solute[7,14].

Table (2) shows the compositional changes of lactating pregnant mother's milk that occur during their gestational ages. Total solids, fat and lactose contents were significantly decreased and total protein and non protein nitrogen were increased with increase of gestational age, while the ash was variable, but with no significant changes in relation to the gestational ages. A decline in fat content has been reported by many authors who studied the effect of gestational age on milk components [2,21]. Changes in fat content in the breast milk of lactating pregnant women may be attributed to the maternal body weight changes as a result of weight loss during breastfeeding and weight gain during pregnancy. Weight loss, because of mobilization of body fat, is reported in breastfeeding women, even among malnourished women. Controlled studies in well-nourished women suggest on average, women increase body weight between conception and one-year postpartum by 0.5–3.3 kg[6]. In another study, maternal fat stores (3.3 kg on average) are gained predominantly between the 10th and 30th week of gestation and provide an energy reserve that can be mobilized when fetal growth demands are high in late pregnancy. NHMRC[25] stated that a third of weight gain should occur in the second trimester and two-thirds in the

Table 1 : Gross chemical composition of breast milk from both control and study groups.

Components	Control group n = (45) Mean ± SD(Range)	Study group n = (45) Mean ± SD(Range)	Significance of p value
Total solids (gm/100)	12.22 ± 1.6 (10.54 – 15.39)	10.62 ±1.72 (9.05 – 13.2)	P > 0.05
Fat (gm/100)	3.97 ± 1.39 (2.0 – 9.4)	2.93 ± 0.78 (1.4 – 4.8)	P < 0.05
Protein (gm /100)	1.06 ± 0.28 (0.72 – 1.46)	1.55 ± 0.62 (0.75 – 3.20)	P < 0.05
Non-protein nitrogen (mg /100)	34.71 ± 6.76 (24.30 – 47.82)	50.39 ± 15.56 (28.5 – 86.9)	P < 0.01
Lactose (gm/100)	7.61 ± 0.87 (6.15 – 8.87)	6.16 ±0.53 (5.03 – 6.94)	P < 0.05
Ash (gm /100)	0.28 ± 0.04 (0.19 – 0.38)	0.16 ± 0.03 (0.10 – 0.28)	P < 0.05
Sodium (mg /100)	14.19 ± 1.81 (10.08 – 16.65)	42.34 ± 9.99 (29.67 – 66.41)	P < 0.01
Potassium (mg /100)	51.28 ± 5.34 (40.82 – 62.19)	38.11 ± 7.49 (28.25 – 61.66)	P < 0.01
Calcium (mg /100)	35.5 ± 1.6 (32.4 – 38.2)	20.28 ± 5.4 (11.9 – 28.7)	P < 0.01
Magnesium (mg /100)	5.55 ± 0.94 (3.8 – 7.4)	0.0 ± 0.0 (0.0 – 0.0)	P < 0.01

Table 2: Gross chemical contents in the breast milk of pregnant lactating women (follow up sub-group) according to the gestational age.

Components	1st trimester (n) = 9 Mean ± SD (Range)	2nd trimester (n) = 9 Mean ± SD (Range)	3rd trimester (n) = 9 Mean ± SD (Range)	Significance of p value
Total solids gm /100	11.94 ± 1.01 (10.24 – 13.02)	11.39 ± 0.95 (9.73 -12.63)	10.29 ± 1.03 (9.1 – 12.22)	P> 0.05
Fat gm/100	3.31 ± 0.83 (1.6 – 4.4)	3.14 ± 0.87 (1.6 – 4.2)	2.37 ± 0.89 (1.4 – 4)	P> 0.05
Protein gm /100	1.25 ± 0.36 (0.75 – 1.79)	1.57 ± 0.19 (1.3 – 1.81)	1.96 ± 0.29 (1.28 – 2.42)	P> 0.05
Non-protein nitrogen mg /100	36.64 ± 6.19 (29.3 – 45.6)	43.32 ± 8.33 (35.20 – 58.8)	63.78 ± 13.70 (47.2 – 84.10)	P> 0.01
Lactose gm/ 100	6.62 ± 0.29 (5.99 – 6.90)	6.44 ± 0.42 (5.69 – 6.88)	6.29 ± 0.49 (5.35 – 6.79)	P> 0.05
Ash gm /100	0.16 ± 0.02 (0.13 – 0.18)	0.15 ± 0.02 (0.12 – 0.17)	0.15 ± 0.02 (0.12 – 0.19)	P< 0.05
Sodium (mg /100)	39.19 ± 7.54 (33.94-58.39)	41.49 ± 5.72 (33.96-54.26)	53.68 ± 8.38 (42.52-65.94)	P> 0.01
Potassium (mg /100)	42.03 ± 8.24 (36.41-61.66)	35.39 ± 2.64 (31.1-39.2)	28.63 ± 3.17 (23.3-32.9)	P> 0.01
Calcium (mg /100)	26.4 ± 1.97 (22.4-28.3)	21.3 ± 2.03 (18.5-24.1)	15.8 ± 1.78 (13.4-18.3)	P> 0.01

Magnesium: Non detected

third trimester. Studies have shown that there is a strong relationship between weight gain during pregnancy and milk fat content[24]. Li *et al.*[20] showed that excessive weight gain during pregnancy had a negative effect on breastfeeding practice.

Protein and non protein nitrogen levels were continuously increased throughout progress of gestational age. This may be due to the female sex hormones. Badraoui *et al.*[4] found that immediate administration of depot medroxyprogesterone acetate (DMPA) postpartum in 772 lactating women followed from delivery to 1 year postpartum resulted in an increase in protein level. In another study 591 Egyptian women took a 6-monthly 300 mg DMPA as a mean of contraception. The total protein content of DMPA users showed a significant increase in their values[12] . Most experts have traditionally delayed starting hormonal contraception to the 6-week postpartum, after lactation is well established. This delay has been based on the theoretical concern that hormonal contraceptives containing estrogen and progestin may impair lactation through their effect on the action of prolactin on the breast. Placental estrogen and progesterone inhibit prolactin activity during pregnancy. After delivery, when estrogen and progesterone levels markedly decrease, prolactin level increases and milk production is initiated[5]. There is a theoretical concern that giving hormones before 6 weeks postpartum, or before breastfeeding is well established, could interfere with optimal lactation[32]. Although protein level was higher in pregnant mother's milk, the decline of other valuable nutrients creates a nutritional deficit when it comes to the needs of the infant. Kalhan[14] reported that the pregnancy data show a decrease in total α-amino nitrogen, a lower rate of urea synthesis, and a lower rate of branched-chain amino acid transamination. The exact mechanism of this adaptation may be related to pregnancy-induced resistance to insulin action that is evident early in gestation. Adaptive responses in nitrogen metabolism during pregnancy are aimed at nitrogen and protein accretion initially by the mother and later by the mother and the fetus. So, the female sex hormones appear to increase the efficiency of utilization of protein and lipid during pregnancy and to direct lipid towards energy stores due to a direct effect on mammary tissue or indirectly by affecting blood components.

Lactose level was significantly decreased with gestational age. A similar result was observed by Maas *et al.*[21]. Earlier studies showed that lactose synthesis in the mammary gland is dependent on the hormonally controlled synthesis of the two protein components of lactose synthetase, α-lactalbumin and a galactosyltransferase. Prolactin induces the synthesis of both proteins in mammary gland explants treated with insulin and hydrocortisone. Progesterone appears to take part in the control of lactose synthesis and acts to repress the formation of α-lactalbumin throughout pregnancy[26]. This may be a reason for reduction of lactose in our study group.

With increasing gestational age, sodium was highly increased while, calcium and potassium were decreased but magnesium was undetectable over time. It was reported that the levels of sodium and magnesium were higher and calcium and potassium were lower in colostrum than in milk. Our study group milk resembled colostrum, but the results were in harmony in sodium which is highly increased, calcium and potassium which are decreased, but differ in magnesium which was undetectable. Mercier[23] found an inverse relationship between the level of lactose and that of sodium in breast milk, suggested that it is part of more complex disturbance of lactation. So, any fall in lactose level will result in rise in sodium. Peters[27] reported that sodium chloride and lactose levels combine reciprocally to maintain the breast milk' osmosis at a level similar to that of blood. Allen *et al.*[2] showed that the correlation between lactose level and sodium, chloride and potassium levels during pregnancy provides evidence that paracellular pathways between mammary alveolar cells are open during pregnancy and are at least partially closed during lactation. Failure of these paracellular pathways to close might be one mechanism involved in elevated breast milk sodium level.

Prosser *et al.*[28] compared changes in the milk of two lactating pregnant women through the first two months of pregnancy to changes in the milk of two women who were gradually weaning. Milk composition changes during pregnancy were similar to those during gradual weaning. The levels of sodium and total protein increased while the levels of potassium and lactose decreased from commence weaning to the end of the study.

Protein Fractions: The relative proportion of different protein fractions of breast milk in follow-up subgroup was found to vary throughout gestation (Fig. 1). The most variable fractions were α_{s1}-casein, α_{s2}-casein and β-lactoglobulin. These fractions were decreased in the second trimester, and raised again in the third trimester. B-casein showed a downward trend in the second trimester and remained almost constant thereafter. k-casein and α-lactalbumin were increased with pregnancy advancement.

Kunz and Lönnerdal[17] observed that casein synthesis is low or absent in early lactation, then increases rapidly and subsequently decreases. The concentration of whey proteins decreases from early lactation and continues to fall. These changes result in a whey protein/casein ratio of about 90: 10 in early lactation, 60: 40 in mature milk and 50: 50 in late lactation. These observations indicate that the synthesis and/or secretion of caseins and whey proteins are regulated by different mechanisms. In addition, the relative proportion of the different β- and k-casein fraction was found to vary throughout lactation

Immunologic Components: The secretory sIgA, lactoferrin and lysozyme concentrations increased significantly with increasing the gestational age (Table 3). Lawrence[19] stated that the sIgA is very high in colostrum then decline rapidly and disappearing almost completely by the fourteenth day. Goldman[8] reported that the concentrations are remarkably high, ≈ 100 – 200 mg/100 in early lactation, and remain at 50 – 100 mg/100 up to 2 years of lactation in non pregnant women.

Harzer *et al.*[11] reported that with the analysis of more than 550 human milk samples lactating non pregnant women they showed sharp decrease in IgA content, and a moderate decrease for lactoferrin.

Hartman[10] found the concentration of lactoferrin in lactating non pregnant women is very high in colostrum. The result of our study which revealed that the increase in the lactoferrin according to the gestational age not exceed that recorded by Hirai *et al.*[13] who found that the lactoferrin concentrations in colostrum, the transitional milk and mature milk of lactating non pregnant women were 670, 370 and 260 mg/100, respectively.

Hamosh *et al.*[9] found that the concentrations of immunologic components in breast milk of lactating non pregnant women during several phases of lactation (2-3 days, 1 mo, 6 mo, 12 mo, 13-15 mo and 16-24 mo) were lactoferrin decreased until 12 months then start to increase up to 24 months of lactation, the secretory IgA decreased with the progress of lactation up to the 6 months then increased slightly at 12 month to reach a plateau up to 24 months and lysozyme show a high variability as it decreased up to 1 month then increased up to 15 months and lastly it decreased up to 24 months of lactation.

The present study had several limitations. First, no pregnancy registrar existed from which a representative sample of women could have been selected. A second limitation was the self-selection of the breast-feeding behavior itself and lack of follow up interest. Finally, this topic is not found readily in the available literature.

Conclusion: Since studies evaluating pregnancy effect on breast milk composition have been limited to breastfeeding mother's milk, our study is necessary to determine the optimal duration of breast-feeding period during pregnancy. The results of this study provide health professionals and mothers with valuable information on breastfeeding during pregnancy to decide whether to wean or continue breastfeeding. Further in-depth studies are needed to determine the cumulative effects associated with a breast-feeding during pregnancy overlap on infant, fetus and maternal outcomes.

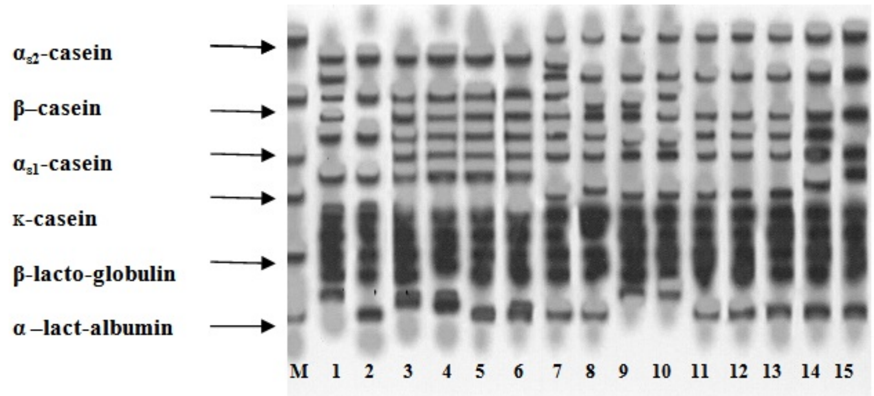

Fig. 1: Electrophoretic patterns of protein fractions on SDS 10% polyacrylamide gel of breast milk of pregnant lactating women (follow up subgroup) according to gestational ages. 1-5: at the first trimester, 6-10: at the second trimester and 11-15: at the third trimester. Molecular mass marker is indicated on the left.

Table 3: The variation in the immunologic components (mg/100) in brestmilk of pregnant lactating women in relation to gestational age (follow up sub-group)

Components (mg/100)	First trimester n = (5) Mean ± SD (Range)	Second trimester n = (5) Mean ± SD (Range)	Third trimester (Range) n = (5) Mean ± SD	Significance of p value
sIgA	144.92 ± 13.32 (132.05–165.25)	170.61 ± 20.93 (144.21-192.13)	212.63 ± 27.98 (185.26 - 251.74)	P < 0.01
Lactoferrin	187.15 ± 7.48 (175.91-196.46)	194.09 ± 14.1 (179.35-215.44)	204.16 ± 22.6 (183.54-232.08)	P < 0.01
Lysozyme	99.47 ± 9.54 (89.42-110.28)	115.12 ± 16.7 (95.24-136.05)	123.33 ± 7.04 (114.65-132.33)	P < 0.01

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