Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.13, 2013
www.iiste.org

Different Factors Effects In Lactating Mother's Milk Compositions

Haider Zaidan[1], Mona Al-Terehi [2], Mohammed Baqur Al-Shuhaib[3], Ali Al-Saadi[1], Mufeed Ewadh[4*]
1.University of Babylon, College of Science
2. University of Kufa, College of Science
3. The Green University of Al-Qasim, College of Agriculture
4. University of Babylon, College of Medicine
*E-mail : mewadh@yahoo.com

Abstract
This study was carried to estimate the effects of different factors on the total protein and lipid concentrations in lactating mother's milk, such as; home, age, body mass index, lactating stage and negative smoking habitat. Total Protein concentration and lipid concentration were estimated using Bradford method and Gravimetric method respectively. Results showed that lipid concentration(L.C) increased with age, BMI, lactation stage, and affected by negative smoking and increased in rural, while protein concentration (P.C)was less effected by age, and decreased in obese and in negative smoking mothers, also it decreased in urban mothers. Protein profile showed slight differences between nonsmoking and negative smoking milk using SDS-PAGE electrophoresis.
Key words: negative smoking, BMI, lactating mother's milk.

1.Introduction

Human milk assumed to be optimal food for infants for 6 month or longer, it has all essential components for growth and development of infants. The nutritional components of human milk are derived from three sources: some of the nutrients of milk are originated by synthesis in the leucocytes, some are dietary in origin, and some originated from maternal stores. The overall nutritional quality of human milk is highly conserved, but attention to maternal diet is important for some vitamins and the fatty acid composition of human milk (Ballard and Ardyth, 2013).

Human milk is the natural and superior food for infants containing the optimal composition to meet their nutritional needs in early life and providing associated immunological, psychological and economic advantages. Evidence for the health advantages of breast feeding and scientific evidence to support this practice has continued to increase. WHO can now say with full confidence that breastfeeding reduces child mortality and has health benefits that extend into adulthood. On a population basis, exclusive breast feeding for six months is the recommended feeding mode for the vast majority of infants, followed by continued breast feeding with appropriate complementary foods for up to two years or beyond (World Health Organization (WHO), 2006). Usually, the human milk is the only source of food for infants during the first four to five months of their life. Many chemicals can be transferred from the body stores and from blood into the breast milk of a lactating mother (Condon, 2005).

Environmental factors increasingly gain importance in public health. Children are affected more than adults from environmental deterioration and harmful effects. Children's exposure to environmental changes may cause permanent damage and will continue during adulthood and general physiological systems of children (Landrigan *et al*., 2002).The proteins of human milk are divided into the whey and casein fractions or complexes, each comprising a remarkable array of specific proteins and peptides.The most abundant proteins are casein, secretory IgA, and serum albumin(Jensen, 1995; Lonnerdal,2005).Total levels of protein in human milk is approximately 0.9-1.2%, 70% of proteins are whey and 30% are casein, it also contain α-lactoalbumine, β-lactoalbumine, lactoferrin, , lysozyme and immunoglobulin (Guo and Hendricks, 2008)

Human milk fat is characterized by high contents of palmitic and oleic acids, the former heavily concentrated in the 2-position and the latter in the 1- and 3-positions of the triglycerides. Fat is the most highly variable macronutrient of milk. Hind milk, defined as the last milk of a feed, may contain 2 to 3 times the concentration of milk fat found in foremilk, defined as the initial milk of a feed. Milk also contains micronutrients such as vitamins, and bioactive factors like immunoglobulin's , cytokines, chemokines, growth factors , hormones and metabolic hormones (Ballard and Ardythe, 2013).

Study on human milk compositions show variations in milk component and these variations differ according to the factors that effect on milk components, protein conformation level, presence or absence of some components. Smoking effects on protein profile of lactating mothers using secretory IgA levels protein\lipids ratio and polyacrylamide electrophoresis was studied (Bachour *et al*., 2012). Add to that, the association between fat content and time of milk collection in morning and evening was highlighted (Stafford *et al*., 1994). Moreover,

the exposing of mothers to xenobiotic effect on milk quality and the quantity of polyaromatic hydrocarbon such as caffeine and nicotine in smoking mothers was demonstrated (Gao and Hendricks, 2012)
Lactating period also effect on human milk because it must be suitable to infants development according to studying stage of lactation which is suitable with infant development. The newborn gastrointestinal tract undergoes maturational changes in the first weeks after birth and human milk has been shown to stimulate gastrointestinal mucosal proliferation and maturation in animal models and it's thought to protect the neonatal infant from harmful environmental factors by affecting and promoting the mucosal barrier (Wagner *et al*., 1996; Takeda *et al*., 2004). Further, the growth factors in human milk, such as epidermal growth factor (EGF), transforming growth factor alpha (TGF) and insulin-like growth factors (IGFs), stimulate the proliferation of intestinal cells and the formation of the mucosal barrier (Corps and Brown, 1987; Wagner and Forsythe 2000).Among them, EGF is thought to have the most significant effect on the proliferation of cells that line the intestine and the promotion of the covering mucosal layer (Grosvenor, 1992)

2.Materials and methods

1- Milk was manually collected from lactating mothers, 10 ml of milk was collected in test tubes then it stored in -20°C until tests were performed.

2- Data were recorded from lactating mother using questionnaire that consists of; name, age, weight, length, home, lactating stage and if she a smoker, negative smoker or not.

3- Body Mass Index (BMI) was calculated according to the following equation; Weight in Kilograms / Height in meter square, then results were classified according to fallowing : BMI between 18.5 – 24.9 = normal weight, BMI between 25- 29.9 = overweight , BMI more than 30 obesity (Gadzik, 2006)

Protein concentrations were detected using Bradford method (Bradford, 1976) and as the following:
A- Standard curve; different concentrations of bovine serum albumin were used to draw standard curve.

B- Coomassie brilliant blue G-250 dye solution; it was prepared by dissolving 100 mg of dye in 50 ml of 95% ethanol, then 50 ml of phosphoric acid 85% was added gradually, mixture filtered by Whatman filter paper and kept at 4 C°.

C- Bovine serum albumin (BSA); Graduated concentration of (0, 20, 40, 60, 80, 100, 120) µg\ml BSA was prepared by titer stock solution which is prepared by dissolving 0.2 g of BSA in 50 ml of DW then it was completed to 100 ml.

D- Protein concentration assay: mixture of 0.05 ml of protein and 0.45 ml of phosphate buffer saline was added to 2.5 ml of dye solution that is prepared previously with shaking. The mixture was left 10 min in room temperature then optical density was measured at 595nm,then the concentration was calculated using the linear equation; **Y=126.33X+0.4966.**

E- Lipid concentrations determination; it was determined using gravimetric method with modification. In brief, 1ml of milk was subjected to a first liquid –liquid extraction with mixture of 2.4 ml dichloromethane\1.2 ml methanol\0.6 ml of 7% NaCl. The aqueous phase was re-extracted with 1.2 ml of dichloromethane. Organic phase were pooled and washed by 7% NaCl and evaporated at 26°C overnight, and then the residual fat matter was weighted (Folch *et al*., 1975; Del *et al*., 2005).

F- Statistics analysis of results; they performed using SPSS V. 17, mean, standard error and standard deviation was calculated, significant at $p \leq 0.05$ for t-test and ANOVA-one way.

G- Polyacrylamide gel electrophoresis (PAGE-SDS); Electrophoresis of milk protein profile performed using 6% staking gel (1 ml of 30:0.8%acrylamide\bisacrylamide; 630µl of 1M tris-HCl pH6.8; 25µl of 20% SDS; 3.6ml of DH2O; 25µl of fresh 10% ammonium persulfate and 5µl of TEMED) and 10% separating gel (2.5ml of 30:0.8%acrylamide\bis acrylamide, 3 ml of 1M tris-HclpH8.8, 38 µl of 20% SDS, 1.9 ml of deionized water, 36µl of 10% ammonium persulfate and 5µl of TEMED), 10 µg of samples loaded by mixing 1:1 V\V with sample loading buffer (0.09 M of Tris-HCl pH 6.8, 20% glycerol, 2% SDS ,1% of bromophenol blue , 0.1 M of beta-mercptaethanol, then it was completed to 100ml with DW and store at -20 °C).

Samples that loaded were electrophoresed in 1X of running buffer (3 gm tris-OH, 14.4 gm glycine and 1 gm SDS were dissolved in 1000 ml DW) in vertical electrophoresis tank at 90V for 60 min in 35 mA (Clever Scientific –UK). Then, the polyacrylamide gel was stained using 1 gm of Coomassie brilliant blue R-250 (500 ml methanol, 100 ml glacial acetic acid, and 400 ml ddH_2O) for 2 hours. Then, the de-staining buffer was used to remove excessive stain (100 ml methanol, 100 ml glacial acetic acid and 800 ml ddH_2O), protein profile was detected using protein size marker (Bioneer Cat# D-2010) and the sizes of electrophoresed proteins were compared with it proportionally.

3.Results

The results show that protein concentrations (P.C) decrease with propagation in age especially in the second and third category, while it increases in last category of age classification, all concentrations were non-significant. Lipid concentrations (L.C) were non- significantly increased parallel with age as it's shown in table (1).

According to body mass index, PC was non-significantly increased in overweight while it decreased in obese. Also LC has same result of proteins as it shown in table (2). Results show the effects of lactating stags on protein and lipid concentrations, PC was decreased within 6-12 and over than 12 months than early periods of lactating (table 3) Negative Smoking affects on lactating mother's milk clarified in table (4) which showed negative smoking and nonsmoking mothers; non-significant decrease in P.C and non- significant increase in L.C.
Also table (5) show effects of residence of lactating mothers on P.C and L.C, two variables that are represented by non-significant increase in rural lactating mother's milk.

4.Protein profile

As shown in Figure (2), (3) and table (6), there are some slight differences in properties of proteins profile of nonsmokers and negative smoking samples between the two SDS-PAGE gels, in such away the upper differences recorded in lysozyme 62.5 , 51.3 in 10 KDa proteins, 22.5 in beta-galactosidase and 11.11, 11.2 Phosphorylate b and A protein respectively.

5.Discussion

Lactating mother's milk considered as an optimum nutrient for new infant because it all the essential nutrients for development of infant, these components are affected by many factors associated with lactating mothers. Thus, this study focuses on the factors that have direct effects on milk components in Iraqi mothers.
Some previously published papers are conducted on smoker lactating mothers, while the present study is conducted with negative smoking effects, in order to clarify the role of cigarette smoking on mother's milk and infant healthy.
Results showed that mean of mothers age was 26.40±6.092, when it's classified according to age, found large percentage was ≥25 years its 47.69%while lower percentage was 7.69% in ≥38. Protein concentration is non –significantly affected by age of mothers. This results comes in accordance with the same results obtained in Lebanon lactating mothers (Bachour *et al.*, 2012), since it was found that total protein concentration don't affected by mothers age in more than 60 samples. Also, the present results are similar to results that reported by Brasil *et al.* (1991) that studied the influence mother's age on human mother's milk composition. The little variables in protein concentrations between age categories may be attributed to the effect of other factors such as nutrients, occupations and education of mothers that inflected habitat, and mothers hygiene. Lipid concentrations increased with age in present study, this may be belongs to physiological changes in mothers that associated with age such as increased cholesterol level, saturated and non-saturated fatty acid that increased with age and caused healthy problem.
When body mass index has an effect on studying milk components, we found that overweight sample percentage was the largest compared with other categories, normal and obese. This factor occasionally hasnon-significant effect on protein and lipid concentration but P.C decreased in obese mothers. Nommsen *et al* (1991) clarified that the reasons of low variables of protein concentration between BMI category, and the concentration of human milk protein don't affected by maternal diet. But P.C decreased in obese, thus, it needs other methods to detect specific proteins such as Kjeldhal method that detects total nitrogen because milk contain nitrogenous protein and non –nitrogenous protein (Lonnerdal., 2003). In Iran, Mehdavi *et al* (2009) found positive correlation between maternal nutritional status and human milk lipid contents. The differences between present study and others resulted from to population habitat in nutrient and genetic expression. Moreover, lipid concentration is

affected by fat diet of maternal and its metabolism and consumption of body activity (Ballard and Ardythe, 2013).

In early stages of lactation it was found a reduction in protein concentration in large portions of samples in present study, as it was noticed that protein level concentration was reduced in eleven infants with the increasing of their age.

Table (4) shows a direct smoking or negative smoking effect on human milk composition. In present study, 40.62% of sample was suffered from negative smoking, as the direct smoking causes harmful effect, this habitat also affect on health and milk composition, since it causes decreasing in total protein concentration but it has little effect on lipid concentration, Boucher and his colleges reported that smoking causes significant reduction in protein concentration in smoker mother's milk (Boucher *et al*., 2012). Also, the present results agree with other several demonstrations about the significant effect of smoking on lactation (UN, 1998; Montagne *et al*., 1999).

The residence effect on human milk composition (that shown in table No.5) that refers to the differences between urban and rural lactating mother's milk was significantly coupled. This might be happened because milk composition doesn't being affected by residence urban and rural. Some researches depend on this factor when they study milk pollutions. For previous estimates of protein concentrations it was found that these factors are affected and the sort of effect is needed to be estimated. So, every protein type alone such as immunoglobulin, interleukins, lactoferein, serum albumin and beta-casein has to be individually estimated.

Protein profile of nonsmoker and negative smoker milk samples show differences. This reflects harmful effect of cigarette smoker on protein conformation, structure, types and its density that is somewhat clarified using SDS-PAGE electrophoresis. These differences might be attributed to the smoke that passes through many toxins and carcinogens into the milk of exposed mothers. These toxins may causes changes in protein structure, conformation and density. Electrophoresis may give an indicator about protein profile so it considered as semi quantitative method for protein profile but it need to be quantified.

References

1. Ballard, O. and Ardythe, L.(2013) Human milk composition. PeditrClin. *60*,49-74.
2. World Health Organization (2006). The International Code of Marketing of Breast-milk Substitutes.
3. Condon M. (2005). Breast is best, but it could be better: What is in breast milk that should not be? PediaterNurs. ***31***, 333-338.
4. Lonnerdal B. (2005). Human milk proteins: key components for the biological activity of human milk. Adv. Exp. Med Biol, *554*:11–25.
5. Landrigan J. P.H. ,Sonawane B. ,Mattison D., and McCally, M. (2002). Chemical contaminants in breast milk and their impact on children health; an overview. Enviro.Health, prespect.***110***, 313-315.
6. Jensen RG. (1995). Handbook of milk composition. San Diego (CA): Academic Press,Inc.
7. Gao, X.; McMahon, R.J.; Woo, J.G. (2012) Temporal changes in milk proteomes reveal developing milk functions. J Proteome Res .***11***, 3897–3907.
8. Bachour, P.; Yafawi, R.; Jaber, F.; Choueiri, E. and Abdel-Razzak1, Z. (2012) Effects of Smoking, Mother's Age, Body Mass Index,and Parity Number on Lipid, Protein, and Secretory Immunoglobulin A Concentrations of Human Milk. Research in medicine***, 7***, 179-188.
9. Stafford, J.; Villalpando, S.; Urquieta, ;Aguila, B. and Circadian. (1994). Variation and changes after a meal in volume and lipid production of human milk from rural Mexican women. Ann βαNutr Metab; ***38***:232–237.
10. Wagner C.L., Anderson D.M. and Pittard WB. (1996). Special properties of human milk. ClinPediatr, ***35***: 283-93.
11. Takeda T., Sakata M., and Minekawa R. (2004). Human milk induces fetal small intestinal cell proliferation –involvement of a different tyrosine kinase signaling pathway from epidermal growth factor receptor. J Endocrinol , ***181***: 449-57.
12. Corps A.N., and Brown K.D.(1987). Stimulation of intestinal epithelial cell proliferation in culture by growth factors in human and ruminant mammary secretions. J Endocrinol, ***113:*** 285-290.
13. Wagner C.L., and Forsythe D.W.(2000). Effect of human milk and recombinant EGF, TGFalpha, and IGF-I on small intestinal cell proliferation. AdvExp Med Biol.***847***: 373-4.
14. Grosvenor C.E., Picciano M.F., and Baumrucker C.R. (1992). Hormones and growth factors in milk. Endocr. Rev.***14***: 710-28.
15. Gadzik J. (2006). How much should weight? Quantities equation, upper weight limits and body mass index prime. Connect medicine ***70***,81-88.
16. Bradford, M.M. (1976), "Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", Anal. Biochem. ***72:*** 248–254,

17. Folch J, Lees M, Sloane and Stanley GH.(1975) A simple method for the isolation and purification of total lipids from animal tissues. J BiolChem; 226:497–509.
18. Del Bubba M, Zanieri L, Galvan P,(2005). Determination of polycyclic aromatic hydrocarbons and total fats in human milk. Ann Chim; ***95***:629–641.
19. Brasil AL, Vitolo MR, Lopez FA, *et al* (1991). Fat and protein composition of mature milk in adolescents. J Adolesc Health; ***12***:365–371.
20. Nommsen LA, Lovelady CA, Heinig MJ, *et al* (1991). Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 months of lactation: The DARLING Study. Am J ClinNutr*.*, ***53***:457–465.
21. Mehdavi, N.; Arefhoesseini, S.; Khiabani, M. (2009). Association between fat content of breast milk and maternal nutritional status and infant's weight in Tabriz, Iran. Mal.J. Nutr, ***15***. 37-44.
22. United Nations Food and Agriculture Organization and the Information Network on Post-Harvest Operations, Rome (1998).
23. Montagne P, Cuillie `re ML and Mole ´ C, (1991). Immunological and nutritional composition of human milk in relation to prematurity and mother's parity during the first 2 weeks of lactation. J PediatrGastroenterolNutr ;***29***:75–80.

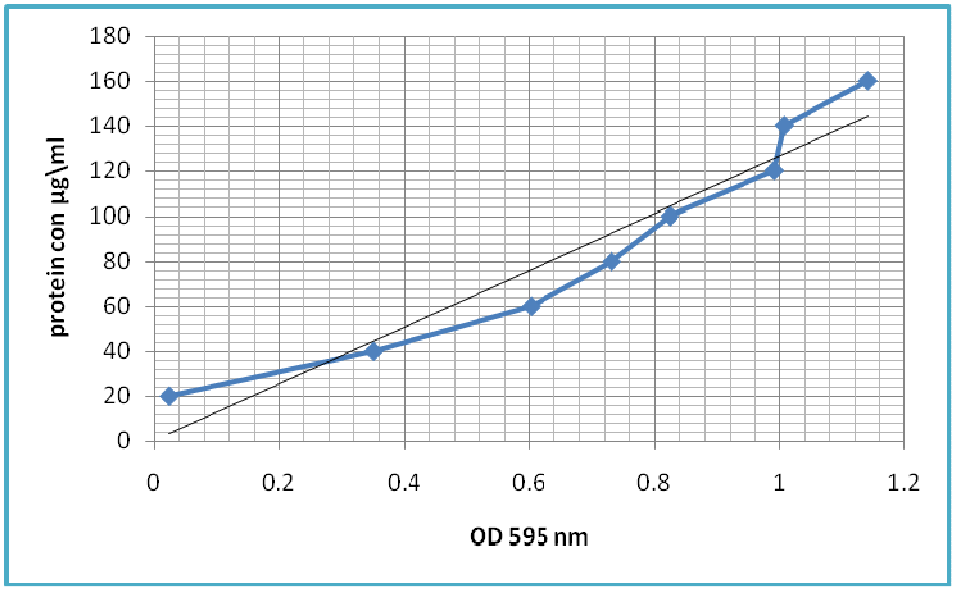

Figure (1). Standard curve of protein concentration (µg\ml).

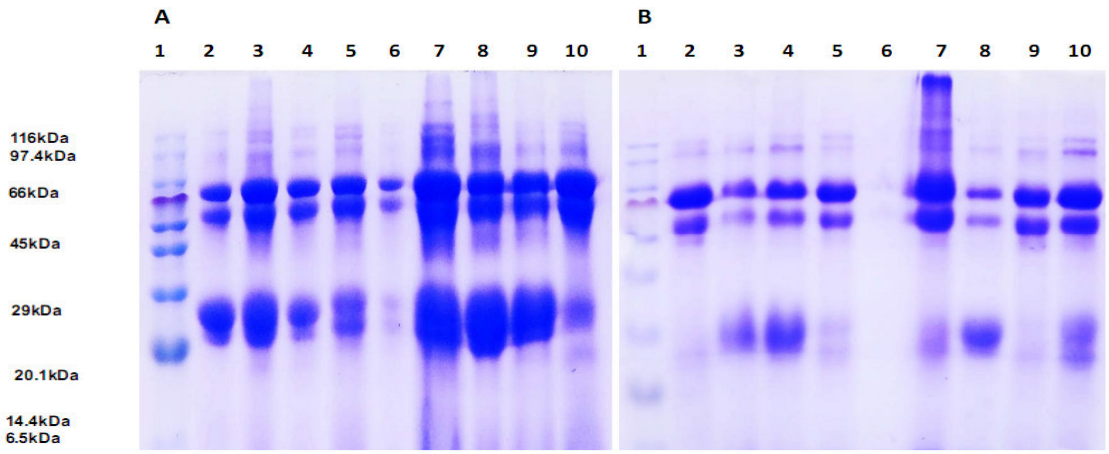

Figure (2) Comparison between SDS-PAGE protein profile of nonsmoking (**A**) and negative smoking (**B**) human milk samples. Slight differences were observed between the two gels.

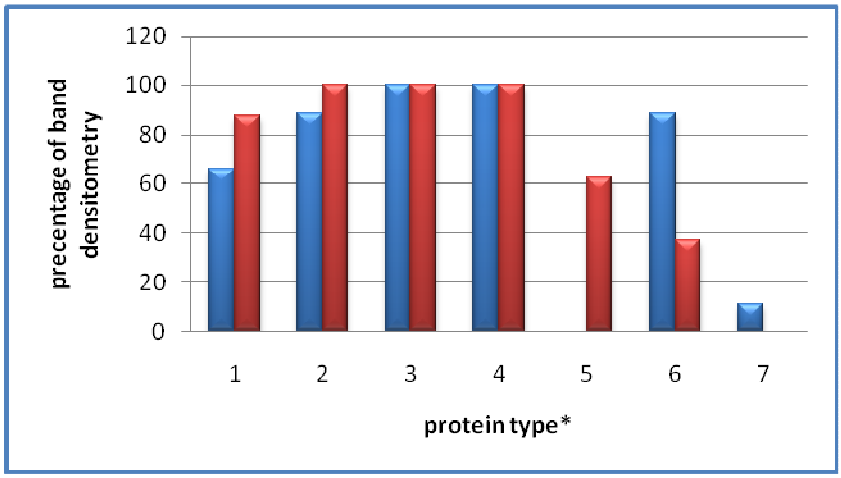

Figure (3) percentage of protein bands occurrence in nonsmoking and negative smoking milk. *protein type clarified in table (6).

Table (1) Total protein (µg\ml) and lipid (mg\ml) concentration in human lactating mother milk according to mother's age.

Age (year)	% sample no.	P.C µg\ml	L.C mg\ml
0.246±0.044	1134.3 ± 56.72	47.69%	≥25
0.221±0.049	994.12 ±59.08	35.38%	26-31
0.349±0.66	911.74 ± 121.86	9.23%	32-37
0.44±0.092	1116.900 ± 93.77	7.69%	≤38

Mean ±SE

Table (2) Total protein (µg\ml) and lipid (mg\ml)concentrations in human lactating mother milk according to mothers BMI.

L.C mg\ml	P.C µg\ml	% sample no.	BMI
0.207± 0.134	1006.98 ±44.24	33.33%	normal
0.220±0.039	1021.306 ± 70.46	43.33%	Overweight
0.216±0.132	960.12± 79.34	23.33%	obese

Mean ±SE

Table (3) Total protein (µg\ml) and lipid (mg\ml) concentrations in human lactating mother milk according to lactating stags.

L.C mg\ml	P.C µg\ml	% sample no.	Stags months
0.138±0.042	**904.27 ± 54.37**	65.56%	≥ 6 month
0.100±0.026	**817.600 ± 100.93**	17.68%	6-12 month
0.350±0.170	**850.47 ±76**	16.76%	≤12 month

Mean ±SE

Table (4) Total protein (µg\ml) and lipid (mg\ml)concentration in human lactating mother milk according to smoker.

L.C mg\ml	P.C µg\ml	% sample no.	Habitat
0.194**±0.030**	**875.85±61.90**	37.14%	Non-smoking
0.210**±0.080**	**743.0922±65.52**	62.85%	Negative smoking

Mean ±SD.

Table (5) Total protein (µg\ml) and lipid (mg\ml)concentration in human lactating mother's milk according to residence

L.C mg\ml	P.C µg\ml	% sample no.	Residence
0.210**±0.195**	**1021.1±46.58**	67.18%	Urban
0.246**±0.227**	**1105.195±78.12**	31.18%	Rural

Mean ±SD

Table (6) proteins type and differences values between nonsmoking and negative smoking milk samples.

Differences value	Negative smoker %	Normal %	KDa	Type of protein	Sample no in figure(3)
22.5	87.5	66	116	Beta-galatosidase	**1**
11.2	100	88.8	97.4	Phosphorylate b	**2**
0	100	100	65	Albumin	**3**
-	-	-	45	Ova albumin	**4**
0	100	100	29	Carbonic anhydrous	**5**
-	-	-	20	Trypsin inhibitor	**6**
62.5	62.5	-	14.4	Lysozyme	**7**
51.3	37.5	88.8	10	-	**8**
11.11	-	11.11	6.5	A protein	**9**