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Antibodies of Human Milk within the First Week of Birth

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Research Article

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ABSTRACT

IgA, IgM and IgG values of breast milk had higher significantly within first 12 hrs with increasing the time of lactation and then dropped markedly with other day's advantages. The mean values with SD were 36.53 ± 6.72, 14.05 ± 2.78 and 0.88 ± 0.42 g/L at 0-12 hrs parturition, respectively; while at 7th day, being 4.23 ± 0.61, 0.37 ± 0.03 and 0.13 ± 0.07 g/L in order. In addition, the total amino acids levels of human milk immunoglobulins (Igs) took the same trend in these times.

INTRODUCTION

Breastfeeding is an unique system of giving the perfect food for healthy growth and development in newborns, which the human milk is the biological form contains a large number featured bioactive particles that ensure against infection and inflammation that due to immune maturation, organ improvement and healthy microbial colonization. Some of these bioactive particles had examined as a new therapeutic agent. Hence, worldwide public health recommendations advocate breast-feeding alone for the first 6 months of life. The components of the human milk are variable due to feeds, between mothers and increase lactation [1,2]. Breast milk not just gives the consummately adjusted nutritious supply for the newborn child. Additionally, it represents a vital portion of advancing healthy growth and development. Thus, protection, support and advancement of breastfeeding are a public health problem [3].

However, colostrum is the "pre-milk" biological fluid secreted from the mammary glands of female mammals shortly after giving birth within first few days (1-3) of your newborn's life. The fluid has a distinct composition different from the milk produced afterwards. It is yellowish, thick, sticky and low in amount but highly in nutritional level. It contains numerous components, which believed to be important in the baby's immune protection such as Igs, lactoferrin, growth factors and lysozyme. Immunoglobulins, also known as antibodies, are blood molecules present in blood, colostrum and milk of all lactating species, which made by the body's natural immune system "produced by plasma cells (white blood cells)". In human, some level of defensive passive immunity is transferred to the fetus within pregnancy to a higher degree than in numerous different other mammals, and the human colostrum further supports the continued advancement of the newborn immunity, while, it comes without immunological protection in numerous different mammals. Much research offers indication for transfer of cytokines, Igs, growth factors, antimicrobial compounds and maternal immune cells to the newborn *via* the feeding of colostrum [4-8]. Also, breast milk is the greatest suitable nourishment for a newborn, where is rich in proteins (including Igs), other immune molecules, an abundance of nutrients as well as vitamins and micronutrients that meets all infant's needs at all stages of lactation due to its significant advantages [9].

The human Igs family comprises five classes three major, IgA, IgM and IgG as well as two minor, IgD and IgE. The main classes in bovine and human milk are IgG, IgM and IgA. The various Ig fractions levels in colostrum and milk differ impressively as per species and are often unique compared with blood. For example, the IgA class contains around 90 and 15-20% of total Igs in human colostrum and milk as well as blood respectively [10]. For cattle, Igs are grouped into four isotypes, IgG (IgG_1 and IgG_2), IgA, IgM and IgE, based on the heavy chain they possess [10-14]. IgG, IgA and IgM molecules are existent in high levels in milk, especially in colostrum. The IgG is principal in colostrum, milk and blood that represent around 80-90, 60-70 and 90% of total Igs, respectively), while in human milk the IgA represent the major Ig class [15,16].

The objective of this research was to precipitate the immunoglobulins from human milk samples to determine the concentrations of IgA, IgM and IgG as well as the essential and non-essential amino acids of human Igs during the first week of birth at 0-0.5,1,2,3,4,5,6 and 7 days postpartum.

MATERIALS AND METHODS

Samples Collection

This study conducted to estimate the concentration of antibodies in human milk within the first week birth. Individual milk samples obtained from six lactating mothers of El-Sadeen village, Menia Al-kamh Center, Sharkia Governorate, Egypt. Colostrum and milk samples collected in 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum. Samples (about 25-35 ml) were collected in sterilized bottles by supervised manual expression at the end of the feeding and transported to the laboratory in an ice box. All samples were stored at -20°C immediately at arrival and kept frozen until analyzed.

Determination of Human Milk Immunoglobulins

Samples preparation

Human milk samples were defatted by centrifugation at 4000 rpm/3 min. Milk whey was prepared from the skim milk by adjusting pH at 4.6 using one *N* Hcl solution and centrifuged at 10000 rpm/15 m to remove casein deposition. A total Igs were equipped with whey samples using saturated ammonium sulfate solution compatible [17]. Extraction of ammonium sulphate dialyzed with many changes of distilled water for 24 h in the refrigerator during this period. The dialyzed extraction was keeping at -20°C until analyzed.

Immunoglobulins quantification by single radial immunodiffusion (srid) technique

Kits of SRID (from the Binding site limited, England) utilized for determination of Igs portions (IgA, IgM and IgG) in human colostrum and milk samples within 7 days of parturition. It derived mainly from the work of [18,19]. Some samples used with dilution; each sample was placed in a well-punched gel in the layers, the lid is tightly closed and the flat plate is stored on 2-8˚C. It is essential that the gel is should not be allowed to dry during incubation. To reduce evaporation during incubation, it is proposed that the plates must be resealed in the foil pouches or stored in a wet box (a sealed plastic box contains moist tissue paper). The minimum incubation time for full diffusion is 48 h (72 h for IgM), then the diffusion is measured and can be reading directly from the RID reference table to calculate the immunoglobulin content in each sample giving to the manufacturer manual.

Amino acids analysis

Amino acids levels were determined as defined by [20,21]. The analysis achieved in Central Service Unit, National Research Centre, Egypt, using LC3000 amino acid analyzer (Eppendorf-Biotronik, Germany). The procedure established on the separation of the amino acids using strong cation exchange chromatography followed by the ninhydrine color reaction and photometric detection at 570 nm. Samples were hydrolyzed with 6 *N* HCl at 110˚C in Teflon capped vials for 24 h. After vacuum exclusion of Hcl, the remainders dissolved in a lithium citrate buffer, pH 2.2. Twenty µl of the solution uploaded on to the cation exchange column (pre-equilibrated with the same buffer), at that time, buffers of four-lithium citrate were successively were used to the column with flow rate 0.2 ml/min and pH values of 2.2, 2.8, 3.3 and 3.7, in the same order. The pressure of zero to 150 bars and the flow rate of ninhydrine was 0.2 ml/m. The buffer pressure was from zero to 50 bars and reaction temperature was 130ºC.

Statistical analysis

Statistical analysis of the obtained data carried out using SPSS version 20-computer program [22]. All data represented by means ± standard deviations (SD) of three replicates and compared them using one way ANOVA and least significant difference (LSD). Values with different letters during the same column differ significantly at $p<0.01-0.05$.

RESULTS AND DISCUSSION

Individual milk samples obtained from six lactating mothers within 7 days postpartum. Samples obtained at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days parturition. The human milk Igs counted by the SRID technique. The relation between the Igs concentrations and the diameter of the precipitated antigen-antibody reaction can be founded. It noticed that the Igs levels were higher in colostrum, which reduced drastically with the first few days of birth. Igs values tend to drop even lower with increasing the time of lactation. This finding was corresponding by [23]. Total Igs levels in human milk samples varied between the 0-0.5, 1, 2 and other days postpartum. It was highest significant during 0-0.5 day when compared with the other days. The mean level of total Igs was 51.47 ± 6.64 g/L during 0-0.5 day, then reduced by 36.88% at 1st day postpartum (32.49 ± 4.79 g/L) and 61.61% at 2nd day (19.76 ± 4.47 g/L). While at 3, 4, 5, 6 and 7 days parturition, the mean levels of total Igs were 14.05 ± 3.47, 10.59 ± 1.38, 8.24 ± 1.91, 6.17 ± 1.10 and 4.73 ± 0.57 g/L, respectively. These concentrations were declined by 72.70, 79.43, 83.99, 88.01 and 90.82% at 3, 4, 5, 6 and 7 days, respectively when compared with the total Igs at 0-0.5 day postpartum **(Table 1)**. These results are in quite agree with those of [14] who stated that in the transition from colostrum to mature milk, Igs levels decrease sharply during the first five days postpartum. Total Igs in this study were higher than that of [24] namely, 33.66, 9.77 and 1.73 g/L at 0, 3 and 6 days postpartum, respectively. In addition, the Igs levels of colostrum were 19.4 and 1.14 mg/ml in mature milk [25].

Human milk IgA values during the first week parturition were higher significantly during 0-0.5 day than those of other days parturition, being were 36.53 ± 6.72 g/L during 0-0.5 day postpartum. It reduced to 23.98 ± 3.09 and 14.25 ± 1.50 g/L at 1st and 2nd days postpartum in the same order. Also, a gradually decrease could be noticed on the following days, being 10.16 ± 2.06, 8.57 ± 1.23, 6.71 ± 1.84, 5.24 ± 1.11 and 4.23 ± 0.61 g/L at 3, 4, 5, 6 and 7 days postpartum, respectively **(Table 1 and Figures 1a-1c)**. These results are in agreement with those obtained by [23-28]. However, the IgA was principal in human colostrum and milk, which it tends to drop even lower with increasing time of lactation, where individual variation in Igs is 0considerable. On the contrary, IgG is the main immunoglobulin class present in ruminant milk [29]. On the other hand, in this study, it were higher than those of [23] namely at 1st (1735 mg/100 ml) and 4th (100 mg/100 ml) days of lactation. The IgA concentrations ranges of human milk samples were 1.5-83.7, 0.63-32.8, 0.40-3.14 and 0.50-1.1 g/L with mean levels of 32, 9, 1.45 and 0.75 g/L at 0, 3,6 and 14 days postpartum, respectively [24]. In this study, the mean concentrations of IgA at 1st and 2nd days were lower than those of [30] being 33.5 ± 25.8 and 21.1 ± 18.0 g/L, in the same order. On the contrary, it was higher at 3,4,5,6 and 7 days parturition (9.5 ± 8.2, 4.3 ± 2.5, 3.1 ± 2.1, 2.9 ± 2.3 and 2.2 ± 1.2 g/L, respectively). The median content of IgA was 17.4 and 1 mg/ml in colostrum and milk in order [25]. The IgA content of human colostrum was 1234 mg/100 ml [31]. Colostrum of Spanish women contain the largest IgA level, followed by transitional milk and finally mature milk with means 290.4 ± 258.2, 63.4 ± 63.5 and 50.3 ± 67.2 mg/dL, with statistically significant differences between them [32]. The IgA levels were 17.35 and 1 g/L of human colostrum and milk with ratio 90 and 87% of total Igs [29]. The mean level of IgA in breast milk samples (83.71 ± 38.03 mg/dl), while mean level of IgM (29.95 ± 15.73 mg/dl) [33].

Table 1 and Figures 2a-2c shows the median levels of IgM, it was significantly higher during 0-0.5 day (14.05 ± 2.78 g/L) than those of other days postpartum and dropped to 7.99 ± 3.04 g/L at 1st day parturition. A gradually decrease could be noticed on the following days, being 5.14 ± 2.96, 3.59 ± 1.86, 1.77 ± 1.76, 1.32 ± 1.18, 0.76 ± 0.28 and 0.37 ± 0.03 g/L at 2, 3, 4, 5, 6 and 7 days postpartum, in order. On average IgM represented 28.56, 24.58, 26.01, 25.57, 16.73, 15.99, 12.28 and 7.77% of the total Igs at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days parturition, in order. In this study, IgM contents were higher than those of [23] namely at 1st (19 mg/100 ml) and 4th (10 mg/100 ml) days of lactation. In addition, it was higher than that of [24] who found that the mean concentrations of IgM were 1.13, 0.58, 0.25 and 0.15 g/L at 0, 3, 6 and 14 days postpartum, respectively. However, they found that a gradually decreasing in IgM content during the first few hours postpartum and the following days after birth. The IgM concentration was 1.6 and 0.10 mg/ml in colostrum and milk in order [25]. In addition, the IgM concentration was 1.59 and 0.10 g/L in human colostrum and milk with ratio 8.0 and 10% of total Igs respectively [29].

Table 1. Average changes in human milk immunoglobulins content within the first week of lactation.

Constituents (g/L)	Lactation period (days)								LSD**
	0-0.5	1	2	3	4	5	6	7	
Total Igs									
Mean ± SD*	51.47 ± 6.64ᵃ	32.49 ± 4.79ᵇ	19.76 ± 4.47ᶜ	14.05 ± 3.47ᵈ	10.59 ± 1.38ᵈᵉ	8.24 ± 1.91ᵉᶠ	6.17 ± 1.10ᵉᶠ	4.73 ± 0.57ᶠ	4.455
% of change	0.0	- 36.88	- 61.61	- 72.70	- 79.43	- 83.99	- 88.01	- 90.82	
IgA									
Mean ± SD	36.53 ± 6.72ᵃ	23.9 ± 83.09ᵇ	14.25 ± 1.50ᶜ	10.16 ± 2.06ᵈ	8.57 ± 1.23ᵈᵉ	6.71 ± 1.84dᵉᶠ	5.24 ± 1.11ᵉᶠ	4.23 ± 0.61ᶠ	3.547
% of total Igs	70.98	73.80	72.12	72.35	80.95	81.37	84.93	89.53	
IgM									
Mean ± SD	14.05 ± 2.78ᵃ	7.99 ± 3.04ᵇ	5.14 ± 2.96ᶜ	3.59 ± 1.86ᶜᵈ	1.77 ± 1.76ᵈᵉ	1.32 ± 1.18ᵈᵉ	0.76 ± 0.28ᵉ	0.37 ± 0.03ᵉ	2.520
% of total Igs	28.56	24.58	26.01	25.57	16.73	15.99	12.28	7.77	
IgG									
Mean ± SD	0.88 ± 0.42ᵃ	0.53 ± 0.21ᵇ	0.37 ± 0.15ᵇᶜ	0.29 ± 0.13ᵇᶜᵉ	0.25 ± 0.14ᶜᵉ	0.22 ± 0.12ᶜᵉ	0.17 ± 0.08ᶜᵉ	0.13 ± 0.07ᵉ	0.239
% of total Igs	1.71	1.62	1.87	.08	2.32	2.64	2.79	2.70	

Means ± SD*: From six mothers with Standard deviation and the various letters in the same column are significantly different. **LSD**:** The least significant difference.

Figure 1a. Single radial immunodiffusion analysis of IgA in human colostrum and milk during the first week postpartum. Wells No: 1,2,3,4,5,6 and 7 represent samples of mother number 1 at 0-0.5, 1, 2, 3, 4, 5 and 6 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13, 14 and 8 represent samples of mother number 2 at 0-0.5, 1, 2, 3, 4, 5 and 6 days postpartum, respectively.

Figure 1b. Single radial immunodiffusion analysis of IgA in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6, 7 and 8 represent samples of mother number 3 at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13 and 14 represent samples of mother number 4 at 0-0.5, 1, 2, 3, 4 and 5 days postpartum, respectively.

Figure 1c. Single radial immunodiffusion analysis of IgA in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6, 7 and 8 represent samples of mother number 5 at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13 and 14 represent samples of mother number 6 at 0-0.5, 1, 2, 3, 4 and 5 days postpartum, respectively.

Figure 2a. Single radial immunodiffusion analysis of IgM in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6 and 7 represent samples of mother number 1 at 0-0.5, 1, 2, 3, 4, 5 and 6 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13, 14 and 8 represent samples of mother number 2 at 0-0.5, 1, 2, 3, 4, 5 and 6 days postpartum, respectively.

Figure 2b. Single radial immunodiffusion analysis of IgM in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6, 7 and 8 represent samples of mother number 3 at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13 and 14 represent samples of mother number 4 at 0-0.5, 1, 2, 3, 4 and 5 days postpartum, respectively.

Figure 2c. Single radial immunodiffusion analysis of IgM in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6, 7 and 8 represent samples of mother number 5 at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13 and 14 represent samples of mother number 6 at 0-0.5, 1, 2, 3, 4 and 5 days postpartum, respectively.

Finally, IgG contents were significantly higher during 0-0.5 day than those of other day's parturition, being 0.88 ± 0.42 g/L, then it dropped to 0.53 ± 0.21 and 0.37 ± 0.15 g/L at 1st and 2nd days postpartum in the same order. Also, a gradually decrease could be noticed on the following days, being 0.29 ± 0.13, 0.25 ± 0.14, 0.22 ± 0.12, 0.17 ± 0.08 and 0.13 ± 0.07 g/L at 3, 4, 5, 6 and 7 days postpartum, respectively **(Table 1 and Figures 3a-3c)**. In our study, IgG contents were higher than those of [23] being at 1st (43 mg/100 ml) and 4th (4 mg/100 ml) days of lactation. In contrast it was lower than that of [24] who found that the IgG contents were 0.53, 0.19 and 0.03 g/L at 0,3 and 6 days postpartum, respectively. Additionally, they established that a gradually decrease in the IgG content within the first few hours parturition and the following days after delivery. IgG level was 0.063 mg/ml in colostrum of Mayan India women (1-4 days postpartum) [34]. While IgG content in human colostrum was 10 mg/100 ml [35].

Generally, it concluded that IgA is the main immunoglobulin of all the analyzed samples, IgM was the second most dominant Igs and IgG was detected with small levels in milk compared to IgA and IgM **(Figure 4)**. This is the same trend with the works of [23-26,28,29,36-38].

Amino acids determination is very important to the newborn because the nutritive value of the protein depends primarily on their content of the essential amino acids that human cannot synthesize. Hence, the essential and non-essential amino acids of human Igs during the first week postpartum were determined in the present study. Human colostrum has a variation of proteins that illustrate and contribute completely to the nutrition quality, and being an important source of special amino acids required for rapid growth of the baby [39]. It is very important for growth after birth, especially, serine, glutamine and glutamic acid that give a considerable protein of the total amino acids. High level of glutamic acid in the milk can use as a key position in the amino acids metabolism. On the other hand, it converted into α-keto glutamic acid that can be entering the tricarboxylic acid cycle. Through analysis the amino acids composition of the mother's milk protein, it recognized that about 20% of it is glutamic acid, while it has in the smallest level of histidine, cysteine and methionine [40] **(Table 2)**.

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Figure 3a. Single radial immunodiffusion analysis of IgG in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6 and 7 represent samples of mother number 1 at 0-0.5, 1, 2, 3, 4, 5 and 6 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13, 14 and 8 represent samples of mother number 2 at 0-0.5, 1, 2, 3, 4, 5 and 6 days postpartum, respectively.

Figure 3b. Single radial immunodiffusion analysis of IgG in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6, 7 and 8 represent samples of mother number 3 at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13 and 14 represent samples of mother number 4 at 0-0.5, 1, 2, 3, 4 and 5 days postpartum, respectively.

Figure 3c. Single radial immunodiffusion analysis of IgG in human colostrum and milk during the first week postpartum. Wells No: 1, 2, 3, 4, 5, 6, 7 and 8 represent samples of mother number 5 at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively. Wells No: 9, 10, 11, 12, 13 and 14 represent samples of mother number 6 at 0-0.5, 1, 2, 3, 4 and 5 days postpartum, respectively.

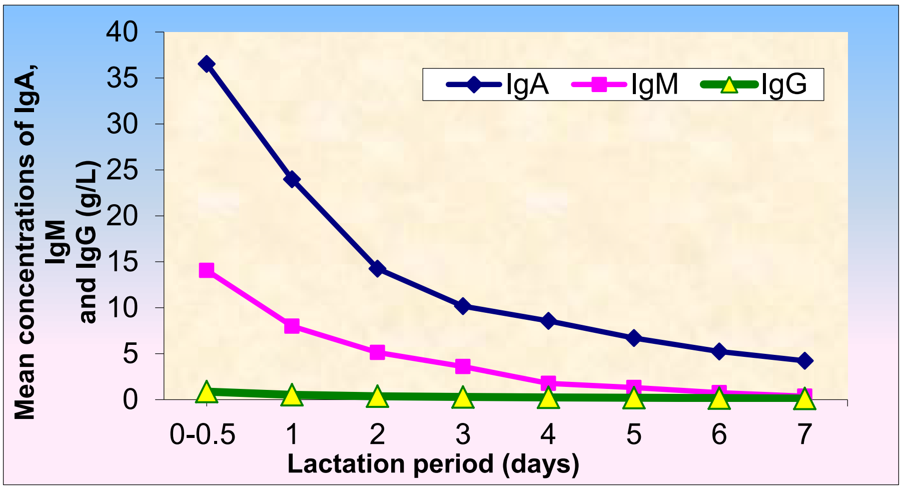

Figure 4. Mean concentrations of IgA, IgM and IgG in human milk samples during the first week postpartum.

Table 2. Amino acids values of human milk Igs within the first week postpartum.

Amino acids (mg/100 ml)		Lactation period (days)							
		0-0.5	1	2	3	4	5	6	7
Essential	Valine	1.33	0.63	0.59	0.48	0.42	0.36	0.40	0.19
	Leucine	1.88	1.17	1.10	1.02	0.99	0.83	0.72	0.50
	Isoleucine	0.56	0.35	0.34	0.35	0.31	0.31	0.23	0.16
	Threonine	0.78	0.49	0.43	0.43	0.34	0.30	0.21	0.17
	Methionine	0.30	0.20	0.15	0.19	0.16	0.11	0.16	0.09
	Phenylalanine	1.47	0.79	0.82	0.61	0.64	0.52	0.52	0.49
	Histidine	0.91	0.43	0.49	0.39	0.57	0.51	0.26	0.20
	Lysine	1.11	0.68	0.64	0.72	0.83	0.63	0.43	0.36
	Total	**8.34**	**4.74**	**4.56**	**4.20**	**4.25**	**3.58**	**2.92**	**2.16**
Non-essential	Aspartic	1.22	0.81	0.79	0.85	0.72	0.67	0.58	0.35
	Serine	1.00	0.68	0.58	0.53	0.44	0.39	0.38	0.21
	Glutamic acid	2.42	1.72	1.55	1.58	1.27	1.24	0.81	0.65
	Glycine	0.37	0.20	0.17	0.15	0.12	0.12	0.12	0.07
	Alanine	1.12	0.76	0.71	0.57	0.51	0.47	0.46	0.23
	Cystine	1.66	0.61	0.57	0.44	0.45	ND	ND	0.22
	Tyrosine	1.16	0.49	0.66	0.38	0.54	0.47	0.27	0.25
	Arginine	1.22	0.83	0.82	0.52	0.48	0.42	0.40	0.20
	Proline	1.95	1.48	1.32	0.87	0.86	1.04	1.42	0.43
	Total	**12.13**	**7.58**	**7.17**	**5.89**	**5.39**	**4.82**	**4.44**	**2.62**
Generally total		**20.47**	**12.32**	**11.74**	**10.09**	**9.65**	**8.40**	**7.36**	**4.78**

Note: ND: Not detected.

Amino acids, threonine, valine, isoleucine, leucine, serine, glutamic, glycine, alanine and arginine concentrations were gradually decreased during the first week of lactation, values being 0.78-0.17, 1.33-0.19, 0.56-0.16, 1.88-0.50, 1.00-0.21, 2.42-0.65, 0.37-0.07, 1.12-0.23 and 1.22-0.20 mg/100 ml at 0-0.5 and 7 days postpartum, respectively. Whereas, methionine, phenylalanine, histidine, lysine, aspartic, cysteine, tyrosine and proline concentrations were the highest at 0-0.5 day then decreased at 7th day postpartum but these decreases were un-gradually. These values at 0-0.5 and 7th days postpartum were methionine (0.30 and 0.09), phenylalanine (1.47 and 0.49), histidine (0.91 and 0.20), lysine (1.11 and 0.36), aspartic (1.22 and 0.35), cysteine (1.66 and 0.22), tyrosine (1.16 and 0.25) and proline (1.95 and 0.43) mg/100 ml, in order. It is evident that the total of amino acids values were highest at 0-0.5 day and dropped markedly with time progress of lactation, these values were 20.47, 12.32, 11.74, 10.09, 9.65, 8.40, 7.36 and 4.78 mg/100 ml at 0-0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively.

CONCLUSION

Moreover, it is very obviously that the glutamic acid (non-essential) predominates in human milk Igs, it forms 13-30% of the general total amino acids with an amount of 1.41 mg/100 ml. This finding is corresponding with the same trend by [41] they found that the content of glutamine and glutamic acid is high in breast milk but varies considerably between mothers. Whereas, the leucine (essential amino acid) had the largest amount (9.72%) with an average of 1.03 mg/100 ml. Additionally, the serine, valine, alanine, threonine, glycine, arginine and cysteine levels of buffalo's milk Igs were gradually decreased within the first two weeks of lactation, while aspartic, phenylalanine, tyrosine and methionine concentrations remained almost constant during the same period of lactation. On the other hand, the concentrations of glutamic, proline, isoleucine, leucine and lysine increased during the early lactation period [42].

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