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SHORT COMMUNICATION

FREE GLUTAMIC ACID CONTENT OF MILK IN INDIAN MOTHERS

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Abstract : Free amino acids levels in the milk of 11 healthy Indian mothers were determined using automatic precolumn derivatization procedure. The aim of the study was to find out the relative concentration of glutamic acid and glutamine in the milk. Glutamic acid is the dominant free amino acid found in the milk of Indian mothers. Glutamic acid and glutamine together formed the major nonessential amino acids present in the human milk. Although glutamic acid has been shown to be the major amino acid in human milk in many studies, to the best our knowledge, this is the first report to confirm that glutamic acid is the most abundant amino acid in milk in Indian mothers.

Key words : human milk free amino acid glutamate glutamine

INTRODUCTION

The free amino acids (FAA) composition of human milk has been widely studied (1–5). Most of the FAA seemed to decrease significantly while the sum of glutamate and glutamine has been reported to increase with advancing lactation (6) Such kind of abundance may reflect a distinctive role of glutamic acid and glutamine during the early postnatal development period. Availability of increasing amounts of glutamine and glutamic acid to the breast-fed infants with progression of lactation period indicates its biological significance during postnatal development. However there is no information regarding the free glutamate and glutamine level in the milk

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of Indian mothers. The only report available on protein and FAA contents of human milk in India do not provide the free amino acid level of individual amino acids (7). The present study was undertaken to find out the free glutamic acid and glutamine level in the milk of Indian mothers.

METHODS

Breast milk samples were obtained from 11 healthy, well-nourished mothers of term infants at days 10–14 of parturition. Collections of milk samples were done with close collaboration of the hospital staff. Mothers were instructed to express hind milk manually into sterile polypropylene bottles. The samples were immediately frozen and delivered at Food Research and Analysis Centre, New Delhi for analysis of FAA The samples were stored below –20°C until analysis. The milk samples were delipidated by centrifugation at 2000 g for 20 min. For FAA analysis 300 µl of milk was slowly added to 2 ml of methanol. The solution was mixed on a vortex type mixer and centrifuged at 2500 g for five minutes. The supernatant phase was taken out and analyzed for amino acids after filtering with 0.45 µm filter (Millipore). FAA were analysed by an automatic precolumn derivatization with orthophthaldehyde in the presence of 2-mercaptoethanol (Sigma Chemical Co, St Louis, MO, USA) and reversed phase high performance liquid chromatography (HPLC) with ultraviolet and fluorescence detection, excitation at 350 nm and emission at 450 nm. Derivatized amino acids were detected on-line spectrophotometrically and quantified by comparing the area under the sample peak against that of an amino acids standard solution (Sigma Chemical, St Louis, MO) of known concentration. The amino acids analysed were aspartate (Asp), glutamic acid (Glu), glutamine (Gln), glycine (Gly), serine (Ser), alalnine (Ala), tyrosine (Tyr), valine (Val), methionine (Met), phenyl-alanine (Phe), iso-leucine (Ile), leucine (Leu), threonine (Thr), arginine (Arg) and histidine (His). According to availability of the standards we could analyse only 15 amino acids.

RESULTS

The human milk samples from 11 mothers collected during 10–14 days of parturition were analyzed for 15 amino acids. The average age of the mothers is 24.3 ± 2.2 yrs. The FAA level of 15 amino acids in the milk of 11 subjects are described in Table I. The percentage of individual amino acids with respect to total amino acids is described in Fig. 1. The total amount of 15 free amino acids present in the samples ranged from 76.6 to 244.2 µmol/100 ml of milk (Mean ± S.D : 165.0 ± 54.1). The percentages of essential and non-essential amino acids were 31.7 and 68.2 respectively. The percentage of glutamate and glutamine calculated together was 26.5. Amongst the free amino acids, the level of glutamate was found to be highest in the breast milk. The level of glutamate and glutamine taken together was highest amongst all the amino acids in all the subjects. The other amino acids that were in abundance were glycine, isoleucine and serine. Leucine was found to be in lowest concentration.

TABLE I : Free amino acids (FAA) level (µmol/100 ml) in the milk of Indian mothers (n = 11) collected in between 10–14 days of parturition.

FAA	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Mean*	*SD*
Asp	10.6	18.17	8.56	12.38	10.86	15.39	17.98	19.75	11.07	9.38	9.68	13.07	4.01
Glu	22.26	35.38	18.74	25.78	22.19	40.49	28.79	36.88	18.27	17.89	25.59	26.57	7.92
Gln	3.49	37.26	3.58	2.79	4.1	28.26	43.79	39.23	2.04	3.81	4.29	15.69	17.38
Ser	0.09	7.12	0.12	0.09	0.05	28.79	40.38	42.2	0.32	28.05	46.33	17.59	19.54
Gly	8.99	43.47	8.39	7.58	10.99	42.79	45.39	42.23	6.17	8.87	8.71	21.23	17.68
Ala	14.63	12.3	14.37	13.86	15.66	17.59	11.98	7.33	11.46	11.05	0.53	11.89	4.64
Tyr	10.56	4.61	7.84	9.36	14.49	6.84	4.12	2.87	4.84	6.46	0.37	6.58	3.91
Val	18.59	5.02	15.83	12.79	27.59	4.89	6.39	3.78	3.47	0.29	7.52	9.65	8.18
Met	3.72	8.13	3.58	2.36	5.22	5.62	8.58	10.19	6.99	7.77	4.54	6.06	2.45
Phe	15.14	0.32	13.14	17.18	13.15	0.49	0.28	0.24	0.02	0.46	4.17	5.87	7.13
Ile	23.14	26.13	18.78	25.98	24.65	24.78	23.79	29.82	4.6	10.05	13.17	20.44	7.88
Leu	0.05	0.21	0.03	0.05	0.08	0.21	0.18	0.23	0.11	1.41	2.13	0.43	0.69
Thr	2.34	2.32	1.88	2.79	2.35	2.09	1.79	3.09	2.18	1.74	3.63	2.38	0.58
Arg	4.14	0.24	5.18	4.78	2.45	0.19	0.38	0.14	2.86	2.45	14.11	3.36	4.03
His	3.5	4.36	2.99	3.26	4.31	2.79	3.99	6.27	2.29	12.73	0.04	4.23	3.21
Total FAA	141.24	205.03	123.01	141.03	158.14	221.81	237.81	244.25	76.69	122.41	144.81	165.06	54.14

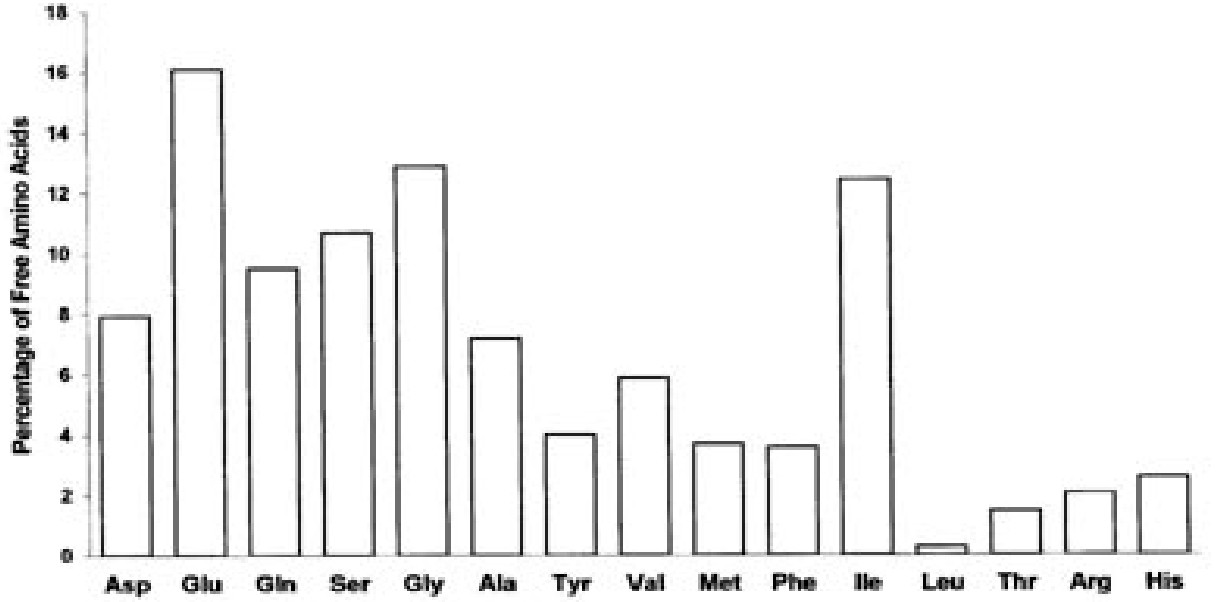

Fig. 1 : Percentage of free amino acids level in the milk of Indian mothers (n = 11) collected in between 10–14 days of parturition.

DISCUSSION

The results of the present study show that glutamic acid is the most abundant amino acid in the milk of Indian mothers. The other FAAs, which were in high concentration, were glycine, isoleucine, glutamine and serine.

Glutamic acid and glutamine together formed the major nonessential amino acids present in the human milk in India. Our finding is in accordance with the report of Agostoni et al that glutamaic acid and glutamine account for most of the FAA in human milk and their sum represents the 25-50% of total FAA (6).

Glutamic acid has been shown to be the most abundant amino acids in the milk of American, Mexican, Spanish, Italian and Taiwanese mothers (1–5). However relatively lower amount of glutamate observed in our study could be due to the time window (10–14 days after parturition) during which the samples were collected Glutamate content of human milk varies from individual to individual (1). Moreover the amount of glutamate and glutamine has been reported to increase with progressing lactation while most other FAA seemed to decrease significantly with progression of lactation period (6). The dietary status and habit of the mother may also influence the concentration of glutamate in the milk (8). It has been clearly shown in all the studies reported, similar to our observation, that glutamate is the most abundant amino acids in human milk. Glutamic acid is not only the dominant amino acid in human milk, but also it is the major free amino acid in the milk of non-human primates, elephants, horses and cows (9). Glutamic acid is shown to be 40 fold higher in human milk with respect to plasma content (10).

Milk of each species had a distinctive FAA pattern that may reflect the relative importance of the FAA during early postnatal development. The presence of high amounts of free glutamic acid in human milk may have important physiological functions. Most dietary glutamate is rapidly metabolized in the gut and used as an energy source (11). Glutamic acid acts as a source of ketoglutaric acid for the citric acid cycle (12). Glutamic acid and glutamine are the major energy substrate for the intestinal cells (13, 14). Mothers' milk may be playing a crucial role as a substrate for glutamate in nervous tissue for synaptogenesis during postnatal period (6). It is proposed that availability of increasingly high amount of glutamate throughout the period of lactation may be enhancing the flavour of the mother's milk for the infants (15, 16).

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