IMMUNE FACTORS IN BREAST MILK: A study and review

Abdulla EM[1], Zaidi FE[2] & Zaidi A[3]

ABSTRACT

Objective: An established source of energy, vitamins, minerals, growth factors, enzymes and hormones, only recent key research has also documented the ability of mothers milk to reduce in infants the incidence of diarrhea, RTI, UTI, otitis media, neonatal septicemia, necrotizing enterocolitis, Crohn's disease, allergic disorders, SIDS, type I diabetes, and lymphoma. However, the apparently scanty data from Pakistan on relevant antimicrobial constituents has encouraged this evaluation of selected immune factors in the early milk of some Karachi lactating mothers.

Design: Milk specimens expressed in sterile containers from lactating women 36-48 hours after parturition were scrutinized for DLC, TIBC and iron, and its centrifuged supernatants for IgA, IgG, IgM, CRP, C_3, C_4, and Secretor status; blood was drawn for Hb% and ABO(H)/Rh grouping.

Setting: A total of 25 colostrums, eleven of which were collected in January 2000 at Karachi's Imam General Hospital and 14 from voluntary lactating women 36-48 hours after parturition in the maternity ward of the city's Civil Hospital during Feb-Dec 2003, were processed at Dr. Essa's Lab & Diagnostic Centre, Karachi. All donors had uncomplicated pregnancies, and no signs of mastitis at the time of sampling.

Results: TIBC ranged from 283 to a high 795ug/dL in one mother with IDDM; CRP was mildly elevated (10-12 mg/L) in 22 subjects; IgG was not detectable in any specimen, but IgM was present (20-455 mg/dL), and sIgA, the major immunoglobulin in secretions, was significantly manifest (165-3090 mg/dL). Both C_3 (30-287 mg/dL) and C_4 (7-54 mg/dL), key components of the complement cascade, were evident. The lowest sIgA amount (48 mg/dL) with only traces of IgM, C_3 and C_4 were noticed in our solitary blood group 'A2' subject incidentally found to be a non-Secretor.

Conclusions: Selected antimicrobial factors in early breast milk expressed with informed consent from 25 Karachi lactating mothers were estimated. The ongoing study has hinted at individual variations from mother to mother in the blend of some of the pertinent constituents assessed, which encourages and necessitates additional sampling for confirmation.

KEY WORDS: Breast Milk, Colostrum, Immune factors, Immunoglobulins, CRP

Pak J Med Sci April-June 2005 Vol. 21 No. 2 178-86

1. Dr. Essa M. Abdulla
2. Dr. Farah E. Zaidi
3. Dr. Aliya Zaidi

1-3: Dr. Essa's Laboratory & Diagnostic Centre, Karachi-Pakistan

Correspondence:

Dr. Essa M. Abdulla
C-87, Block-15, Gulistan-e-Jouhar,
Karachi-Pakistan
E-mail: emabdulla11@hotmail.com

* Received for publication: June 19, 2004

Revision received: November 2, 2004

Revision accepted: January 6, 2005

INTRODUCTION

Man has been drinking the milk of cow, buffalo, camel, elk, goat, horse and yak among 4000 mammalian species throughout history without realizing until the late 1970s that the composition of milk varies from species to species and from cow to cow. Human milk, like the milk of many other mammals, is specially adapted to the needs of the newborn. Before birth, the mother transfers nutrients and bioactive components through the placenta: after birth, these substances are conveyed in colostrum and milk.[1]

Most of the major progress in understanding the unique and complex features of human breast-milk has emerged in just the past two decades.[2] Key researches has examined such aspects as its composition and the effects of maternal and environmental factors on human milk, its influence on the infant, including the protection against disease that breast-milk can confer on the newborn. Research suggests that lactation is robust and that a mothers breast-milk is adequate in essential nutrients, even when her own nutrition is inadequate.[3,4]

Mature breast-milk usually has regular levels of about 7g/dL carbohydrates, of which lactose (5.5-6.0 g/dL) is the most constant nutrient and about 0.9g/dL proteins[2,5] and is an established source of energy, enzymes, minerals and vitamins. In addition to its value as a nutrient source, interest has arisen in the ability of milk to kill a broad spectrum of bacteria, and in how this knowledge can be applied to child health.[6,7] Apparently every teaspoon of breast milk offers a complex blend of over 3 million germ-killing cells in it along with, among others, cytokines, lactoferrin, lysozyme and mucins that manipulate the wellbeing of the recipient baby toward a lower incidence of neonatal and infant infection.[8-15] (Table-I).

The principal immunoglobulin in human colostrum and ensuing milk is Secretory IgA (sIgA), which is resistant to the proteolytic effects of enzymes in the neonatal gut. The protective role of sIgA induces bactericidal activity, virus neutralization, the aggregation of antigens, and prevention of bacterial adherence to epithelial cells.[16-18]

Milk is often referred to as "white blood" since the cells it contains are important in phagocytosis and can provide the first line of defense in the baby's gut.[19] There is indeed

Table-I: Immune Benefits of Breast Milk

Component	*Action*
B-cells	Produce antibodies & continue doing so as plasma cells
Th-cells	Produce various cytokines, including interferon gamma
Tc-cells	Attack virally infected and malignant cells
Macrophages	Kill microbes; produce cytokines, lysozyme, etc
Neutrophils	Are also major phagocytic cells
Anti-Staph factor	Inhibits staphylococcal growth
B_{12} binding protein	Deprives bacteria of a vitamin it requires to grow
Bifidus factor	Promotes growth of *Lactobacillus bifidus* in baby's gut
C_3 & C_4	Key components of the Complement system
CRP	A Cytokine that coats a number of microbial surfaces
Fatty acids	Destroys many viruses by disrupting outer envelope
Fibronectin	Enhances antimicrobial activity of macrophages
Hormones	Stimulate baby's digestive tract to mature quicker
Interferon gamma	Enhances antimicrobial activity of immune cells
Lactoferrin	Bactericidal: binds to iron, depriving bacteria
Lactoperoxidase	An anti-streptococcal agent
Lysozyme	Cause lysis of bacteria by disrupting their cell walls
Mucins	Prevent bacteria and viruses from attaching to mucosa
sIgA antibodies	Thwart microbes from passing through walls of the gut

evidence that such protection is provided by macrophages in breast-milk.[20] Necrotizing enterocolitis is caused by bacterial penetration of the gut and occurs in infants with poor local defense; the condition is relatively uncommon in breast-fed recipients.[21] Immunocompetent lymphocytes are also present in human milk, and though there is no evidence that these react against the neonate in a harmful way, CD4+ T-cells can carry HIV from an infected mother.[6,9]

An anti-allergenic role has been proposed for breast-feeding. The neonatal gut shows increased permeability to some macromolecules so that susceptible infants could become sensitized to ingested foreign proteins. Re-exposure at a later date may allow minute quantities of absorbed antigens to trigger allergic symptoms; these include intestinal disturbances, eczema and asthma.[2,22,23]

Hanson (1998) in a review of the literature observed that while sIgA antibodies play a major role as "intestinal paint," several other factors, including the iron-binding lactoferrin participate as defense factors.[11] Indeed protection against infections has been well evidenced during lactation against acute and prolonged diarrhea,[24-28] respiratory tract infections,[29,30] otitis media,[31,32] Urinary Tract Infection (UTI),[33] neonatal septicemia[34,35] and possibly Sudden Infant Death Syndrome (SIDS).[36]

Human milk is characterized not only by a complex host defense system that prevents the colonization and proliferation of common microbial pathogens that may pervade the alimentary and respiratory tracts of the infant, but also by an array of anti-phagocytic factors.[37] The major anti-inflammatory agents include enzymes that degrade mediators of inflammation, as well as anti-proteases and a number of antioxidants such as cysteine, ascorbate, alpha-tocopherol and beta-carotene. Most of these factors are either absent or poorly represented in cow's milk, or indeed in other artificial substitutes for breast-feeding, and thus the provision of these antioxidants may help to protect the infant's developing immune system which is apparently quite susceptible to oxidant damage.[7,22,37]

In an excellent study on the influence of age, parity and maturity of pregnancy on antimicrobial proteins in human milk, Lewis-Jones et al (1985) investigated sequential samples of colostrum, transitional and mature milk from 47 women. The concentrations of sIgA, IgG, IgM, alpha$_1$-antitrypsin, lactoferrin, lysozyme, C_3 and C_4 were found to be high in colostrum with a decline in concentration as lactation proceeds; parity and age did not appear to influence significantly the variation in key protein concentrations.[38]

The manifestation of key components of the complement system in human breast-milk by Bush and Beer (1979)[39] and Cole et al (1982)[40] emphasized the presence of nonspecific factors in innate immunity which work in conjunction with IgG and IgM immunoglobulins. Indeed, knowledge about the protective value of passive acquired immunity provided by maternal IgG via placenta and sIgA in breast-milk is currently being expanded.[16] The slow development of IgA in the neonate seems to be compensated by selective transfer of secretory IgM into exocrine secretions; apparently sIgA and IgM against *E. coli* and poliovirus are found in the neonate in proportion with maternal contact experience.[11,23]

Recent interest has also included the presence of cytokines in human milk. Studies have demonstrated purified polypeptide growth factors[41] lymphokine production by milk lymphocytes[42] Tumor Necrosis Factor (TNF)[43] chemokines and colony stimulating factors (CSF)[44] in milk which reasonably can modulate the immunological development of the recipient infant.

Colostrum, also called "liquid gold" is a complex, usually lemon-yellow, nutrient-rich fluid produced towards the end of pregnancy, and possibly 3-5 days after parturition; though low in quantity (about a teaspoon in amount at each feeding), it contains all the ingredients that a neonate needs. Briefly, these include:

-- sometimes as much as 5000 mg / dl of sIgA that coat the baby's intestine, protecting the

mucosal surfaces against pathogenic bacteria and enteroviruses;

-- a variety of antibodies including those produced in the mother against any microorganism that she may have harbored or contacted at the time;

-- over 50 different health supportive processes, including growth and tissue repair factors;

-- high protein and salt, low fat and sugar content, ideal for the neonate's immature digestive system.[2]

Among a wealth of data in the literature, Vitolo et al (1993) observed that the colostrum of adolescent mothers showed significantly higher levels of sIgA and IgM compared to that of adults, and also reported on the chemical composition of colostrum of high and low socio-economic level mothers;[45] Louriero et al (1998) demonstrated IgA antibodies reactive to Entero-pathogenic *E. coli* (EPEC),[46] while Vassilev and Veleva (1996) showed that colostrum contains IgA and IgM antibodies to a number of antigens, including native DNA, myoglobin, transferrin and thyroglobulin, suggesting that this could explain the lower frequency of allergy, autoimmune disease, and lymphomas seen in later life in formula-fed infants.[47]

Researchers also report that colostrum stimulates the maturation of B-cells and primes them for the production of antibodies, and encourages over 50 different heath supportive processes.[48] Available data also sheds light on the composition of human milk,[49] the distribution of trace elements and minerals in human and cow's milk,[50] and also the adverse side effects of cow's milk in humans. Indeed, circulating antibodies to cow's milk proteins have been demonstrated in children with acute diarrhea,[51] and the immunogenicity of 5 cow's milk proteins in man have been measured;[52] Dean (1995) has further identified 20 constituents in cow's milk which were potentially capable of giving rise to an IgE-mediated allergic response in the pediatric population.[53] The various mechanisms whereby cow's milk affects gastrointestinal functions resulting in diarrhea, vomiting, GIT bleeding and mucosal surface damage have also been defined.[54]

With such a wealth of information on the constituents and effects of milk accumulating in recent years, it was unexpected that there was apparently scanty published data on similar studies attempted in Pakistan. Aside from a functional review on the immunology of breast-feeding in a local symposium by Fahmida Jalil in 1987 and her thoughtful paper along with a Swedish team on antibody-mediated immunity in the neonate in 1990,[16] and our preliminary report in 2000,[55] articles have covered mainly the attitudes and breast-feeding practices among Pakistani women.[56-58] It was therefore considered of interest to initiate this study on selected antimicrobial and immune factors in early breast milk collected from Karachi lactating women and to continue the learning by assessing the influence of environment, diet, parity, age and maturity of pregnancy on such components in sequential samples of colostrum, transitional, and mature milk. Logically the relevance of this learning is to fathom the contribution of defensive factors in breast-milk towards the health and wellbeing of the growing Pakistani child.

SUBJECTS AND METHODS

Donors and Specimen Collection:

A total of 1-2 ml of colostrum from both nipples was expressed manually into sterile containers, and 5-6 ml of blood drawn from the forearm from a total of 25 voluntary consenting lactating donors 36-48 hours after parturition. Eleven of these specimens were collected during January 2000 at Imam General Hospital, Karachi, and a further 14 from the maternity ward of the city's Civil Hospital between Feb-Dec 2003. The subjects were aged 19-30 yrs, represented lower and middle socio-economic groups, and were mainly housewives; just one was a school teacher, one a lab technician, another a bank officer. Menarche

was largely reported at 12-13 years of age; a solitary subject said that it occurred at 18. One donor (parity 7+1) had gestational diabetes, while another had IDDM and was on insulin. There were no signs of mastitis at the time of sampling. Only three milk specimens appeared white / pale yellow in color and watery in consistency, while the rest (n=22) were lemon-yellow and turbid.

Processing:

Blood hemoglobin was estimated on the automated Sysmex K-4500; TIBC and iron concentrations in milk samples were approximated on Hitachi 902 auto-analyzer using Roche reagents.

The Differential Leukocyte Count (DLC) was determined by listing 100 cells from the tail end to the body of each direct smear of intact milk.

The quantity of each milk for study was first accurately measured and the samples then diluted by adding up to 5ml of sterile saline (1 in 5 dilution) for expedience, and also because sIgA contents were expected to be significantly higher than that of blood levels and thus too concentrated for routine Radial-Immunodiffusion (RID) quantitation; the final resultant study parameter values were adjusted and quoted accordingly. The thinned specimens were centrifuged at 5000 RPM for 10 minutes to separate the fat and cell pellet, and part of the supernatant used to quantitate CRP values on Cobas Core Immunoassay Analyzer. The sIgA, IgM, IgG, C_3 and C_4 components were estimated using 0.005 ml amounts of each supernatant micropipetted in the gel layer of commercial NOR-Partigen RID plates (Behring, Germany), with the zone diameters measured using calipers after 48 hours of incubation in a moist chamber at room temperature.

Blood grouping was done using the DianaGel (Grifols, S.A.) ABO / Rh Cards on each subjects' blood and also spun milk supernatants, the latter in order to establish ABO secretor status. Specimens suggestive of group "O" were confirmed by using *Ulex europaeus* anti-H lectin (TransClone / Bio-Rad, France).

RESULTS

A. USING CENTRIFUGED SUPERNATANTS OF 25 COLOSTRUMS

1. Immunoglobulin Quantitation:

The amounts of sIgA in general averaged between 960-1024mg / dL (992 $\pm$ 32) in 21 of 23 spun supernatants analyzed; however, a comparatively low content (605mg / dL) was seen in the specimen of one housewife (Mrs. MP), and the highest quantity (3090mg / dL) in another (Mrs. SR, with gestational diabetes), wife of a milkman. Furthermore, scanty sIgA (165mg / dL) with negligible IgM (20mg / dL) and hints of C_3 and C_4 were recorded in the sample of a wife of a Honda salesman (Mrs. KH), while barely traces of sIgA (48mg / dL), IgM (23mg / dL), C_3, C_4 and CRP were seen in the white, watery colostrum of Mrs. SM, a bank cashier.

IgM amounts were also significant (except for the two cited above) and ranged from 115-262mg / dL (188 $\pm$ 73) in a majority (n=19) of the supernatants, with the highest amounts (n=4) between 455 to 550mg / dL, the latter peak recorded in the milk of a 20-year-old (Mrs. MA) wife of a businessman (Table II).

IgG was barely measurable, seen in irrelevant traces in all 25 supernatants.

2. C_3 and C_4 Quantitation:

While Complement components C_3 and C_4 amounts were negligible in the two samples cited above (Mrs. SM and KH), and low comparative levels of C_3 (30mg / dL) and C_4 (07mg / dL) in one other specimen (Mrs. MP), nevertheless, C_3 (95-287mg / dL: 191$\pm$96) and C_4 (27-53 mg / dL: 40$\pm$13) were significantly apparent in 22 others.

3. CRP Quantitation:

The magnitude of C–reactive protein was the lowest (<6mg / L) in 3 milk samples, one of which was of Mrs. SR, while in the majority (n=22) of the supernatants it averaged 8-12mg / L; incidentally, the normal range established in adult blood is quoted as 6-8mg / L, and that in milk is yet to be reported.

Table-II: Comparison of findings in six (6) representative specimens

Donor (Mrs.)	*Age (Yrs)*	*Personal Details*	*IgA*[1]	*IgM*	C_3	C_4	*CRP (mg/L)*	*Hb (g/dL)*	*Iron (ug/dL)*
KH	27	Housewife	165	20	Traces	Traces	10	12.3	42
MP	26	Housewife	605	90	30	07	12	11.2	30
MA	20	Housewife	960	550	210	27	08	11.8	29
SM	20	Cashier	48	23	Traces	Traces	Traces	10.3	74
SR	30	w/o Milkman	3090	115	143	53	<6	11.2	59
SF	27	IDDM[2]	1010	262	170	11	10	10.2	33

[1]Immunoglobulin / C_3 & C_4 amounts quoted in mg / dL [2] Insulin Dependant Diabetes Mellitus

B. USING INTACT COLOSTRUM SAMPLES

1. Iron Concentration:

Total iron ranged from 29 to 74ug / dL (56±17) in 24 intact milk specimens; the highest amount was detected in the sample of Mrs. SM that was also noted to be deficient in immunoglobulin and complement components.

2. TIBC Assessment:

The Total Iron Binding Concentration ranged from 283 to 795ug / dL (539±256) in contrast to the reported average of 250-350ug / dL in the blood of adult women.

3. Differential Leukocyte Count:

Results of DLC microscopy indicated a range of 53-80% neutrophils, 20-35% lymphocytes, along with 1-3 monocytes in each smear. Also noted were 80% lymphocytes, 20% neutrophils and 2-4 RBCs / hpf in the colostrum of an IDDM donor on insulin.

C. USING BLOOD/MILK SUPERNATANT

1. Hb Estimation:

Hemoglobin ranged from 9.8 to 12.3g / dL, with an average of approximately 10.7g / dL in a majority (n=16) of the 25 blood samples.

2. Blood Grouping:

Blood group 'B' was identified in a total of 12 subjects, 'A1' in 4, 'A2' in a single donor, and group 'O' in 8 of the study women, the latter established using anti-H lectin to confirm the terminal fucose determinant of 'O' grouping.

The corresponding blood groups were recognized in 21 of the 25 centrifuged milk supernatants: the proportion of ABH secretors was thus about 84%. Also, one of the 4 non-secretors was Mrs. SM, who was the sole group 'A2' subject.

D. STATISTICAL ANALYSIS

A statistical analysis of results of this ongoing exercise was not considered suitable at this point with outcome collected from just 25 donors; the statistical software package (SPSS) will be employed when an appropriate sampling bulk has been evaluated for statistical confidence.

DISCUSSION

The breast: symbol of femininity, celebrated throughout history by poets, painters and sculptors, provides a near-perfect food on which all higher forms of life depend in order to bridge the gap between pre-natal life and independent living on earth. There is but one raw material the breast can draw on to make milk – blood — and possibly 400 ounces of it must circulate through the breast to make a single ounce of milk.[1, 2]

Furthermore, no other fluid or tissue in the human body is as immunologically complicated as human milk. Throughout the course of breastfeeding, every feed offers a leukocyte "transfusion" along with a variety of anti microbial, anti-inflammatory and immunomodulating agents that not only compensate for the infant's immature immune system but also plays a role in modulating the rate of its development. During the first 10 days there are more white cells per ml in milk than there are in blood, and the macrophages and neutrophils they contain, aside from releasing cytokines, surround and destroy harmful bacteria by their phagocytic activity.[19,37,42,44] Our first-round scrutiny of stained smears of intact colostrum samples, as expected, showed abundant phagocytic neutrophils along with ample lymphocytes, the nature and subtypes of which will be an interesting target of further study.

In our ongoing exercise, although the 3 principal immunoglobulins along with CRP and the complement components C3 and C4 in milk were focused on, additional parameters also evaluated included blood hemoglobin and the colostrums TIBC and iron concentrations. While hemoglobin averaged 10.7g/dL in a majority (n=16) of the 25 blood samples, TIBC ranged from 283 to 795ug/dL in milk, in contrast to the reported average of 250-350ug/dL in the blood of adult women. The total iron concentration ranged from 29 to 38ug/dL in the intact milks, with notably the highest amount (38ug/dL) in the sample of one donor that was deficient in immunoglobulin and complement components. Also, her colostrum appeared unusually watery and colorless in contrast to the common viscous, golden-yellow specimens seen, reminding the authors of data that milk ingredients indeed can vary from feed to feed and even during each feed.[9,14]

The normal range of CRP that is established in adult blood is 6-8mg/L; our quantization detecting 8-12mg/L in a majority (n=22) of the milk supernatants is apparently the first report of this important immune factor in milk. Though there were no signs of mastitis in our subjects, the significance of its presence is profound: CRP reacts nonspecifically with diverse bacterial, yeast, fungal and protozoal surfaces, thus acting as an opsonin enhancing phagocytosis,[59] and in addition by creating an interface with properdin that launches C_3b, accordingly triggering the alternate complement pathway that targets offending microbes.[60]

Both the complement components C_3 and C_4 were significantly apparent in a majority (n=22) of donor milks; indeed their array (C_3: 95-287mg/dL; C_4: 27-53mg/dL) indicates a higher level of presence in colostrum than the average range in adult female blood (90-180mg/dL and C4: 15-40mg/dL IFCC respectively). No doubt their occurrence offers both the classical complement cascade when triggered by antibodies present, and the alternate pathway, through C_3b.

Antibodies indeed in the form of immunoglobulins A, G, M and D have been reported in human milk.[2,9] While IgD is found in barely traces in adult circulation, it is, along with monomer IgM, recognized as specific surface receptors on B-cells,[6] and hence logically is attached to any B-lymphocytes in milk. IgA, IgG and IgM immunoglobulins, though, are classical antibodies and reasonably are the same ones that are amply circulating in the mother's system and directed against many germs she has encountered in her home environment and other places she frequents. However, we failed to detect IgG in any of the milks analyzed, although it is comparatively small in size, can traverse the placenta, and is the most prevalent immunoglobulin class in the circulation (average adult range: 800-1700 mg/dL). On the other hand, IgA, the secretory immunoglobulin, was, as expected, amply present (960-1024mg/dL) and in significantly higher amounts than the average range in mature female blood (85-450mg/dL). Moreover, IgM, which ranged from 115-263mg/dL in a majority (n=19) of colostrums, compared favorably with normal female adult blood levels (60-370mg/dL). Because of its large size, IgM is logically not likely to diffuse well and is said to be found in low concentrations in intercellular tissue fluids. Nevertheless, the J-chain allows

IgM to bind to receptors on secretory cells which transport it across epithelial linings to the external secretions that bathe mucosal surfaces, thus in milk,[7, 11,16,45,47] wherein it also provides an accessory role to IgA as a "secretory" immunoglobulin.

An interesting observation was concerning the single donor of blood group A2 who was an ABH non-secretor; her colostrum contained the lowest comparative amount of IgA (48mg/dL), and barely traces of IgM, CRP, C3 and C4. Since the other three non-secretor mother milks were average in their contents, it seems unlikely that the secretor status played a role in the deficiency;[61] probably the specimen's appearance (white, watery) suggests that it was an "early" one.

Our groundwork scrutiny of some immune factors is incomplete; no doubt the vast mix of potentially obliging material in milk that guard the recipient baby against a variety of illnesses needs – and indeed encourages – supplementary study, and, frankly, the interpretation of results entails employing a larger sampling for statistical confidence and potential clinical connotations.

REFERENCES

1. Fred EL. Breast feeding: Time to teach what we preach. JAMA Pak 1993;4(12):691-3.
2. Hamosh M. Breast-feeding: Unraveling the mysteries of mother's milk. Medscape Women's Health 1996;1(9):4-20.
3. Brown KH, Bennett B. Lactational capacity of marginally nourished mothers. J Red 1986;78:909-19.
4. Almroth S, Bidinger P. No need for water supplementation for exclusively breast-fed infants under hot and arid conditions. Trans R Soc Trop Med Hyg 1990;84: 602-4.
5. Winikoff M, Baer EC. The obstetrician's approach to the breasts and breast feeding. J Reprod Med 1975;14:98-116.
6. Chapel H, Haeney M. in 'Essentials of Clinical Immunology' (third edition), Blackwell Sc Publ Oxford, London. pp 296-7,1993.
7. Chenishov VP, Sluvkin II. Mucosal immunity of the mammary gland and immunology of mother/newborn interrelation. Arch Immunol Therap Experimentalis 1990;38(1-2):145-64.
8. Goldman AS et al. Immunologic components in human milk during weaning. Acta Paed Scand 1983;73:133-4.
9. Goldman AS, Goldblum RM. Immunologic systems in human milk: characteristics and effects, in Lebenthoul E (ed) 'Textbook of Gastroenterology and Nutrition in Infancy' (second edition), NY Raven Press, pp 135-42, 1989.
10. Levy J. Immuno-nutrition: the pediatric experience. Nutrition 1998;14:641-7.
11. Hanson LA, Ahlstedt S, Andersson B, Carlsson B, Fallstrom SP, Mellander L, et al. Protective factors in milk and the development of the immune system. Pediatrics 1985;75(1):172-6.
12. Goldman AS, Chheda S, Garafolo R. Evolution of immunologic functions of mammary gland and the postnatal development of immunity. Pediatr Res 1998;43(2):155-62.
13. Oske FA, McMillan JA. Don't discount breastfeeding. N Engl J Med 1991;325:60-1.
14. Duerbeck NB. Breast-feeding: what you should know so you can talk to your patients. Compr Ther 1998;24(6):310-8.
15. Xanthou M, Bires J, Walker WA. Human milk and intestinal defense in the newborn: an update. Adv Pediatr 1995;42:178-208.
16. Hanson LA, Adlerbeth I, Carlsson B, Zaman S, Hahn-Zoric M, Jalil F. Antibody-mediated immunity in the neonate. Pediatr Pathol 1990;25(5):371-6.
17. Schroten H et al. Secretory IgA is a component of the human milk fat globule membrane. Pediatr Res 1999;45(1):82-6.
18. Kit YY et al. Secretory IgA from human milk possesses affinity to oligo-nucleotides and nucleic acids. Biochemistry (Moscow) 1999;64(1):40-6.
19. Bhaskaram P, Reddy V. Bactericidal activity of human milk leukocytes. Acta Paedtr Scand 1981;70(1):87-90
20. Lucas A, Cole TJ. Breast-milk and neonatal necrotizing enterocolitis. Lancet 1990;336:1519-23.
21. Avery VM, Gordon DL. Antibacterial properties of breast milk: Requirements for surface phagocytosis and chemi-luminescence. Eur J Clin Microbiol Infect Dis 1991;10(12):1034-9.
22. Hayward AR. The human fetus and newborn: development of the immune response. Birth Defects 1983;19:389-94.
23. Hanson LA. Breastfeeding provides passive and likely long-lasting immunity. Allergy Asthma Immunol 1998;81(6):523-33.
24. Rogers HJ, Sunge C. Bacteriostatic effect of human milk on *Escherichia coli*: the role of IgA. Immunology 1978;34(1):19-28.
25. Zetterstrom R, Bennet R, Nord KE. Early infant feeding and micro-ecology of the gut. Acta Paediatr Japan 1994;36(5):562-71.
26. Khin-muang. Effect and clinical outcome of breast feeding during acute diarrhea. Brit Med J 1985;290: 587-9.
27. Brown KH, Bennett B, Lopez AM. Infant feeding practices: relationship with diarrheal and other diseases in Lima, Peru. J Pediatr 1988;83:31-44.

28. Mitra AK, Rabbani F. The importance of breast-feeding in minimizing mortality and morbidity from diarrheal disease, the Bangladeshi perspective. J Diarrheal Dis Res 1995;1391:1-7.
29. Lopez AM, Villal PS. Breast-feeding lowers the frequency and duration of ARI and diarrhea in infants under six months of age. J Nutr 1997;127:436-43.
30. Wright AL, Holberg CJ, Martinez FD et al. Breast-feeding and lower respiratory tract illness in the first year of life. Br Med J 1989;299:946-9.
31. Ogra PL, Losonsky GA, Fishaut M. Colostrum derived immunity and maternal-neonatal interaction. Ann NY Acad Sci 1983;409:82-5.
32. Duncan B, Holberg CJ et al. Exclusive breast-feeding for four months protects against Otitis Media. Pediatrics 1993;91:867-72.
33. Piscane A et al. Breast-feeding and urinary tract infection. J Pediatr 1992;120:87-9.
34. Estrada B. Human milk and the prevention of infection. Infect Med 2003;20(6):270-72.
35. Hanson LA, Mellander L, Porras O, Soderstrom T. Breastfeeding protects against infections and allergy. Breastfeeding Review Nov 1988;pp 19-22.
36. Saadi AT, Gordon AE, MacKenzie DA et al. The protective effect of breast feeding in relation to sudden infant death syndrome (SIDS): I. human milk and infant formula preparations on binding of *Staphylococcus aureus* cells. FEMS Immunol Med Microbiol 1999;25(1-2):155-65.
37. Goldman AS, Goldblum RM, Hanson LA. Anti-inflammatory systems in human milk. Adv Exp Med Biology 1990;262:69-76.
38. Lewis-Jones DI et al. The influence of parity, age and maturity of pregnancy on antimicrobial proteins in human milk. Acta Paediatr Scand 1985;74(5):655-9.
39. Bush JF, Beer AE. Analysis of complement receptors on B-lymphocytes in human milk. Am J Obstet Gynecol 1979;133(6):708-12.
40. Cole FS et al. Complement biosynthesis in human breast milk macrophages and blood monocytes. Immunology 1982;46(2):429-41.
41. Shing Y, Davidson S, Klagsbry M. Purification of polypeptide growth factors from milk. Methods Enzymol 1987;146:42-8.
42. Keller MA et al. Lymphokine production by human milk lymphocytes. Infect Immunol 1981;32(2):632-6.
43. Mushtaba AA et al. Chemokinetic agents for monocytes in human milk: Possible role of tumor necrosis factor-alpha. Pediatr Res 1989;25(6):629-33.
44. Garafolo RP, Goldman AS. Cytokines, chemokines, and colony-stimulating factors in human milk: the 1997 update. Biol neonate 1998;74(2):134-42.
45. Vitolo MR, Brasil AL, Lopez FA. Colostrum composition in adolescent mothers. J Am Coll Nutr 1993;12(5):574-50.
46. Loureiro I, Frankel G, Dougan G et al. Human colostrum contains IgA antibodies reactive to enteropathogenic *E. coli* virulence-associated proteins. J Pediatr Gastroenterol Nutr 1998;27(2):166-71.
47. Vassilev TL, Veleva KV. Natural poly-reactive IgA and IgM auto-antibodies in human colostrum. Scand J Immunol 1996;44(5):535-9.
48. Murakami K, Lagarde M, Yuki Y. Identification of minor proteins of human colostrum and mature milk by two-dimensional electrophoresis. Electrophoresis 1998;19(14):2521-7.
49. Jenness R. The composition of human milk. Semin Perinatol 1979;3(3):225-39.
50. Fransson GB, Lonnerdal B. Distribution of trace elements and minerals in human and cow's milk. Pediatr Res 1983;17(11):912-5.
51. Fino TD, Ramonet M, Toccalino H. Circulating antibodies to cow's milk proteins in children with acute diarrhea. Arch Gastroenterol 1979;16(1):39-43.
52. Ferguson A. Immunogenicity of cows milk in man. Influence of age and of disease on serum antibodies of five cow's milk proteins. Clin Lab 1977;7:211-9.
53. Dean T. Cow's milk allergy: therapeutic options and immunological aspects. Eur J Clin Nutr 1995;49 (suppl 1):19-25.
54. Eastham EJ and Walker WA. Effect of cow's milk in the gastrointestinal tract: a persistent dilemma for the pediatrician. Pediatrics 1977;60(4):477-81.
55. Abdulla EM, Zaidi Farah E, Zaidi Aliya. Breast milk: Natural & Immune factors in Karachi lactating mothers. A paper presented at 15th Annual Symposium of Society of Gyn Obs of Pakistan, Feb 18-20 2000, Karachi
56. Sarwar SA, Mazhar A, Azhar M. Attitude of mothers towards breast-feeding their babies. Pak Pediatr J 1988;17(4):155-62.
57. Khichi GQK, Channar MS, Wararaich E, Bajwa SN. Patterns of breast-feeding in children under two years of age in Bahawalpur. Pak J Med Sci 2001;17(2):94-98
58. Arif MA, Mehdi Z. Knowledge and attitude of school teachers regarding breast feeding. Pak J Med Sci 2002;18(2):99-197.
59. Bryant NJ in 'An introduction to Immunohematology' (second edition) WB Saunders, Philadelphia 1982 p 82.
60. Young B, Gleeson M, Cripps A. C-Reactive protein: a critical review. Pathology 1991;23:118-24.
61. D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. Altern Med Rev 2001;6(4):390-405.