Clin Chem Lab Med 2005;43(2):198–201 © 2005 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2005.034

Chitotriosidase activity in colostrum from African and Caucasian women

Maria Musumeci[1], Lucia Malaguarnera[1], Jacques Simpore[2], Rita Barone[3], Michael Whalen[4] and Salvatore Musumeci[5,*]

[1] Department of Biomedical Sciences, University of Catania, Catania, Italy
[2] Centre Medical Saint Camille, Ouagadougou, Burkina Faso, Africa
[3] Institute of Chemistry and Technology of Polymers – National Research Council, Catania, Italy
[4] Department of Bioinformatics, Shardna Life Sciences, Cagliari, Italy
[5] Department of Pharmacology, Gynecology and Obstetrics, Pediatrics, University of Sassari, and Institute of Population Genetic, National Research Council (CNR), Alghero (SS), Italy

Abstract

Chitotriosidase (ChT), a protein produced by activated macrophages, belongs to the chitinases, a group of enzymes able to hydrolyze chitin, a structural component of fungi and nematodes. A codominant inherited deficiency in ChT activity is frequently reported in plasma of Caucasian subjects, whereas in the African population this deficiency is rare. This study compares ChT activity in colostrum of 53 African women and 50 Caucasian women. Samples were collected at 24–48 and 72 h after delivery. We found elevated ChT in colostrum of African women on the first day after delivery (1230±662 nmol/mL/h) which decreased to 275±235 nmol/mL/h on the third day. The ChT activity on the first day after delivery in the colostrum of Caucasian women, however, was significantly lower (293±74 nmol/mL/h) and decreased to 25±20 and 22±19 nmol/mL/h on the 2nd and 3rd day, respectively. The ChT activity in plasma of African women was also higher (101±80 nmol/mL/h) than that of Caucasian women (46±16 nmol/mL/h), but no correlation was found between plasma and colostrum ChT activity. The elevated ChT activity in colostrum of African women suggests the presence of activated macrophages in human milk, consistent with the genetic characteristics of the African population.

Keywords: African women; Caucasian women; chitotriosidase; colostrum.

*Corresponding author: Prof. Salvatore Musumeci, Cattedra di Pediatria Generale e Specialistica, Università di Sassari, Viale San Pietro 12, 07100 Sassari, Italy
Phone: +39-0360-285505, Fax: +39-095-7179690,
E-mail: smusumeci@tiscalinet.it

Introduction

Human milk is often regarded only as a food for infant growth and development, but the first milk (colostrum) can also serve as a vehicle for mother-to-neonate transfer of molecules that regulate the immune system and intestinal function (1, 2). In fact, a wide array of biologically active compounds such as hormones, cytokines and enzymes present in milk, especially in colostrum, control the proliferation, survival, differentiation and function of the neonate intestine (3). These compounds are ingested by neonates during a period of rapid maturation of gut-associated development of peripheral lymphoid tissues (4), so they have also an immune modulation function (5). Furthermore, it is also possible that colostrum may also exert direct action against pathogens present in the mouth of neonates.

Chitotriosidase (ChT) was the first human homologue of the chitinases of the plant kingdom to be discovered, and it is encoded by a gene located on chromosome 1q31-32 (6). This enzyme is produced by activated macrophages and is relatively abundant in the gastrointestinal tract and lung, supporting a possible role for chitinases in human defense mechanisms. There is also a second chitinase gene in the gastrointestinal tract that is called acidic mammalian chitinase (AMCase) (7). Recently, a codominant inherited deficiency in ChT activity, due to the duplication of 24 bp in exon 10 (ChT null allele), was reported (6). It is frequently encountered in Caucasian populations (35% are heterozygous carriers for ChT deficiency), whereas in the African population it is very rare (1%) (8).

Since chitinase is capable of cleaving chitin present in the cell wall of fungi and nematodes, it is possible that this enzyme continues to play a role in defense mechanisms against parasites (9). Elevated plasma ChT levels have been found in various infectious diseases, including neonatal systemic candidiasis (10), but contrasting results have been reported in the susceptibility of ChT-deficient individuals to *Candida albicans* (11) and to human *Wuchereria bancrofti* filarial infections (12, 13). Since this enzyme is thought to express macrophage activation, the difference in frequency of genetic deficiency between Caucasian and African populations deserves interest (8).

The aim of this study was to measure the ChT activity in colostrum of 53 African and 50 Caucasian women on the first 3 days after delivery in order to test if ChT activity is present in breast milk and if there are important differences in ChT activity between Caucasian and African populations, reflecting the genetic characteristics of ChT inherited deficiency.

Table 1 Characteristics of mothers who donated colostrum on the first 3 days after delivery, and levels of ChT activity.

Mother	n	Age, years	Deliveries	Gestational age, weeks	Spontaneous delivery	ChT activity, nmol/mL/h		
						24 h	48 h	72 h
African	53	26 (17–40)	4 (1–9)	40.0 (39–41)	53/53	1230±662*$	773±582*$	275±235$
Caucasian	50	27 (20–30)	2 (1–3)	39.5 (38–40)	20/50	293±74*	25±20	22±19

ChT values were corrected for protein concentration (mg/mL) in each sample. Results for age, deliveries and gestational age are given as median (range). *24 h, 48 h, 72 h, Student t-test for paired data, $p<0.001$. $African, Caucasian, Student t-test for unpaired data, $p<0.001$.

Materials and methods

Study area

Colostrum samples from African women were collected between July and October 2002 in the maternity ward of Centre Medical Saint Camille (CMSC) in Ouagadougou (Burkina Faso, Africa), where approximately 25–30 deliveries occur daily. Burkina Faso (formerly Upper Volta) was once a French colony but gained its independence in 1960 and is currently one of the poorer countries of the West African region between the Sudan and Guinea. The population of 11–12 million comprises several ethnic groups (Mossi, Peuhl, Gurunsi, Bobo, etc.). They are primarily shepherds or non-nomadic farmers and live in sod and thatch huts in small rural villages. Their socio-economic status is poor and their hygienic/sanitary conditions are defective, with a poor water supply. The direct consequence of these conditions is that the oro-oral transmission of infectious diseases is easy, beginning in the 1st day of life.

Caucasian women were from Catania, which is located in East Sicily, Italy. Ethical approval for the study was given by the institutional Review Boards at the CMSC Ouagadougou and at the St. Bambino Hospital in Catania.

Subjects

Data on personal characteristics (age, number of pregnancies, gestational age, etc.) and clinical findings (history, symptoms, body temperature) were collected from all the participants in the study. The food of the mother was in accordance with the traditional habits of their countries (millet, vegetables, fruit, and little beef for African women; grain, vegetables, fruit, fish and beef for Caucasian women).

Milk and blood sample collection

This study was approved by the local Ethical Committees in both Italy and in Burkina Faso. Mothers for colostrum donation were chosen randomly and they tested previously for HIV and sexually transmitted diseases. The exclusion criteria included HIV infection, sexual transmitted diseases and mastitis. All individuals participating in the study signed informed consent before starting the study. Table 1 summarizes the characteristics of breast milk sample donors. Colostrum was collected at 24, 48 and 72 h post partum in both the maternity ward of CMSC in Ouagadougou (Burkina Faso) and in the maternity ward of St. Bambino Hospital in Catania (Italy).

Milk samples were collected by the same teams in both Sicily and Burkina Faso using a standardized procedure: in the morning after awakening, before breastfeeding their babies, breast milk was collected by manual expression into a sterile polystyrene tube up to a volume of 5 mL. The time taken for manual expression was t maximum of 10 min and this operation was repeated for 3 consecutive days at 24-h intervals. Milk samples were immediately refrigerated at 4°C after collection, transported on ice to the local laboratory and stored at −20°C. Then the colostrum samples were transported at −80°C in dry ice to the Department of Pediatrics, University of Catania, Italy, After thawing, colostrum samples were first centrifuged at $680\times g$ for 10 min at 4°C. The liquid component was removed and re-centrifuged at $10{,}000\times g$ for 30 min at 4°C. In this way the floating lipid layer and cellular sediments were removed. After separation, the serum fraction of colostrum samples was stored in 1.5-mL polypropylene tubes and frozen at −20°C until assay of the ChT activity. A normalization of colostrum samples was made by measuring the total protein content in each tube by the Lowry method (14) and the activity of ChT was corrected for the protein concentration (mg/mL).

Blood samples (5 mL) were obtained by venepuncture on the same morning as colostrum collection in an EDTA-containing tube. Plasma and packet cells were separated for centrifugation at $1500\times g$ for 10 min and frozen for chitotriosidase activity determination and 24-bp duplication identification. All donor mothers remained in the maternity ward (Sicily and Burkina Faso) for 3 days after delivery. After being discharged from the hospital, all the mothers continued to breastfeed their babies ad libitum at home.

ChT activity in colostrum

ChT activity was assayed in plasma and colostrum using an artificial chitin-like substrate as described by Barone et al. (15). A 5-μL sample of undiluted milk serum or plasma was incubated with 100 μL of a solution of 22 μmol/L 4-methylumbelliferyl-β-D-N,N′,N″-triacetylchitotriose (Sigma Chemical Co, St. Louis, MO, USA) in 0.5 mol/L citrate phosphate buffer, pH 5.2, for 15 min at 37°C. The reaction was stopped using 2 mL of 0.5 mol/L Na_2CO_3-$NaHCO_3$ buffer, pH 10.7. The fluorescence was read on a Hitachi 2500 fluorimeter (Hitachi Europe Ltd, Herts, UK), with excitation at 365 nm and emission at 450 nm. ChT activity was measured as nanomoles of substrate hydrolyzed per mL per hour (nmol/mL/h).

Samples with ChT levels >110 nmol/mL/h were re-assayed after dilution of 1:10 or 1:50 with distilled water.

DNA analysis

Genomic DNA was isolated from 5 mL of peripheral blood as previously described (16). A 30-ng sample of DNA was used as template in subsequent polymerase chain reaction (PCR). The duplication mutation analysis was performed using specific primers [Chs9 (AGCTATCTGAAGCAGAAG) and Chas8 (GGAGAAGCCGGCAAAGTC)] and fragments of 75 and 99 bp were amplified from the normal and null ChT gene, respectively. Electrophoresis in metaphore gel (4%) allowed the detection of both fragments. In the case of carriers for the duplication, a mixture of both fragments was detected.

Statistical analyses

Demographic, clinical and treatment profiles were recorded on computer file and analyzed by standard software (SPSS 10 for Windows). We used the two-tailed Student t-test for parametric data. A p value <0.01 was considered statistically significant.

Results

Colostrum chitotriosidase levels

ChT levels in colostrum of African women were elevated in the first 24 h (1230 ± 662 nmol/mL/h) and this level progressively decreased on the 2nd day (773 ± 582 nmol/mL/h; $p<0.001$) to a value of 275 ± 235 nmol/mL/h on the 3rd day ($p<0.001$) (Table 1). The values for ChT were significantly lower ($p<0.001$) in colostrum of Caucasian women (293 ± 74 nmol/mL/h) and this level rapidly decreased to a minimal level on the second and third days (25 ± 20 and 22 ± 19 nmol/mL/h, respectively; $p<0.001$) (Table 1). The level of ChT in plasma of African women was 101 ± 80 nmol/mL/h, while in Caucasian women this value was 46 ± 16 nmol/mL/h, but no correlation was found between colostrum and plasma ChT activity in both African and Caucasian women. The frequency of ChT null allele in the group of Sicilian women was 42%, but no woman was homozygous for the ChT null allele. Since the ChT genetic deficiency seems to be uncommon (1–2%) among women in Burkina Faso (8), this study was omitted in African women. Caucasian mothers who were heterozygous for the ChT null allele had a reduced level of ChT in their colostrum from the 1st day (181 ± 56 nmol/mL/h) post-partum.

Discussion

In the present study we demonstrated that the mean levels of ChT activity were elevated in the colostrum of the African women, especially on the first 2 days after delivery. Interestingly, the ChT level decreased by the 3rd day to the level of ChT found in the colostrum of Caucasian women on the 1st day (Table 1). The absence of correlation between colostrum and plasma ChT activity in both African and Caucasian women suggests that high ChT activity in colostrum is a consequence of local secretion of ChT by activated macrophages independently from plasma ChT concentration.

The significance of intestinal functionality of ChT in colostrum is unclear: as is the case for many other proteins in breast milk, it could be inactivated or destroyed before arriving in the intestine. However, the specific high secretion in colostrum could reflect possible protection against pathogens (17).

The differences encountered between colostrum ChT levels in African and Caucasian women are in accordance with the different distribution of ChT genetic polymorphism in sub-Saharan areas and Mediterranean countries. In fact, in our recent study carried out in Benin and in Burkina Faso, both regions endemic for *Plasmodium falciparum* malaria and infections due to intestinal parasites, we found a low incidence of the ChT null allele (heterozygous 0% and 1-2%, respectively), and no homozygous subject for ChT deficiency (8) was detected. In contrast, we observed that in Sicily and in Sardinia, the heterozygote frequency for duplication was 44% and 32.71%, whereas homozygote frequency was 5.45% and 3.73%, respectively. As a direct consequence of the elevated frequency of the ChT null allele in the group of Sicilian women (42%) we observed that the ChT activity was lower in the colostrum of Caucasian women from the 1st day, while the level of ChT activity in colostrum of African women was initially higher.

In human plasma, ChT activity has been proposed as a biochemical marker of macrophage activation and our results show that in colostrum ChT activity could also represent a marker of macrophage activation, which we think has a role in the protection of babies against pathogens on the first days of life. In fact, the prevalence of oral candidiasis is very low in the first month of life in African compared to Caucasian babies, despite large diffusion of oral and intestinal candidiasis later on (unpublished observation).

The possible role of human ChT in protection against *Candida albicans* and nematodes has been sustained by the consideration that this enzyme shows chitinase activity towards an artificial chitin substrate and some authors have attributed an endocellular antibiotic function to ChT (10, 18).

Nevertheless, contrasting results on the presumptive anti-parasitic activity of ChT have recently been reported. Masoud et al. (11) did not find any difference in the percentage of homozygotes for the ChT null allele among survivors of *Candida* sepsis with respect to a control population. Hise et al. (13), studying the polymorphism of three innate immunity genes suspected of contributing to susceptibility to infections and lymphatic pathologies, showed in residents of Papua New Guinea that the ChT null allele and polymorphisms of toll-like receptor-2 and toll like receptor-4 genes did not correlate with human *Wuchereria bancrofti* filarial infection with respect to a control population. These results are clearly in contrast with a previous paper by Choi et al. (12), who showed that in India the homozygous condition for the defective allele and the consequent decreased ChT activity was associated with elevated susceptibility to human *Wuchereria bancrofti* filarial infection.

All in all, these observations suggest that the role of the homozygotic defective allele in susceptibility to human *Wuchereria bancrofti* filarial infection and survival of *Candida* sepsis is unclear. However, the lower level of ChT activity in colostrum of Caucasian women, which is in agreement with the high carrier frequency of the ChT null allele (44%) in the Sicilian population (8), suggests that the ChT enzyme could be redundant (i.e., not an essential defense mechanism) in both plasma and colostrum of the Caucasian population, possibly reflecting improved environmental conditions in these areas. On the contrary, the low

frequency or absence of the ChT null allele in sub-Saharan regions may represent an important protective mechanism through the colostrum in African newborns still living in difficult environmental conditions, where the wild-type ChT gene continues to be an advantage. Based on this study, it appears that improved environmental conditions in Mediterranean countries are responsible for the diversity of colostrum ChT activity between Caucasian and African populations, but really these improvements have occurred very recently. This statement would be supported if it were possible to compare subjects from Burkina Faso who have been resident in Italy for several generations with those still living in Burkina Faso. In African subjects, comparison of ChT activity with other biological substances (cytokines, growth factors, prolactin, etc.) present in the colostrum would be interesting to demonstrate if the first 3 days after delivery are determinant in guaranteeing efficient natural protection to the newborn in such disadvantaged surroundings.

Acknowledgements

We thank the participants as well as the obstetricians of the maternity ward of Centre Medical Saint Camille, Ouagadougou (Burkina Faso) and the maternity ward of St. Bambino Hospital, Catania (Italy) and in particular Father Salvatore Pignatelli, Chief of the Centre Medical St. Camille, Sister Bernarda Omassi and Elisabeth Tiemtore for indispensable help in this study. We thank Mr. Giuseppe Rapicavoli, Department of Pediatrics, University of Catania, for his skilful technical assistance in laboratory work.

References

1. Hennart PF, Brasseur DJ, Delogne-Desnoeck JB, Dramaix MM, Robyn CE. Lysozyme, lactoferrin, and secretory immunoglobulin A content in breast milk: influence of duration of lactation, nutrition status, prolactin status, and parity of mother. Am J Clin Nutr 1991;53:32–9.
2. Ellis LA, Mastro AM, Picciano MF. Milk-borne prolactin and neonatal development. J Mammary Gland Biol Neoplasia 1996;1:259–69.
3. Hirai C, Ichiba H, Saito M, Shintaku H, Yamano T, Kusuda S. Trophic effect of multiple growth factors in amniotic fluid or human milk on cultured human fetal small intestinal cells. J Pediatr Gastroenterol Nutr 2002;34:524–8.
4. Wagner CL, Forsythe DW. Effect of human milk and recombinant EGF, TGF alpha, and IGF-1 on small intestinal cell proliferation. Adv Exp Med Biol 2000;478:373–4.
5. Ellis LA, Mastro AM, Picciano MF. Do milk-borne cytokines and hormones influence neonatal immune cell function? J Nutr 1997;127(5 Suppl):985S–8S.
6. Boot RG, Renkema GH, Verhoek M, Strijland A, Bliek J, de Meulemeester TM, et al. The human chitotriosidase gene. Nature of inherited enzyme deficiency. J Biol Chem 1998;273:25680–5.
7. Boot RG, Blommaart EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, et al. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J Biol Chem 2001;2:6770–8.
8. Malaguarnera L, Simporè J, Prodi DA, Sassu A, Persico I, Barone R, Musumeci S. A 24-base pair duplication in exon 10 of human chitotriosidase gene from the sub-Saharan to the Mediterranean area: role of parasitic diseases and environmental conditions. Genes Immun 2003;4:570–4.
9. Renkema GH, Boot RG, Au F, Danker-Koopman WE, Strijland A, Muijsers AO, et al. Chitotriosidase, a chitinase, and the 39 kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. Eur J Biochem 1998;251:504–9.
10. Den Tandt WR, Van Hoof F. Marked increase of methylumbelliferyl-tetra-N-acetylchitotrioside hydrolase activity in plasma from Gaucher disease patients. J Inher Metab Dis 1996;19:344–50.
11. Masoud M, Rudensky B, Elstein D, Zimran A. Chit deficiency in survivors of Candida sepsis. Blood Cells Mol Dis 2002;29:116–8.
12. Choi EH, Zimmerman PA, Foster CB, Zhu S, Kumaraswami V, Nutman TB, et al. Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with Wuchereria bancrofti in South India. Genes Immun 2001;2:248–53.
13. Hise AG, Hazlett FE, Bockarie MJ, Zimmerman PA, Tisch DJ, Kazura JW. Polymorphisms of innate immunity genes and susceptibility to lymphatic filariasis. Genes Immun 2003;4:524–7.
14. Lowry OH, Roseburgh NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951;193:265–78.
15. Barone R, Di Gregorio F, Romeo MA, Schilirò G, Pavone L. Plasma chitotriosidase activity in patients with beta-thalassemia. Blood Cells Mol Dis 1999;21:1–8.
16. Ciulla TA, Sklar RM, Hauser SL. A simple method for DNA purification from peripheral blood. Anal Biochem 1988;174:485–8.
17. Wold AE, Adlerberth I. Breast feeding and the intestinal microflora of the infant – implications for protection against infectious diseases. Adv Exp Med Biol 2000;478:77–93.
18. Borregaard N. Antibiotic molecules: intracellular. Inv: Encyclopedia of Life Sciences. Nature Publishing Group, 2001:1–8 (www.els.net).

Received September 11, 2004, accepted November 10, 2004