Inhibition effect of Colostrum and breast milk against *Shigella flexneri* infection

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Abstract:

In this study was undertaken to demonstrate whether colostrum and breast milk exerts any inhibitory effect on bacterial pathogens ***Shigella flexneri*** after induction of rabbit orally at concentration 10^8 cfu/m1, diarrhea appeared in 4-5 days. These cases of diarrhea treated with colostrum and breast milk, we sought the recovery after 12hr. compared with antibiotics (Ampicillin and Cloxacillin) which gives the same result with in 24hr.

التاثير التثبيطي للبأ والحليب ضد الاصابة بـ *Shigella flexneri*

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ملخص البحث:

تضمنت دراستنا استخدام اللبأ وحليب الصدر كمادة مثبطة للجراثيم المرضية كـ ***Shigella flexneri*** وذلك بعد احداث اصابة فموية للارانب بتركيز 10^8 وحدة تكوين المستعمرة/ مل ومن ثم ظهور علامات الاسهال بـ 4-5 ايام وتم علاج هذه الحالات المرضية بـ اللبأ وحليب الصدر، ظهرت الاستجابة للعلاج بعد 12 ساعة من التجريع باللبأ وحليب الصدر، مقارنة بالمضادات الحيوية (Ampicillin and Cloxacillin) التي أعطت نفس النتائج ب24 ساعة.

Aim of the study:

The aim of the present study was to investigate the effect of colostrum and breast milk in management in rabbits of the infection caused by ***Shigella flexneri*** isolates

Introduction:

The colostrum and breast milk contain of material named of Lactoferrin. Also, it is known as Lactotransferrin which is aglobular multifunctional protein with antimicrobial activity (bacteriocide, fungicide) and it is part of the innate immunity, mainly at mucosa, also Lactoferrin is found in colostrum, milk and many mucosal secretions such as tears, saliva, bile, uterine fluid, vaginal secretion, seminal fluid, pancreatic juice, small intestine secretion and nasal secretion. Lactoferrin is also presented in secondary granules of polymorphonuclear neutrophils (will, 2000; Liuque., *et al*.2010; David, *et al*, 2012). Human colostrums has the highest concentration, followed by breast milk of human, then cow milk. Lactoferrin belongs to the transferrin family protein (melanotransferrin, ovotransferrin,etc.). It's molecular mass is 80 , 000 u(78-80 KDa), it generally contains two bound Fe^{+2} ions. It contains 4 identical domains, with two surrounding each iron atom. Lactoferrin antimicrobial activity is due partly to it's high affinity for Fe^{+3} (ferric state), it proteolysis produces Lactoferricin, Kaliocin-1 small pepties with antimyocarodial activity. The combination of iron and lactoferrin in mucosal secretions modulate the ability and aggregation of pathogenic bacteria, and inhibit both bacteria and viruses by binding to host cell/viral particles, this inhibits the ability of bacteria and viruses to attach to cell membrane, it is also an antifungal agent (Oral, 2001; L.AdLerova , *et al* 2008; Kanthawong,*et al* 2009.)

Lactoferrin is a glycoprotein that belongs to the iron transporter or transferrin family that is resistant to proteolytic enzymes, it contains 703 amino acids and is considered a multifunctional or multi-tasting protein. It appears to play several biological roles, the possible antibacterial activity of supplemental Lactoferrin may be accounted for in part, by it's ability to strongly bind iron. Iron is essential to the growth of pathogenic bacteria, Lactoferrin may also inhibit the attachment of bacteria to the intestinal wall.

Lactoferrin classified as a bioactive peptide, may also have antibacterial as well as antiviral, and it causes impaired bacterial multiplication due to it's ability to decrease the availability of iron required for growth (Henry , *et al*. 2002; Grubor , *et al*. 2006; Atef , 2012). Lactoferrin consist of a single polypeptide chain produced during lactation and by epithelial cells at mucosal surfaces. The protein is approminent component of the first line of mammalian host defense and it's expression is upregulated in response to inflammatory stimuli, it is the second most a bundant protein in breast milk. Also the polypeptide structure of lactoferrin comprises two homologous domains that appear to have arisen by intragenic duplication. The crystal structure of the protein has been resolved and each domain binds one ferric and one carbonate anion. In addition, each domain contains one glycosylated

site to which N-linked glycan residues are attached (Orla , 2001; Legrand ., *et al*, 2005; L.Adlerova, *et al*, 2008; Isaacs., *et al*, 2011).

Materials and methods

In this study the antibacterial activity of Lactoferrin that found in both colostrum and breast milk have been substantiated by both invivo and invitro evidence .

A) The samples consist of:

1. Samples of colostrum were collected from 4 nursing mothers beyond 2 days post partam..

2. Samples of breast milk were collected from 5 nursing mothers beyond 4 weeks post partum..

The 9 dornor mothers were in good health and had no clinical evidence of mastitis, delivered to healthy full-term infants, and without intaking of medication with the time prior to collection. Following thorough hand washing and cleaning of the breast and nipple with soap and tap water, these samples were expressed manually into sterile test tubes and frozen at -20°c.

B) Isolates of ***Shigella flexneri*** had been taken from college of science department of biology/university of Mosul, that originated from patients suffering from enteritis in Germany, and diagnosed by biochemical test, isolates were subcultured from a starter culture, incubated at 37°c under aerobic condition for 24 hr. in nutrient broth and stored in refrigerator of 4°c until used. (Findgold and Martin, 1982).

C) The invivo assay test was applied to 8 rabbits (private breed) their ages ranged between 6-8 months, by induction of ***Shigella flexneri*** at concentration 10^8 cfu/ml orally, then 4-5 days the diarrhea appeared. All the (8) infected rabbits were divided into (4) groups (2 rabbits/group)

- The first group (2 rabbits) were treated orally with the colostrums (0.5)ml twice daily morning and evening for 4 days.

- The second group (2 rabbits) were treated orally with the breast milk (1)ml twice daily monring and evening for 4 days.

- The third group (2 rabbits) were treated orally with antibiotics (Ampicillin and Cloxacillin 500 mg) as above.

- The fourth group (2 rabbits) were treated orally with saline. as above (control group).

D) the invitro assay test was included inoculated of ***Shigella flexneri***; in nutrient broth, incubated for 24hr. at 37°c, then nutrient agar plates were seeded with (0.1)ml of liquid inoculum prepared for the isolates of ***Shigella flexneri*** disc impregnated with the tested materials were placed on the surface of the nutrient agar plates, the first disc was impergnated in colostrum by taking swab of it, the second disc was impergnated with breast milk by taking swab of it, the third disc was impergnted with antibiotics (Ampicillin and Cloxacillin) at concentration (500)mg, while the fourth disc was impergnated in saline and used as control, then incubated at 37°c and the zone of inhibition were appeared after 24hr.(WHO, 2004; Clsl clinical and Laboratory standards institute, 2010).

Results and Discussion:

The result of this study estimated the effect of colostrum and breast milk in treatment of the infections specially in our study the effect of two materials upon ***Shigella flexneri*** :-

In this study, the first group treated with colostrum, the recovery within 12hr, compared with the third group which was treated with Ampicillin and Cloxacillin 24hr. Otherwise the second group treated with breast milk, the recovery with in 12hr. compared with the third group which was treated with Ampicillin and Cloxacillin 24hr. These result improved the action of colostrums and breast milk upon ***Shigella flexneri***, this considered as antibiotics, accordance with Susan , *et al*, 2012).

Significant differences in the rate of recovery were observed between control diarrhea treated with saline and diarrhea treated with either colostrum, breast milk and antibiotics, these two materials gave the fastest rate of recovery compared with others, this was the results of invivo study upon experiment, the same results were obtained in vitro study, zone of inhibition against ***Shigella flexneri*** : for the colostrum, breast milk was higher than that inhibition for antibiotics against ***Shigella flexneri*** , but control disc (saline) showed no zone of inhibition by diameter (1.9mm)(1.5mm)(2.5mm)(0mm) respectively (Image 1 , figure(1)).

Image (1) zone of inhibition of colostrum, breast milk, antibiotics, saline against ***Shigella flexneri*** :

A= Colostrum C= antibiotics

B= breast milk D= saline

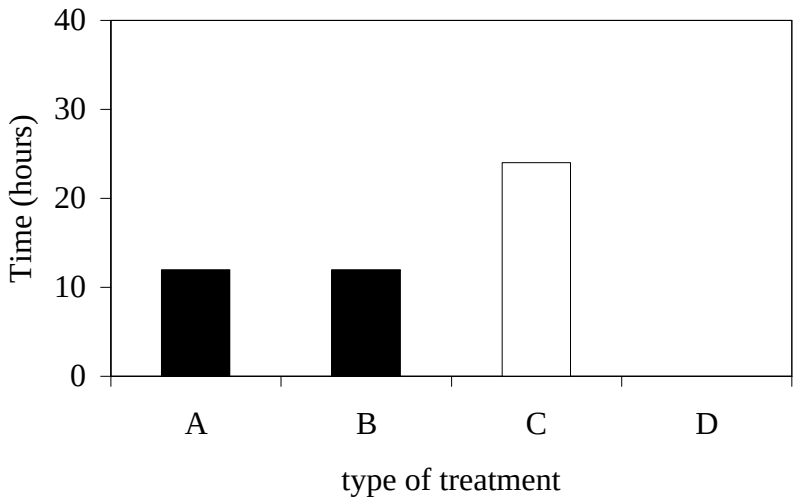

Figure (1). Relation between time of recovery and type of treatment

A, B } Colostrum and breast milk

C= antibiotics

D= saline

The study improved Lactoferrin aglycoprotein present in human main mucosal secretion such as colostrum and breast milk protection from bacteria enteric infection, i.e., it bacteriostasis for enteric pathogens like ***Shigella flexneri***, this accords with many studies as (Esperanza, 1989; Will , 2000; Willer, *et al*, 2004; Legrand , 2005; L. Adlerova, *et al*, 2008; Henry , 2003; Susan , *et al*, 2012).

Lactoferrin, antilipopoly-Saccharide (anti-Lps) and asecretory immunoglobulin A (IgA),Lysozyme and oligosaccharides, may protect of infection of Shigellosis because the virulence of *Shigella* is due to it's ability spread intracellularly and induce inflammation with in the intestinal epithelinm cells. The anti-inflammatory activity occurs through inhibition of binding of Lipopolysaccharide endotoxin to inflammatory cells as well as through interaction with epithdial cells at local sites of inflammation to inhibit inflammatory cytokine production(Oral , 2001; Legrand , 2005; Ando, 2010). There are many antibacterial factors in breast milk that may be responsible for it's protective function., the chief immunoglobulin of breast milk is secretory IgA. Secretory IgA has the ability to attach itself to mucosal epithelinm and prevent the attachment and possible invasion of specific infections agent, this substance binds free iron in breast milk avidly, presumably it also limits iron availability to potentially pathogenic flora by competing with bacterial enterochelin for iron (Arnol, *et al*. 1980; Gomez , 2002; Atef , 2012).

In that respect, it should be noted that Lactoferrin in breast milk is largely unsaturated and therefore could be apotential microbicidal agent. However, in the presence of antibody and bicarbonate, Lactoferrin exerts a strong bacteriostatic effect, probably by causing deformation in transfer RNA (Arnold, 1980; Beers , *et al*, 2002; Borregaard , 2007).

Another studies showed that Lactoferrin include regulation of iron absorption in the intestine, promotion of intestinal cell growth, protection against microbial infection, regulation of myelopoesis and systemic immune responses, this appearts to focus on the activities of Lactoferrin that contribute to host defense, The antibacterial properties of Lactoferrin appear that two different mechanisms involving two separate domains of the protein contribute to the anti-microbial function of Lactoferrin.

The first mechanism is abacteriostatic effect related to the high iron binding affinity of the protein that deprives iron-requiring bacteria of this essential growth nutrient. Since the bacteriostatic properties of Lactoferrin are due to it's iron binding ability, the protein is capable of retarding the growth of abroad range of microorganisms including avariety of gram-negative and gram-positive bacteria and certain yeasts.

The second antibacterial property of Lactoferrin is due to a direct bactericidal function within the protein. Lactoferrin has a direct bactericidal effect against some gram-negative and gram-positive bacteria that can not be attributed to simple iron deprivation (Iyer and Lonnerdal , 1993; Headon , 2000; Kanyshkova, . *et al*, 2001; Ors,. 2004; David , *et al*, 2012).

Finally a study looked at Lactoferrin as a natural antibiotic found that Lactoferrin both invitro and invivo strongly the toxic bacteria (Will , 2000, L.Adlerova, *et al*, 2008, Susan , *et al*, 2012).

References:

1. Ando K, Hasegawa K, shindo K, Furusawa T. Fujino T, Kikugawa K. (2010) Human activites NF-Kappa B through the Toll-like receptor 4 pathway white it interferes with the lipopoly saccharide-stimulated TLR4 signaliny FEBS J. 277(9): 2051-2066 Cross Ref Medline
2. Arnold, R. R. M. Brewer, and J. J. Gauthier (1980) Bactericidal activity of host variety of microorganism. Infect Immun. 28:893-898.
3. Atef E. Fayed (2012) Healthy Multifunctional spectra of milk glycoproteins and fragments-a review article polish Journal of food and nutrition sciences vol. 62, N.3, 125-142.
4. Beers S. A. Buckland AG. Kodur: RS. Cho. W. Gelb MH. Witon DC. (2002). The antibacterial of secreted phospholipases A:2 a major physiological role for the group 11A enzyme that the very high pl of the enzyme to allow penetration of the bacterial cell Wall. J. Biol. Che 1788-1793. Cross. Ref. Medline.
5. Borregaad N. Sorensen OE. Theilgaard-Monch K.(2007) Neutrophil granules: a library, immunity proteins. Trends immunol. 28(8): 340-345. Cross. Ref. Medline.
6. CLSI clinical and Laboratory standards institute. (2010). Performance standards for antisusceptibility testing .Twentieth information supplement. Avaliable from www.cisi.org .
7. David B. Alexander, Masaaki ligo, Koji Yamauchi, Masumi Suzui, Hiroyuki Tsuda (2012) Lactoferrin: an alternative view of its role in human biological fluids Biochemistry and cell Biology 90(3): 279-306, 10.1139/o 2012-o13.
8. Esperanza F. Rivera, M. D. and Ricarchito B. Manera, M.D. (1989) Antimicrobial activity of breast milk against common pediatric pathogens, J. Microbial infect. Dis., 18(2): 67-74.
9. Finegold M. and Martin, J. (1982), Diagnostic microbiology, 6th edition, the c.v. Mosby Company, st. Louis. Toronto. London.
10. Gomez, H. F., I. Herrera-Insua, M. M. Siddiqui, V. A. Diaz-Gonzalez, E. C., Cleary, (2001) protective role of human Lactoferrin against invasion of shigella Biol. 501:457-467.
11. Grubor B, Meyetholz DK, Ackemann. MR. (2006) collections and catonic antimicrobial respiratory epithelia. Vet. Pathol. 43(5): 595-612 Cross Ref. Medline.
12. Headon DR (2000) Human Lactoferrin: Production at large scale, characterization and applications. Lactoferrin structure, function and application, Wiley publ, p415.
13. Henry F. Gomez., Theresa J. Ochoa, Irene Herrera-Insua, Lily G. Cleary, (2002) Lactoferrin protects rabbits from ***Shigella flexneri***: infect enteritis. Infect Immun, December, 70(12): 7050-7053.

14. Henry F., Gomez, Theresa J.Ochea, Lity G, Carline and Thomas G. Cleary, (2003) Human Lactoferrin impairs virulence of ***Shigella flexneri:*** The Journal of infections disease (187), 87-95.
15. Isaacs S. Fakhri S, Luong A, Whited C, Citard; MJ. (2011). The effect of dilute baby sharrow mucociliary clearance in healthy subjects. Am. J. Rhinol. Allergy 25(1): e27-e29 Cross R.
16. Iyer S. Lonnerdal B. (1993): Lactoferrin, Lactoferrin recepctors and iron metabolism. European Journal of clinical nutrition, 47, 232-241.
17. Kanthawong S. Nazmi K, Wongratana-cheewin S, Boischer JG, Wuthiekanun V, Taweek (2009) In vitro susceptibility of Burkholderia psendomallei to antimicrobial peptides. In Antimicrob. Agents 34(4): 309-314 Cross Ref, Medline.
18. Kanyshkova T.G.Buneva V.N.,Nevinsky G.A.(2001):Lactoferrin
and its biological functions,Biochemistry(Moscow), 66,1-7.
19. L. Adlerova, A. Bartoskova, M. Faldyna (2008), Lactoferrin: a review veterinarn: medicina, 53, (9): 457-468.
20. Legrand D., Elass E., Carpentier M, Mazurier, J. (2005): Lactoferrin: a modulator of immune and inflammatory responses.cellular and molecular life sciences, 62, 2549-2559.
21. Liuque. A. Mosquito, S. prada, A.; Durand, D, Mercado, E, Contreras, C, (2010) susceptibility, mechanisms of resistance and virulence factors of shigella strains isolate peruvian children, 59th ASTMH Annual meeting. Atlanta EE. UU.
22. Oral M. Conneely; phD (2001) Antiinflammatory activities of Lactoferrin, Journal of the American college of nutrition, Vol 20, No., 90005, 389s- 395s.
23. Ors: N. (2004). The antimicrobial activity of Lactoferrin: current status and perspectives. Biometals, 17, 189-196.
24. Susan Mosquito, Gianina Zegarra, Claudia Villanueva, Joaquin Ruiz, Theresa J. Qchoa (2012) Effect of bovine lactoferrin on the minimum inhibitory Concentrations of ampicillin and trimethoprim-sulfamethoxazole for clinical ***Shigella*** spp. Strains, Biochemistry and cell Biology, 90(3): 412-416-10.1139/o 11-o 66.
25. WHO. (2004) Antibiotics in the management of shigellosis. WHO weekly Epidemiological(9): 355-356.
26. Will Brink (2000) LE Magazin, October 2000. Report: Lactoferrin: The Bioactive Peptide that fights diseases. file://G:\oct2000-report Lactoferrin.htm
27. Willer, E. dam. Lima R. del, and Giugliano L. G. (2004) In vitro adhesion and invasion inhibition of ***Shigella dysenteriae***, ***Shigella flexneri:*** and ***Shigella sonni:*** clinical strains by human milk proteins. BMC Microbiol. 4,18.