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ORIGINAL RESEARCH

Lactoferrin versus Long-Acting Penicillin in Reducing Elevated Anti-Streptolysin O Titer in Cases of Tonsillopharyngitis

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Background: Beta-Hemolytic streptococci are the most frequent bacteria causing tonsillitis. Lactoferrin may play a role in the treatment of chronic tonsillitis due to its direct antimicrobial activity.

Objective: To assess the possible role of lactoferrin in reduction of raised serum Anti-Streptolysin O Titer (ASOT) in cases of chronic tonsillopharyngitis in comparison to long acting penicillin.

Methods: This study included 117 children with tonsillopharyngitis with high ASOT randomly divided into three groups; group 1 treated with lactoferrin, group 2 treated with long acting penicillin and group 3 treated with both drugs. For all patients ASOT was measured after three and six months of starting treatment.

Results: This study included 60 males and 57 females with the mean age (8.5 ± 2.4). There is statistically significant reduction in ASOT in all groups after three months of treatment. ASOT after 3 months was significantly lower in group1 (370±440) and group 3 (350±450) in comparison to group 2 (420±560) with p value 0.02, 0.004, respectively, with no significant difference in comparing group 1 to group 3 p value 0.4. Also, ASO titre after 6 months was significantly lower in group1 (350±420) and group 3 (340±440) in comparison to group 2 (420±550) with p value 0.02, 0.007, respectively, with no significant difference in comparing group 1 to group 3 p value 0.5. In comparing ASOT at three months and six months of treatment in the three studied groups; it decreased by 2% in group 1, and 1.6% in group 3 and no change in group 2.

Conclusion: Lactoferrin alone or in combination with long acting penicillin is safe and more effective than long acting penicillin alone in reducing ASOT. Treatment for six months with lactoferrin alone or in combination with long acting penicillin could offer a better response.

Keywords: lactoferrin, long-acting penicillin, anti-streptolysin O titer, tonsillopharyngitis

Introduction

Throat infection is one of the most frequent health problems. Tonsillitis is one of the most severe throat infections.[1–3] Chronic tonsillitis is defined as repeated attacks of acute tonsillitis with or without enlargement, and it is characterized by five or more episodes of true tonsillitis per year, which is common among children.[4,5] Numerous numbers of bacteria can cause tonsillitis but Beta-Hemolytic streptococci are the most common.[6,7] ASOT has been used to diagnose Beta Hemolytic Streptococcal infection. It is not uncommon for laboratory personnel and physicians to misinterpret streptococcal antibody titers because of a failure to appreciate that the normal levels of these antibodies are higher among school-age children than among adults.[8]

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Tonsillectomy is the most common surgical procedures for the treatment of chronic tonsillitis in children, it is valuable in reducing the duration and number of attacks of sore throat in children.[9] However, pediatricians prefer long-acting penicillin in treating children with chronic tonsillitis. The American Heart Association, the American Academy of Pediatrics, and the Infectious Diseases Society of America have always recommended either a monthly ten days course of oral penicillin V (2 to 4 times a day) or a monthly single intramuscular injection of benzathine benzyl penicillin as the mainstay therapy in patients without penicillin allergy.[10] Human lactoferrin (LF) is a cationic glycosylated protein consisting of 691 amino acids[11] folded into two globular lobes (80 kDa bi-lobal glycoprotein),[12,13] that are connected by a-helix[14,15] LF was first isolated from bovine milk in 1939,[16] other secretions contain lactoferrin, such as tears and saliva.[17] It is secreted from neutrophils in the blood and inflamed tissues. It has a direct antimicrobial role, as it limits the proliferation and division of bacteria, viruses and parasites, and kills them.[18] Increased level of plasma LF concentration has been suggested to be a predictive indicator of sepsis-related morbidity and mortality.[19] LF also modulates the differentiation of immune system cells, by increasing natural killer (NK) cell activity.[8,20] Studies done by Arnold et al and Velliyagounder et al have shown that LF has bactericidal effect against *S. mutans*.[21,22] In this study, we try to assess the possible role of LF in reducing ASOT and subsequently the recurrence of tonsillitis.

Aim of the Work

To assess the possible role of lactoferrin in decreasing raised serum Anti-Streptolysin O Titer (ASOT) in cases of chronic tonsillopharyngitis in comparison to long acting penicillin.

Patients and Methods

This prospective study was done on 117 children suffering from recurrent tonsillo-pharyngitis with high level of anti-streptococcal antibodies (ASOT) (>400 Todd units), age ranges from 4 to 14 years with mean age 8.5 ± 2.4 years. Informed consent from parents of all patients was taken. An institutional ethical committee approval, Faculty of Medicine, South Valley University, Qena, Egypt, was taken (Ethical approval code: SVU cod 12/54/2020).

Full history taking and clinical examination were done. Subjects were diagnosed by fever, sore throat, vomiting, and headache, with or without tender anterior cervical nodes and signs of inflammation of the tonsils and/or pharynx. The children were classified into 3 groups. Group (1) included 39 children had been treated with lactoferrin 100 mg once daily for 6 months. Group (2) included 39 children had been treated with long acting penicillin intramuscular injection of 1.200.000 IU for children >25 KG and 600.000 IU for those <25 KG every 2 weeks after sensitivity testing every time for 6 months. Group (3) was treated with both long acting penicillin and lactoferrin for 6 months. All patients were followed up for any side effects related to lactoferrin and/or long acting penicillin during the study period. ASOT was assessed at presentation, 3 months and 6 months of treatment for all included patients.

Blood Sample for Laboratory Investigations

Four mL venous blood was collected into a plain tube for serum collection: Blood was allowed to clot for 15 minutes at 37°C and serum was separated by centrifugation at 3000 rpm for 10 minutes then collected serum was inspected to ensure that it was clear and non-hemolyzed or lipemic.

By using (Stat Fax 4700 – awareness technology INC – Palm City - USA) ELISA analyzer quantitative Hs CRP High sensitivity CRP level will be measured by ELISA (solid phase enzyme linked immunosorbent assay) using a Chemux Bioscience, inc kit catalog No. 10603.

ASOT was measured by rapid latex agglutination intended for semi quantitative determination of ASOT in serum using the Avitex ASO test kit. Avitex ASO latex particles are coated with purified and stabilized streptolysin-O. Sera were stored at 2°C to 8°C for up to 48 hours prior to testing by Latex serology test for detection of Streptococcal Antibodies. The reagent was brought to room temperature and mixed gently prior to use. Isotonic saline was then used to prepare serial dilutions of the patient sera (1/2, 1/4, 1/8, and so on). A drop of each serum dilution was then transferred to the test circle on the slide and another drop of the reagent was added to each circle and mixed with the diluted sera. Gently and evenly, the test slide was rocked and rotated for 2 minutes whilst examining the test slide for agglutination. The serum ASO concentration was then calculated by multiplying the dilution factor (ie, 2, 4, 8 …) by the detection limit (200) to give the number of IU/mL concentration.[14]

The serum ASO concentration could then be calculated approximately by multiplying the dilution factor (ie, 2, 4, 8 or 16) by the detection limit. Kit controls were tested with each test run. Sera having titers between 200 lU/mL and 3500 lU/mL were reactive. This method was chosen because it is the widely used technique for ASOT measurement in Egypt. The study was explained to the parents of the patients, written consent was given before enrollment, and the study was approved by the institutional ethical committee.

All routine investigations were done to exclude chronic diseases, and other inflammatory or infectious diseases that affect ASO titre.

Complete Blood Count and ESR

Two mL of venous blood were collected into an ethylene di-amine-tetra-acetic acid (EDTA) tube.

The blood sample was analyzed for CBC within 4 hours after collection, using (XN sampler unit SA-01 – Sysmex corporation - Japan) Automated cell counter and the reference values were: RBCs:3.8–5.2 $\times 10^6/\mu L$, HB:11.5–15.2 g/dl, HCT: 35.0–46.0%, MCV: 77.0–97.0 Fl, MCH: 26.0–34.0 pg., MCHC: 32.0–35.0 g/dl, WBC: 3.5–10.0 $\times 10^3/\mu L$, Neut.: 1.6–7.0$\times 10^3/\mu L$ (40–73%), Lymph.:1.0–3.0$\times 10^3/\mu L$ (18–45%), Mono.: 0.2–0.8 $\times 10^3/\mu L$ (4–12%), PLT: 150–400 $\times 10^3/\mu L$. MPV: 8.0–11.0 FL, PCT: 0.15–0.40 $\times 10^3/\mu L$, PDW:11.0–22.0 FL, P-LCR: 18.0–50.0%.

The blood sample was analyzed for ESR within 2 hours at room temperature. Using the Westergren method, 2 mL of the EDTA blood was added to a tube containing 0.2 mL of tri-sodium citrate. Then, the blood was put in the ESR tube to the 100 mm mark. The tube was placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The result is expressed as millimeters after 1 hour and 2 hours.

Complete blood count, erythrocyte sedimentation rate (ESR), C reactive protein (CRP) were done at presentation and ASOT were done at presentation and repeated at 3 months and 6 months. Venous blood samples were collected from the studied children at presentation, 3 months and 6 months.

The serum ASO concentration was then calculated by multiplying the dilution factor (ie, 2, 4, 8 …) by the detection limit (200) to give the number of IU/mL concentration.[14]

Statistical Analysis

Data are managed and analyzed using statistical package for social sciences (spss) version 26. Descriptive statistics will be done in the form of frequencies, median and range, then analytic statistics will be done using non parametric tests such as Mann Whitney to compare 2 quantitative variables, Krauskall Wallis to compare 3 quantitative variables and Wilcoxon test to compare 2 paired samples. Values will be considered significant when the P value equal or less than 0.05.

Our study complies with the Declaration of Helsinki.

Results

This study included 117 children aged between 4 and 14 years. The children were presented with signs and symptoms suggestive of streptococcal pharyngitis and history of recurrent tonsillitis and high ASOT level. The patients mean age was 8.5 ± 2.4 with 60 males (51.3%) and 57 females (48.7%). There were no reported side effects or complications from lactoferrin or long acting penicillin during the study Table 1.

The patients were classified into 3 groups, group (1) included 39 children mean age was 8.4± 2.7 with 19 males and 20 females treated with lactoferrin, group (2) included 39 children mean age was 7.8 ± 2.4 with 21 male (53.8%) and 18 females (51.3%) treated with long acting penicillin, group (3) included 39 patients mean age was 9 ± 1.6 with 20 males (46.2%) and 19 females (48.7%) treated with combination of the two drugs. All groups were age and sex matched Table 1.

There was no significant difference between the three studied groups regarding ASOT at the time of enrollment. Group (1) ASOT at the time of presentation (median ± range) was 610±2955, and in group (2) it was 720±2420 with p value 0.3 which is not statistically significant, group (3) ASOT at presentation was 700±2510 with no statistically significant difference with other groups.

Table 1 Demographic Data of All Studied Patients

Variable	Group	Frequency	P value
Group frequency	Lactoferrin	39	
	Penicillin	39	
	Penicillin plus lactoferrin	39	
Age	Lactoferrin	8.4 ±2.7	0.08
	Penicillin	7.8 ±2.4	
	Penicillin plus lactoferrin	9 ±1.6	
Male sex	Lactoferrin	19 (48.7%)	0.9
	Penicillin	21 (53.8%)	
	Penicillin plus lactoferrin	20 (51.3%)	
Female sex	Lactoferrin	20 (51.3%)	
	Penicillin	18 (46.2%)	
	Penicillin plus lactoferrin	19 (48.7%)	

After three months of treatment, ASOT showed statistically significant lower titer in group 1 (370 ± 440) and in group 3(350±450) than in group 2 (420±560) with p-value 0.02, 0.004, respectively. While there was no statistically significant difference in ASOT between group (1) and group (3).

After six months of treatment, ASOT showed statistically significant lower titer in group 1 (350±420) and in group 3 (340±440) than in group 2 (420±550) with p-value 0.02, 0.007, respectively. While there was no statistically significant difference in ASOT between group (1) and group (3) Table 2.

In comparing ASOT at the time of presentation and after three months of treatment: in group 1(lactoferrin) it was 610 ±2955 and reduced to 370±440 with 54% decrease and p value 0.000 which is highly statistically significant reduction. In group 2 (long acting penicillin) ASOT was 720±2420 reduced to 420±560 with 51% decrease and p value 0.000 which is highly statistically significant reduction. In group 3 (lactoferrin and long acting penicillin) it decreased from 700±2510 to 350±450 with 59% decrease and p value 0.000 which is statistically significant reduction Table 2, which means that all the three methods of treatment are effective in reducing ASOT.

In comparing ASOT at presentation and its level after six months of treatment, it showed marked decrease. In group 1 (lactoferrin) ASOT decreased from 610±2955 to 350±420 with 55% decrease and highly statistically significant p value. Group 2(long acting penicillin) showed statistically significant reduction in ASOT from 720±2420 to 420±550 presenting 51% decrease with p value 0.000. Also, group 3 (lactoferrin and long acting penicillin) showed highly statistically significant reduction in ASOT level from 700±2510 to 340±440 presenting 60% decrease and p value 0.000 Table 3.

Table 2 Comparision Between Serum Level of ASOT Pre and Three Months Post Treatment and Six Months Post Treatment in the Three Studied Groups

Variable	Lactoferrin (1)	Long Acting Penicillin (2)	Lactoferrin +Penicillin (3)	P value (1&2)	P value (1&3)	P value (2&3)	P value Krauskal wills
Pre-treatment ASOT (median±range)	610±2955	720±2420	700±2510	0.3	0.2	0.9	0.3
Post- treatment ASOT 3 months (median±range)	370±440	420±560	350±450	0.02*	0.4	0.004*	0.008*
Post- treatment ASOT 6 months (median±range)	350±420	420±550	340±440	0.02*	0.5	0.007*	0.01*

Note: *Means significant.

Table 3 Percentage of Change in ASOT After Three Months and Six Months of Treatment in the Three Studied Groups

	ASOT Pre- (1)	ASOT Post-3 (2)	ASOT Post-6 (3)	Change After 3ms	Change After 6ms	Change From 3 to 6 ms.	P value 1 and 2	P value 1 and 3	P value 2 and 3
Lactoferrin	610±2955	370± 440	350± 420	54% decrease	55% decrease	2% decrease	0.000*	0.000*	0.000*
Penicillin	720±2420	420± 560	420± 550	51% decrease	51% decrease	No change	0.000*	0.000*	0.1
Penicillin plus lactoferrin	700±2510	350± 450	340± 440	59% decrease	60% decrease	1.6% decrease	0.000*	0.000*	0.000*

Note: *Means significant.

In comparing between ASOT after three months of treatment and six months post treatment in the three studied groups, group 1(lactoferrin) ASOT was 370±440 after three months of treatment reduced to 350±420 with 2% decrease (p-value 0.000). In group 2 (long acting penicillin) it was reduced from 420±560 to 420±550 with no statistically significant difference (p-value 0.1). In group 3 (lactoferrin and long acting penicillin) ASOT after three months of treatment was 350±450 and after six months was 340±440 with 1.6% decrease (p value 0.000) Table 3.

Discussion

Penicillin is still the treatment of choice for chronic tonsillitis; aminopenicillins were effective in treatment of beta-lactamase producing bacteria.[23] Beta-lactam antibiotics can protect against rheumatic fever and glomerulonephritis, arthritis, myocarditis and death.[24] Lactoferrin is known to have antiviral,[25–28] antibacterial,[29–34] anti-inflammatory,[35] antifungal,[36–38] and anti-carcinogenic[39] activity. One of its properties is the ability to limit iron availability to microbes.[40]

A study was done on patients with acute tonsillitis, to reveal whether lactoferrin (human Lf) binds to Streptococcus pyogenes or not. They reported that human lactoferrin may have a role in the treatment of acute tonsillitis in several ways; by binding to the S. pyogenes pathogens, also by its well-known iron-binding capacity.[41]

Another study done by Velusamy et al 2014 revealed that human lactoferrin treated mice exhibited lower levels of S. mutans CFU (colony forming unit) compared to the non-human lactoferrin treated infected group of mice.[42]

This study included 60 males (51.3%) and 57 females (48.7%) with tonsillopharyngitis, this is in agreement with Lin et al who found that group A streptococcal pharyngitis was slightly higher incidence in boys. But Nirmal Kushwaha found that male to female in patients with tonsillopharyngitis was 1:1.12 (53% female).[43] This study included 117 children with tonsillopharyngitis aged between 4 and 14 years this is in agreement with Fadwa et al 2014, who reported that tonsillopharyngitis is more common in children.[44]

In this study, all treatment regimens showed reduced ASOT at three and six months of treatment. Kumar and Kumari, in 2019, compared the use of long acting penicillin for six months to tonsillectomy for treatment of patients with raised ASOT. They concluded that both long acting penicillin and tonsillectomy reduced ASOT but surgery is significantly better in reduction of ASOT.[45]

In this study, lactoferrin group and both lactoferrin and long acting penicillin group showed significantly lower levels of anti-streptolysin O than patients received long acting penicillin alone after three and six months treatment. Also, this study showed that use of lactoferrin alone or in combination with long acting penicillin for six months induced significant reduction in ASOT in comparison to three months treatment. On the other hand, using long acting penicillin alone for six months could not induce a significant reduction in ASOT in comparison to three months use which questions the value of its use for more than three months. In this study, no side effects or complications of lactoferrin use were reported.

A human study done by Benson et al 2012 found that nutritional supplementation with colostrum was equally efficient in preventing episodes of the flu compared to a vaccine. They concluded that lactoferrin is effective in promoting mammalian cell growth and increasing cell productivity. Human lactoferrin is safe and is considered by the FDA as a GRAS (generally recognized as safe) product with no contraindications in either pediatric or adult patients.[46–50]

So, the use of lactoferrin alone or in addition to long acting penicillin could represent a safe and better option for the treatment of chronic tonsillopharyngitis with raised ASOT.

Conclusion

Lactoferrin alone or in combination with long acting penicillin is safe and more effective than long acting penicillin alone in reducing ASOT. Treatment for six months with lactoferrin alone or in combination with long acting penicillin could offer a better response.

Recommendations

We recommend giving combined long acting penicillin with lactoferrin in patients with high ASOT for three months.

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To members of Ear, Nose and Throat Department, clinical and chemical pathology department, Faculty of Medicine, South Valley University, Egypt.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Lau AS, Upile NS, Wilkie MD, Leong SC, Swift AC. The rising rate of admissions for tonsillitis and neck space abscesses in England, 1991–2011. *Annals Royal Coll Surg Engl*. 2014;96(4):307–310. doi:10.1308/003588414X13946184900363
2. Klug TE. Peritonsillar abscess: clinical aspects of microbiology, risk factors, and the association with parapharyngeal abscess. *Clin Infect Dis*. 2009;49:1467–1472.
3. Borgström A, Nerfeldt P, Friberg D, Sunnergren O, Stalfors J. Trends and changes in paediatric tonsil surgery in Sweden 1987–2013: a population-based cohort study. *BMJOPEN*. 2017;7(1):e013346.
4. Brook I, Gober AE. Increased recovery of Moraxella catarrhalis and Haemophilus influenzae in association with group a b-haemolytic streptococci in healthy children and those with pharyngotonsillitis. *J Med Microbiol*. 2006;55:998–1092. doi:10.1099/jmm.0.46325-0
5. Hammouda M, Abdel-Khalek Z, Awad S, Abdel-Aziz M, Fathy M. Chronic tonsillitis bacteriology in Egyptian children including antimicrobial susceptibility. *Aust J Basic Appl Sci*. 2009;3:1948–1953.
6. Burton MJ, Glasziou PP. Tonsillectomy or adenotonsillectomy versus nonsurgical treatment for chronic/recurrent acute tonsillitis. *Cochrane Database Syst Rev*. 2009;CD001802. doi:10.1002/14651858.CD001802.pub2
7. Awad Z, Al-Yaghchi C, Anwar M, Georgalas C, Narula A. Does tonsillectomy help children with recurrent tonsillitis? *Otolaryngol Head Neck Surg*. 2010;143(l):113–116. doi:10.1016/j.otohns.2010.06.207
8. Kaplan EL, Rothermel CD, Johnson DR. Antistreptolysin O and anti-deoxyribonuclease B titers: normal values for children ages 2 to 12 in the United States. *Pediatrics*. 1998;101:86–88. doi:10.1542/peds.101.1.86
9. Millington AJ, Phillips JS. Current trends in tonsillitis and tonsillectomy. *Annals Royal Coll Surg Engl*. 2014;96(8):586–589. doi:10.1308/003588414X13946184901966
10. Dajani A, Taubert K, Ferrieri P, et al. Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: a statement for health professionals. *Pediatrics*. 1995;96(4):758–764. doi:10.1542/peds.96.4.758
11. Anderson BF, Baker HM, Norris GE, Rumball SV, Baker EN. Apolactoferrin structure demonstrates ligand-induced conformational change in transferrins. *Nature*. 1990;344:784–787. doi:10.1038/344784a0
12. Vogel HJ. Lactoferrin, a bird's eye view. *Biochem Cell Biol*. 2012;90:233–244. doi:10.1139/o2012-016
13. Karav S, German JB, Rouquié C, Le Parc A, Barile D. Studying lactoferrin N-glycosylation. *Int J Mol Sci*. 2017;18:E870. doi:10.3390/ijms18040870
14. Karav S. Selective deglycosylation of lactoferrin to understand glycans'contribution to antimicrobial activity of lactoferrin. *Cell Mol Biol*. 2018;64:52–57. doi:10.14715/cmb/2018.64.9.8
15. Moore SA, Anderson BF, Groom CR, Haridas M, Baker EN. Threedimensional structure of diferric bovine lactoferrin at 2.8A resolution. *J Mol Biol*. 1997;274:222–236. doi:10.1006/jmbi.1997.1386
16. Sorensen M, Sorensen S. *Report of the Works of the Carlsberg Laboratory*. Copenhagen 48- Lactoferrin: industrial production and applications. Rev Mex Pharmaceutical Sciences; 1939.
17. Van Der Strate BWA, Belijaars L, Molema G, Harmsen MC, Meijer DKF. Antiviral activities of lactoferrin. *Antiviral Res*. 2001;52:225–239. doi:10.1016/S0166-3542(01)00195-4
18. Valenti A, Antonini G. Lactoferrin: an important host defense against microbial and viral attack. *Cell Mol Life Sci*. 2005;62:2576–2587. doi:10.1007/s00018-005-5372-0
19. Damiens E, El Yazidi I, Mazurier J, et al. Role of heparan sulphate proteoglycans in the regulation of human lactoferrin binding and activity in the MDA-MB-231 breast cancer cell line. *Eur J Cell Biol*. 1998;77:344–351. doi:10.1016/S0171-9335(98)80093-9
20. Shau H, Kim A, Golub SH. Modulation of natural killer and lymphokine-activated killer cell cytotoxicity by lactoferrin. *J Leukoc Biol*. 1992;51:343–349. doi:10.1002/jlb.51.4.343
21. Arnold RR, Russell JE, Champion WJ, Gauthier JJ. Bactericidal activity of human lactoferrin: influence of physical conditions and metabolic state of the target microorganism. *Infect Immun*. 1981;32:655–660. doi:10.1128/iai.32.2.655-660.1981
22. Velliyagounder K, Kaplan JB, Furgang D, et al. One of two human lactoferrin variants exhibits increased antibacterial and transcriptional activation activities and is associated with localized juvenile periodontitis. *Infect Immun*. 2003;71:6141–6147. doi:10.1128/IAI.71.11.6141-6147.2003

23. Sidell D, Shapiro NL. Acute tonsillitis. *Infect Disord Drug Targets*. 2012;12(4):271–276. doi:10.2174/187152612801319230
24. Demir UL, Cetinkaya B, Karaca S, Sigirli D. The impacts of adenotonsillar Hypertrophy on periodontal health in children: aprospective controlled pilot study. *Am J Otolaryngol*. 2013;34(5):501–504. doi:10.1016/j.amjoto.2013.04.013
25. Lang J, Yang N, Deng J, et al. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. *PLoS One*. 2011;6:e23710. doi:10.1371/journal.pone.0023710
26. Redwan EM, Uversky VN, El-Fakharany EM, Al-Mehdar H. Potential lactoferrin activity against pathogenic viruses. *C R Biol*. 2014;337:581–595. doi:10.1016/j.crvi.2014.08.003
27. Chen JM, Fan YC, Lin JW, Chen YY, Hsu WL, Chiou SS. Bovine lactoferrin inhibits dengue virus infectivity by interacting with heparan sulfate, low density lipoprotein receptor, and DC-SIGN. *Int J Mol Sci*. 2017;18:E1957. doi:10.3390/ijms18091957
28. Carvalho CAM, Casseb SMM, Goncalves RB, Silva EVP, Gomes AMO, Vasconcelos PFC. Bovine lactoferrin activity against Chikungunya and Zika viruses. *J Gen Virol*. 2017;98:1749–1754. doi:10.1099/jgv.0.000849
29. Rosa L, Cutone A, Lepanto MS, Paesano R, Valenti P. Lactoferrin: a natural glycoprotein involved in iron and inflammatory homeostasis. *Int J Mol Sci*. 2017;18:1985. doi:10.3390/ijms18091985
30. Petrik M, Zhai C, Haas H, Decristoforo C. Siderophores for molecular imaging applications. *Clin Transl Imaging*. 2017;5:15–27. doi:10.1007/s40336-016-0211-x
31. Beddek AJ, Schryvers AB. The lactoferrin receptor complex in Gram negative bacteria. *Biometals*. 2010;23:377–386. doi:10.1007/s10534-010-9299-z
32. Pogoutse AK, Moraes TF. Iron acquisition through the bacterial transferrin receptor. *Crit Rev Biochem Mol Biol*. 2017;52:314–326. doi:10.1080/10409238.2017.1293606
33. Wandersman C, Stojiljkovic I. Bacterial heme sources: the role of heme, hemoprotein receptors and hemophores. *Curr Opin Microbiol*. 2000;3:215–220. doi:10.1016/S1369-5274(00)00078-3
34. Huang W, Wilks A. Extracellular heme uptake and the challenge of bacterial cell membranes. *Annu Rev Biochem*. 2017;86:799–823. doi:10.1146/annurev-biochem-060815-014214
35. Lepanto MS, Rosa L, Paesano R, Valenti P, Cutone A. Lactoferrin in aseptic and septic inflammation. *Molecules*. 2019;24:1323. doi:10.3390/molecules24071323
36. Fernandes KE, Carter DA. The antifungal activity of lactoferrin and its derived peptides: mechanisms of action and synergy with drugs against fungal pathogens. *FrontMicrobiol*. 2017;8:2. doi:10.3389/fmicb.2017.00002
37. Liao H, Liu S, Wang H, Su H, Liu Z. Enhanced antifungal activity of bovine lactoferrin-producing probiotic Lactobacillus casei in the murine model of vulvovaginal candidiasis. *BMC Microbiol*. 2019;19:7. doi:10.1186/s12866-018-1370-x
38. Andrés MT, Acosta-Zaldívar M, Fierro JF. Antifungal mechanism of action of lactoferrin: identification of H+-ATPase (P3A-type) as a new apoptotic cell membrane receptor. *Antimicrob Agents Chemother*. 2016;60:4206–4216. doi:10.1128/AAC.03130-15
39. Wang B, Timilsena YP, Blanch E, Adhikari B. Lactoferrin: structure, function, denaturation and digestion. *Crit Rev Food Sci Nutr*. 2019;59:580–596. doi:10.1080/10408398.2017.1381583
40. Nairz M, Schroll A, Sonnweber T, Weiss G. The struggle for iron – a metal at the host-pathogen interface. *Cell Microbiol*. 2010;12:1691–1702. doi:10.1111/j.1462-5822.2010.01529.x
41. Eric Stenfors L, Marie Bye H, Vorland LH, Nord H. Remarkable attachment of lactoferrin to Streptococcus pyogenes during acute pharyngotonsillitis. *Acta Otolaryngol*. 2001;121(5):637–642. doi:10.1080/00016480117451
42. Velusamy SK, Fine H, Velliyagounder K. Prophylactic effect of human lactoferrin against Streptococcus mutans bacteremia in lactoferrin knockout mice. *Microbes Infect*. 2014;16(9):762–767. doi:10.1016/j.micinf.2014.07.009
43. Kushwaha N, Kamat M, Banjade B, et al. Prevalence of group-a streptococcal infection among school children of urban community. *IJIMS*. 2014;1(5):249–256l.
44. Abd Al-Kareem F, Abbas A, Hussein M. Comparative study of the Antibody Responses to Streptococcus pyogenes between school children carriers and patients with Tonsillitis. *Iraqi J Sci*. 2014;55(2A):403–410.
45. Kumar A, Kumari N. Evaluate the effectiveness of tonsillectomy and long-acting penicillin on the levels of the antistreptolysin O titer in children with recurrent tonsillitis. *Int J Res Med Sci*. 2019;7(5):1692–1695. doi:10.18203/2320-6012.ijrms20191660
46. Benson KF, Carter SG, Patterson KM, Patel D, Jensen GS. A novel extract from bovine colostrum whey supports anti-bacterial and anti-viral innate immune functions in vitro and in vivo. *Prev Med*. 2012;54:116–123. doi:10.1016/j.ypmed.2011.12.023
47. Yoshioka Y, Kudo S, Nishimura H, et al. Oral administration of bovine colostrum stimulates intestinal intraepithelial lymphocytes to polarize Th1-type in mice. *Int Immunopharmacol*. 2005;5:581–590. doi:10.1016/j.intimp.2004.11.005
48. Drago ME. Lactoferrina: producción industrial y aplicaciones. *Rev Mex Ciencias Farmacéuticas*. 2007;38:30–38.
49. Goldman IL, Deikin AV, Sadchikova ER. Human lactoferrin can be alternative to antibiotics. In: Proceedings of the World Medical Conference. 2010:27–38.
50. Leon N, Reyes M, Ordaz C, de la Garza M. Microbicidal action of lactoferrin and lactoferricin and their synergistic effect with metronidazole in Entamoeba histolytica. *Biochem Cell Biol*. 2006;84:327–336. doi:10.1139/o06-060