

## Lactoferrin and its nano-formulations in rare eye diseases

Jiya Singh, Mohita Sharma<sup>1</sup>, Neha Jain<sup>2</sup>, Insha Aftab<sup>1</sup>, Naval Vikram<sup>3</sup>, Tej P Singh, Pradeep Sharma, Sujata Sharma

Lactoferrin (LF) is an iron-binding glycoprotein released from mucous secreting cells and neutrophils. LF can be used in a broad range of eye diseases related to the retina, cornea, and optic nerve. The retina is particularly affected by oxidative stress inside the photoreceptor being constantly exposed to light which induces accumulation of reactive oxygen species (ROS) in the retinal pigmented epithelium (RPE) causing damage to photoreceptor recycling. Retinitis pigmentosa (RP) and macular degeneration are inherited retinopathies that consist of different disease-causing genes, that cause mutations with highly varied clinical consequences. Age-related macular degeneration is a chronic disease of the retina and one of the major causes of sight loss. This review provides an application of lactoferrin and LF-based nano-formulations or nanoparticles in the field of retinal diseases or corneal diseases such as retinitis pigmentosa, retinoblastoma, age-related macular degeneration (AMD), keratoconus and uveitis. Several studies have found that lactoferrin's antibacterial activity is not limited to its iron sequestration, but also its ability as a nanoparticle that acts as a carrier to deliver drugs by crossing the blood-retina barrier (BRB) and its involvement in cell cycle control, which is not possible by many transferrin proteins.

**Key words:** Corneal diseases, lactoferrin, macular degeneration, nanoparticles, retinal diseases, retinitis pigmentosa, retinoblastoma

The eye is a highly metabolically active structure composed of a cornea, lens, retina, and optic nerve.<sup>[1]</sup> Retinitis pigmentosa (RP), retinoblastoma (RB), and Usher syndrome are considered rare eye diseases<sup>[2-4]</sup> and are inherited retinal diseases. RP is a degenerative disease that leads to a partial loss of vision; it affects millions of individuals worldwide, and several diseases are included in this set of diseases.<sup>[5,6]</sup> RB is an aggressive intraocular cancer of childhood; loss of one RBL allele leads to retinoblastoma tumor.<sup>[7-11]</sup> The life-threatening white tumor reflects light and blocks view of the red retina. Leukocoria is the initial sign of RB.<sup>[12,13]</sup> The most common treatment for eye inflammation is the use of corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs), either alone or in combination. Alternatives in the treatment of eye diseases must be researched to solve these difficulties. Lactoferrin (LF) is an iron-binding glycoprotein that affects the immune system's innate and adaptive reflexes.<sup>[14]</sup> It is a multifunctional glycoprotein with a molecular weight of approx. 80 kDa, which contains about 690 amino acid residues. LF consists of two homologous globular lobes, C-lobe and N-lobe. LF structure resembles about 60% similarity to other members of iron transporting proteins and is associated with

a broad-spectrum of biological functions within the human body, such as antitumor effect, antioxidant, antimicrobial activity, cell proliferation, differentiation regulation, and antibacterial activity.<sup>[15-18]</sup> Because of its propensity to bind Fe<sup>3+</sup> ions, LF is classified as an iron chelator as a member of the transferrin family.<sup>[19,20]</sup> The production of reactive oxygen species (ROS) is also a part of the inflammatory process.<sup>[21-24]</sup> As a result, chelation may be a viable treatment method for treating inflammatory disorders by removing free iron and decreasing redox processes. Because of enhanced permeability and bioavailability, retinal active delivery via controlled release systems has gained popularity in recent years, offering distinct advantages over conventional pharmaceutical dose forms.<sup>[25]</sup> Several findings show that the production of LF nano-formulations improved cellular absorption and gene transfection even at low LF concentrations when compared to other proteins, such as LF binding to DNA encapsulated liposomes and LF enteric coating.<sup>[26,27]</sup> LF nanoparticles were previously reported in the treatment of retinal diseases due to their ability to cross the blood-retina barrier (BRB). Once LF attaches to its receptor on the cell surface, it will be easily passed across the BRB via receptor-mediated transcytosis. In various studies, subretinal injection of DNA compacted nanoparticles efficiently induces gene expression in retinal

Departments of Biophysics and <sup>3</sup>Medicine, All India Institute of Medical Sciences, New Delhi, <sup>1</sup>Tirupati Eye Centre and Research Institute, Noida, Uttar Pradesh, <sup>2</sup>The Cornea Institute, KAR Campus, L V Prasad Eye Institute, Hyderabad, Telangana, India

**Correspondence to:** Prof. Sujata Sharma, Department of Biophysics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, India. E-mail: sujatasharma.aiims@gmail.com  
Dr. Pradeep Sharma, Department of Biophysics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, India. E-mail: pradeepbdk@gmail.com

Received: 30-Jan-2021  
Accepted: 03-Jun-2022

Revision: 22-Feb-2022  
Published: 30-Jun-2022

## Access this article online

## Website:

www.ijo.in

## DOI:

10.4103/ijo.IJO\_303\_22

## Quick Response Code:



This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow\_reprints@wolterskluwer.com

Cite this article as: Singh J, Sharma M, Jain N, Aftab I, Vikram N, Singh TP, et al. Lactoferrin and its nano-formulations in rare eye diseases. Indian J Ophthalmol 2022;70:2328-34.

neuronal cells and slows degenerative changes in mice with a haploinsufficiency mutation in the retinal degeneration slow gene.<sup>[28]</sup> This review discusses the nano-formulation of LF as a topical ophthalmic medication delivery method for the treatment of retinal and corneal inflammation. Furthermore, both *in vitro* and *in vivo* roles of LF nanoparticles are discussed in this review. The goal of this study was to summarize the existing nano-based knowledge on the use of LF as a potential therapeutic agent for rare eye diseases of the retina and cornea.

## Methods

LF nanoparticles were prepared using the sol-oil method<sup>[29]</sup> by dissolving 0.2% (w/v) of LF powder in phosphate buffer saline (PBS) under agitation for 1 hour at 25°C. After 1 hour of agitation, the LF solution was adjusted to different pH values (4, 7, and 10) and kept at different thermal conditions (60–90°C) with varying holding times (0–60 min) [Fig. 1]. The most favorable condition for LF nanoparticle formation was found at pH 7, holding time of 20 min at 75°C. The particle size distribution and surface morphology of LF nanoparticles were measured using dynamic light scattering (DLS) and transmission electron microscopy (TEM), respectively. For TEM analysis, LF nanoparticles were resuspended in 0.01 M PBS (pH 7.4). The particles were then placed on a carbon-coated copper grid, air-dried for 2 min and negatively stained using a 1% aqueous solution of uranyl acetate for 1 min, and samples were examined using transmission electron microscopy (TEM, Talos L120C, Thermo Fisher, Waltham, MA, USA) for morphology.

## Retinal diseases

The retina is made up of a photoreceptor layer that converts light into nerve impulses and is located in the back of the eye. The neuronal layer and the pigmented layer make up the vast bulk of retinal cells. Photoreceptors, which are made up of 95% rods and 5% cones, absorb excess light and help keep photoreceptors in the brain layer healthy.<sup>[30]</sup> Retinal pigmented epithelium (RPE) is a pigmented layer adjacent to the retina form space for new disc and phagocytosed the older one. RPE contains melanin which sequesters iron ions and provides protection against oxidative damage. The common

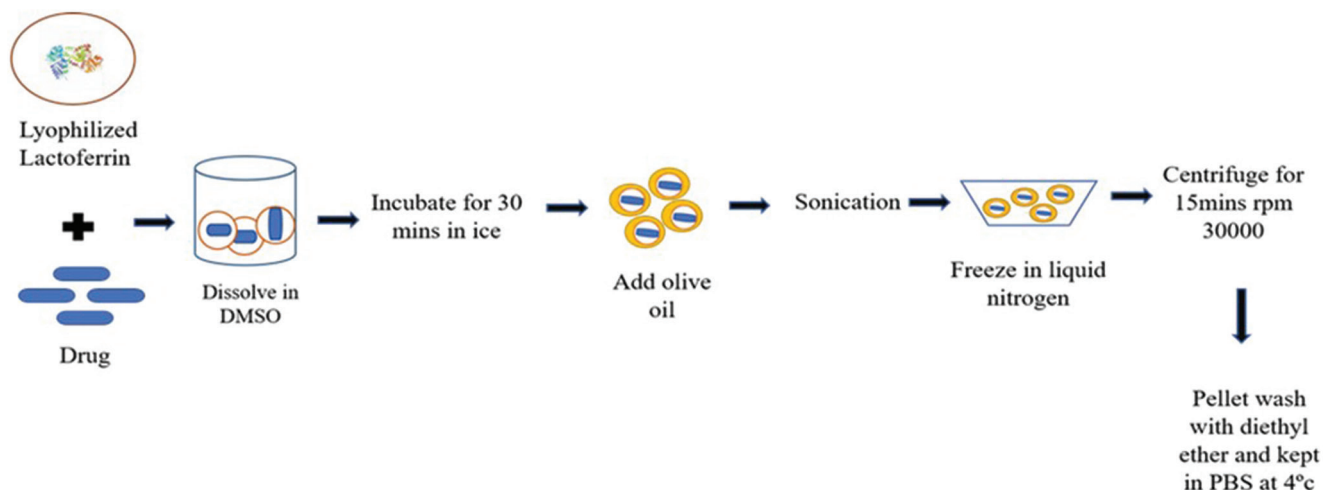
pathology of retinal disease is macular degeneration and retinitis pigmentosa.

## Lactoferrin and Retinitis pigmentosa

Retinitis pigmentosa (RP) is a condition in which photoreceptors undergo apoptosis; it is an inherited disorder that leads to progressive vision loss.<sup>[31,32]</sup> Initially, the rod photoreceptor system is affected resulting in loss of peripheral vision. In later stages, this may affect the cone photoreceptor which leads to complete vision loss.<sup>[33–35]</sup> RP is also with phenotypic heterogeneity due to different penetrance, expressivity, and interaction with oxidative stress factors<sup>[36]</sup> Oxidative stress is caused by an excessive synthesis of reactive oxygen species (ROS) and free radicals, which results in cellular malfunction, necrosis, apoptosis, or death. Normal LF levels can deplete free iron and inhibit the pro-inflammatory effects of the hydroxyl radicals LF nanoparticles which reduces oxidative stress in the retina and prevents degenerative alterations caused by photoreceptor cell degeneration by scavenging ROS.<sup>[37]</sup> Another strategy for treating retinal degeneration in several degenerative disorders, including retinitis pigmentosa, is to provide therapeutic genetic material.

## Phototransduction

Phototransduction is the process by which light impulses are transformed into electrical signals in photoreceptors (rods and cones) in the retina's outer layer.<sup>[38,39]</sup> In the retina, iron is necessary for phototransduction. Photoreceptor cells comprise of three segments: the outer part, inner part, and cell body. The main role of the inner segment is membrane trafficking along with the energy metabolism system. The outer segment is comprised of rhodopsin protein in disc membrane which absorbs photon then changes the conformation of Schiff base bond from *cis* to *trans*, hence initiating vision.<sup>[40]</sup> Desaturase, an iron-containing fatty acid enzyme, is required for the formation of new photoreceptor disc membranes. In the RPE, all-*trans*-retinal is released from opsin in the pigmented layer of the retina and gives rise to 11-*cis* retinal for continuous vision.<sup>[41]</sup> The conversion of all-*trans*-retinal to 11-*cis* retinal is accomplished by enzyme isomer hydrolase. The build-up of excess iron in photoreceptors is involved in the development of age-related macular degeneration. The iron-chelating property



**Figure 1:** Lactoferrin nanoparticle formation by using sol-oil method

of LF, in particular, helps to reduce the pro-inflammatory and tissue-damaging effects of ROS by providing oxygen-free radicals and oxygen scavenging activities. To reduce pathogen-induced inflammation, LF also suppresses classical complement activation and downregulates inflammatory mediators such as tumor necrosis factor (TNF)-alpha and interleukins (ILs).

#### Lactoferrin and macular degeneration

A chronic disease of the central retina, age-related macular degeneration (AMD) was formerly untreatable. In macular degeneration, complete loss of vision occurs at the late stages of the disease<sup>[42–45]</sup> [Fig. 2]. The pathogenesis-related to AMD is incompletely understood; certain evidence suggests that complement activation and several genetic and environmental influences, oxidative stress and iron may play important roles.<sup>[46]</sup> Iron-depleted LF is called apo-LF, iron saturated LF is known as holo-LF. The apo form of LF acts as an iron chelator because it has a higher affinity to bind to iron.<sup>[47,48]</sup> Several studies have demonstrated the role of apo LF in neurodegenerative and infectious diseases. Previous studies have demonstrated higher levels of iron in age-related macular degenerated retinas than in age-matched ones, suggesting that iron-mediated oxidative stress may cause retinal degeneration in AMD. Iron chelators play a considerable role in (AMD).<sup>[49,50]</sup> Chelation therapy is mainly used for acute iron toxicity. LF is known for its ability to cross the blood–brain barrier; LF nanoparticles can also be a different approach for the treatment of retinal disease.<sup>[27,51]</sup>

#### Lactoferrin and Retinoblastoma

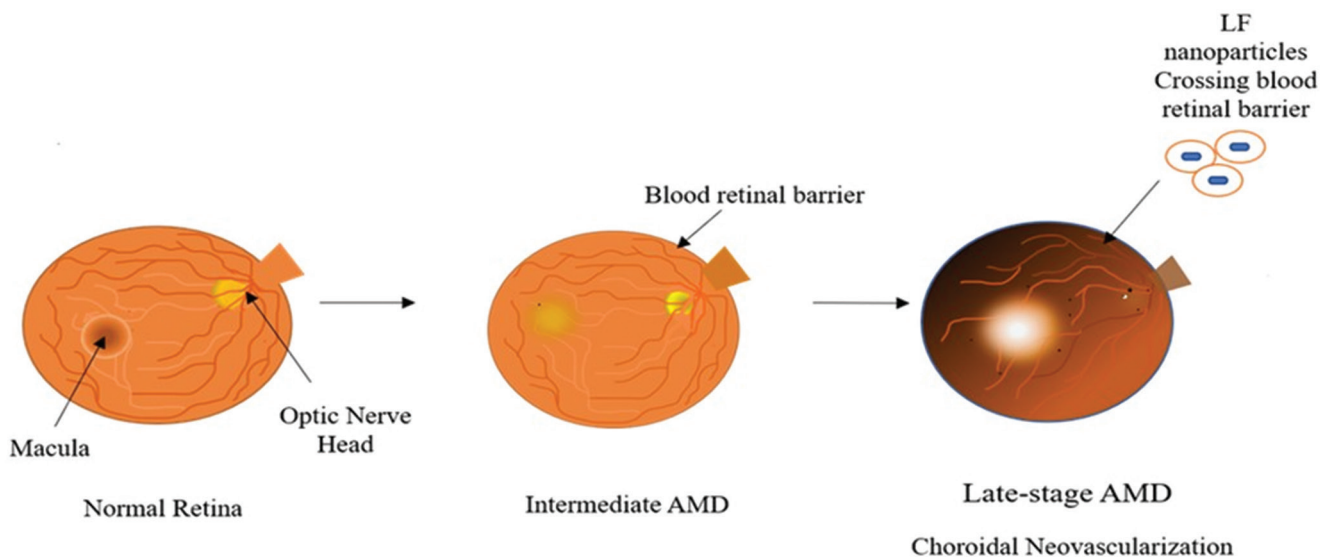
Retinoblastoma is a rare intraocular malignancy of childhood, initiated by the mutation of the RB1 gene (tumor suppressor gene) that leads to cancer.<sup>[52,53]</sup> Retinoblastoma can be bilateral or unilateral.<sup>[54]</sup> Bilateral is heritable cancer whereas unilateral is non-heritable. Leukocoria is the initial sign of retinoblastoma and strabismus is the second sign of RB<sup>[55,56]</sup> [Fig. 3]. When the tumor is small, it is thoroughly intraretinal. As it increases in size, it extends away from the vitreous cavity (exophytic) or towards it (endophytic). Retinal detachment is caused when

the tumor arises from the outer retinal layer (exophytic growth pattern) whereas the inner retinal layer results in vitreous seeding caused by endophytic retinoblastoma.<sup>[57–60]</sup> Tumor recurrence in RB after chemotherapy is common, and it can be caused by several factors, including the presence of cancer stem cells (CSCs), drug resistance, and the reactivation of retinocytoma-like regions within the tumor. Nano-formulated medicines were used to overcome drug resistance in CSCs.

Clinical grade carboplatin and etoposide were delivered using LF as a drug carrier. LF is an iron-transporting glycoprotein. Because of the increased iron need for quickly dividing metabolically active tumor cells, such as Rb Y79 cells, LF receptors are extensively expressed in tumor cells.<sup>[61,62]</sup> Oil-solution technique was used to make drug-loaded LF-NPS. It is a low-cost, simple, and quick process that does not require chemical alteration of the natural LF protein.<sup>[29]</sup> The nanoparticles were scattered evenly, had a spherical shape, and were smaller in size. According to several investigations, LF-NPS loaded with CPT and ETP showed increased drug uptake, retention, and cytotoxicity of LF-CPT (carboplatin) and LF-ETP (Etoposide) than traditional medicines. LF has been shown to impact the expression level of cell cycle regulator proteins such as cyclins and cyclin-dependent kinases (CDKs) in various investigations.<sup>[63,64]</sup> LF treatment causes an increase in CDK inhibitor p21 and a drop in cyclin E levels, resulting in G1 arrest in LF-treated cells.<sup>[65,66]</sup> While some studies demonstrate that LF inhibits E2F1 transactivation, RB binds to E2F1 more efficiently in cells that express LF, effectively blocking E2F1 expression.<sup>[67]</sup> LF anti-proliferative impact can thus be linked to higher amounts of hypophosphorylated RB.<sup>[68]</sup>

#### Lactoferrin in anterior segments of the Eye

Keratoconus, glaucoma, allergic conjunctivitis, anterior uveitis, and cataract are all common illnesses that affect the anterior portion of the eye.<sup>[69]</sup> Drug distribution to the eye remains a difficulty, despite substantial research efforts. The eye's anatomical position provides a distinct advantage for site-specific drug delivery and non-invasive disease assessment. Drug molecules should be able to



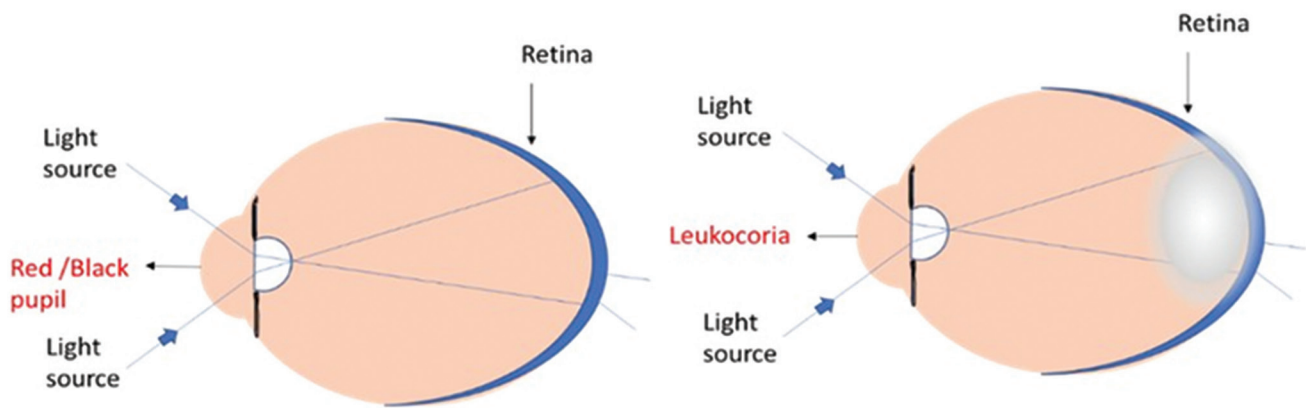
**Figure 2:** Age-related macular degeneration has different stages

bypass protective physiological barriers without causing permanent tissue damage for effective therapeutic efficacy.<sup>[70]</sup> Traditional dosage forms including solutions, suspensions, and ointments, as well as innovative dosage forms like liposomes, and nanoparticles are used to deliver drugs to the anterior segment.<sup>[71]</sup> The development of sustained-release nanocarrier systems with increased precorneal retention is a major focus of study. Such technologies can increase drug bioavailability in the eyes while also ensuring high patient compliance. Because the nano-drug was able to reach higher lens concentrations whereas the free drug was washed away by tears, nanocarriers have been claimed to be effective in the prevention and treatment of cataracts.<sup>[72]</sup> Recent research has focused on improving medication permeability across the cornea via a nanocarrier-mediated tight junction rearrangement effect.<sup>[73]</sup>

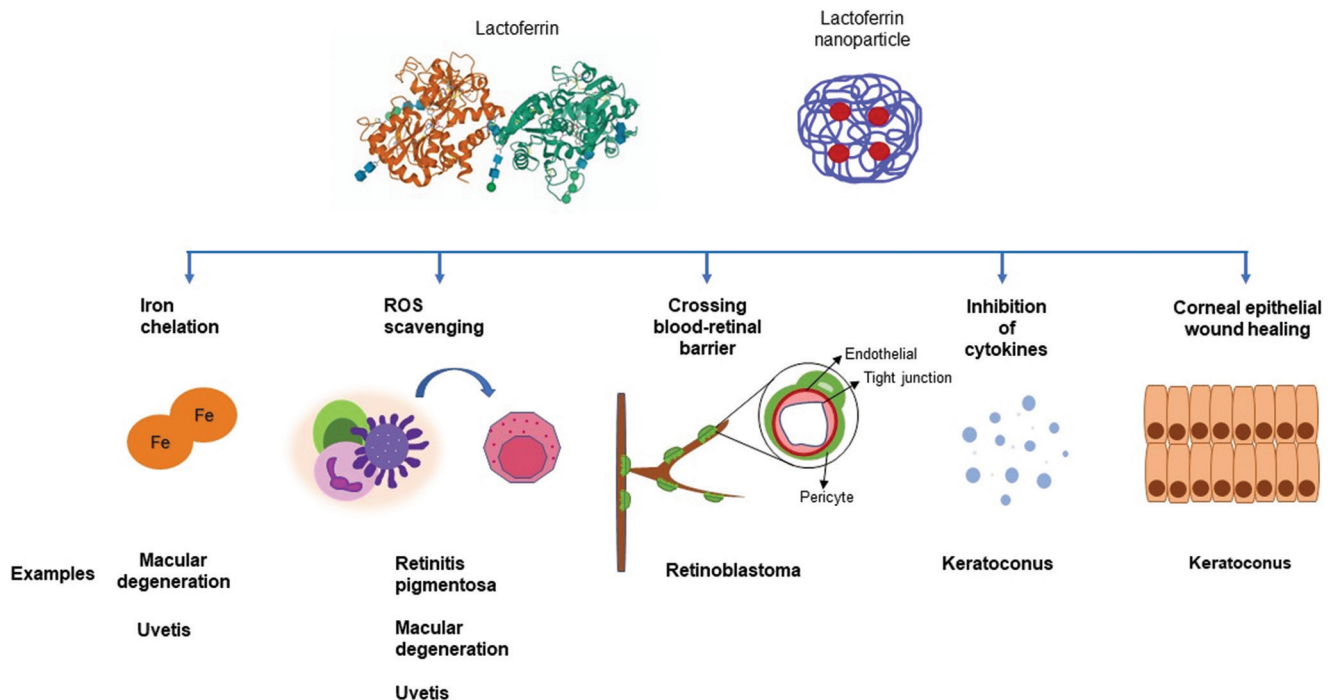
**Corneal diseases**

*Keratoconus*

Keratoconus is a progressive bilateral degenerative corneal ectasia that primarily affects young people and results in considerable visual impairment as well as a negative impact on quality of life and social well-being.<sup>[74]</sup> The worldwide prevalence of keratoconus has risen to greater rates in some countries due to the introduction of topographic and corneal tomographic techniques that allow for an earlier diagnosis.<sup>[75]</sup> No medical or pharmacological treatment can stop or delay the growth of this illness. Inflammatory and immunological mediators such as cytokines, proteases, and other chemicals are found in the breakdown of corneal tissue in progressive keratoconus.<sup>[76]</sup> LF levels in the lacrimal fluid were also shown to be lower in keratoconus individuals.<sup>[77]</sup> LF has been shown to promote corneal epithelial wound healing *in vivo*



**Figure 3:** Leukocoria



**Figure 4:** Functions of lactoferrin in various rare eye diseases



and *in vitro*. By promoting changes in humoral and cellular components, as well as inducing extracellular and intracellular signaling pathways involving toll-like receptors (TLRs), which regulate cytokine and other inflammatory mediator gene expression, LF may influence the immune system's innate and adaptive responses.<sup>[78]</sup> In the experimental conditions, a reduced LF diffusion rate is expected due to its increased molecular weight. As a result, prolonging the period of LF occupancy on the corneal surface and boosting LF penetration into the stroma is crucial. The fact that LF nanoparticles have effective physicochemical properties for topical ophthalmic administration lends support to the idea that they are retained in the corneal layer, extending drug release time from the tissue and thus ensuring patient adherence to treatment because a lower dosage frequency is required.<sup>[79,80]</sup> Furthermore, the adaptability of these systems would allow them to be included in various pharmacological forms for ocular administration based on the patient's demands and characteristics, enabling a fresh way to partially personalize and individualize the treatment plan.

### Uveitis

Uveitis, an inflammatory condition of the uveal tissues, including the iris, ciliary body, and choroid, is another target disease for nanotherapeutics in the eye.<sup>[81,82]</sup> Chronic clinical courses characterize uveitis. As a result, one of the goals in the treatment of uveitis is the control of chronic inflammation through sustained administration of therapeutic drugs.<sup>[83]</sup> Another reason is iron promotes the formation of oxygen free radicals, which can contribute to ocular inflammation, and it is also required for microbial growth.<sup>[84,85]</sup> Given the role of iron in inflammation and microbial infection, we believe that iron chelators could be used as a novel therapeutic in uveitis to assist and improve clinical outcomes.<sup>[86]</sup> The majority of *in vitro* and *in vivo* research has used bovine LF (bLF), which has significant sequence homology and activities similar to human LF. The US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) both consider bLF to be generally recognized as safe (GRAS).<sup>[87-89]</sup> Because LF can remove oxygen free radicals and hydroxyl, it could be used to cure uveitis.

### Conclusion

Lactoferrin has important anti-inflammatory and antioxidant activity which are due to high iron affinity. Iron is involved in several critical metabolic processes and can react with oxygen to form reactive oxygen species (ROS), which are primarily responsible for tissue damage in ocular inflammation. Iron chelators are thought to help remove free iron and reduce the generation of oxygen radicals, which helps to reduce inflammation. It can also stop bacteria from growing. As a result, iron chelation could be a viable new treatment option for inflammatory eye illnesses. LF has some important advantages: firstly, it is easily found in different biological fluids, and exhibits multiple beneficial effects in different pathologies such as cancer and several inflammatory diseases [Fig. 4]. Because of its capacity to pass the blood-brain barrier, LF nano-formulations or nanoparticles have been employed in various trials to deliver drugs to tumors. Efficacious treatment for many retinal illnesses has remained elusive, and enhanced efficacy for those that are treatable would be extremely desirable. LF could be used as a stand-alone treatment or in conjunction with other medications to improve efficacy.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### References

- Willoughby CE, Ponzin D, Ferrari S, Lobo A, Landau K, Omid Y. Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function—a review. *Clinical & Experimental Ophthalmology*. 2010;38:2-11.
- Ali MU, Rahman MS, Cao J, Yuan PX. Genetic characterization and disease mechanism of retinitis pigmentosa; current scenario. *3 Biotech* 2017;7:251.
- Aerts I, Lumbroso-Le Rouic L, Gauthier-Villars M, Brisse H, Doz F, Desjardins L. Retinoblastoma. *Orphanet J Rare Dis* 2006;1:31. doi: 10.1186/1750-1172-1-31.
- Nolen RM, Hufnagel RB, Friedman TB, Turrieff AE, Brewer CC, Zalewski CK, *et al.* Atypical and ultra-rare Usher syndrome: A review. *Ophthalmic Genet* 2020;41:401-12.
- Summaries of Gene and Locus Symbols by Disease Category (One or More Diseases per Gene/Locus). 2015. Available from: <https://sph.uth.edu/retnet/sum-dis.htm>. [Last accessed on 2022 Jan 30].
- Riazuddin SA, Iqbal M, Wang Y, Masuda T, Chen Y, Bowne S, *et al.* A splice-site mutation in a retina-specific exon of BBS8 causes nonsyndromic retinitis pigmentosa. *Am J Hum Genet* 2010;86:805-12.
- Dimaras H, Corson TW, Cobrinik D, White A, Zhao J, Munier FL, *et al.* Retinoblastoma. *Nat Rev Dis Primers* 2015;1:15021. doi: 10.1038/nrdp. 2015.21.
- Dimaras H, Khetan V, Halliday W, Orlic M, Prigoda NL, Piovesan B, *et al.* Loss of RB1 induces non-proliferative retinoma: Increasing genomic instability correlates with progression to retinoblastoma. *Hum Mol Genet* 2008;17:1363-72.
- Shields JA, Shields CL. *Intraocular Tumors: An Atlas and Textbook*. 3<sup>rd</sup> ed. Philadelphia: Wolters Kluwer; 2015.
- Kivelä, T. The epidemiological challenge of the most frequent eye cancer: Retinoblastoma, an issue of birth and death. *Br J Ophthalmol* 2009;93:1129–31.
- Ramasubramanian A, Shields CL. *Epidemiology and magnitude of the problem. Epidemiology and magnitude of the problem. Retinoblastoma*. New Delhi, India: Jaypee Brothers Medical Publishers; 2012.
- Broaddus E, Topham A, Singh AD. Incidence of retinoblastoma in the USA: 1975-2004. *Br J Ophthalmol* 2009;93:21–3.
- Dimaras H, Kimani K, Dimba EA, Gronsdaht P, White A, Chan HS, *et al.* Retinoblastoma. *Lancet* 2012;379:1436-46.
- González-Chávez SA, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin: Structure, function and applications. *Int J Antimicrob Agents* 2009;33:301-e1-8.
- Rusciano D, Pezzino S, Olivieri M, Cristaldi M, Gagliano C, Lupo G, *et al.* Age-related dry eye lactoferrin and lactobionic acid. *Ophthalmic Res* 2018;60:94-9.
- Shinmoto H, Dosako S, Nakajima I. Antioxidant activity of bovine lactoferrin on iron/ascorbate induced lipid peroxidation. *Biosci Biotech Biochem* 1992;12:2079–80.
- Danjo Y, Lee M, Horimoto K, Hamano T. Ocular surface damage and tear lactoferrin in dry eye syndrome. *Acta Ophthalmol (Copenh)* 1994;72:433-7.
- Devendra J, Singh S. Effect of oral lactoferrin on cataract surgery induced dry eye: A randomised controlled trial. *J Clin Diagn Res* 2015;9:NC06-9.
- Fujihara T, Nagano T, Nakamura M, Shirasawa E. Lactoferrin

- suppresses loss of corneal epithelial integrity in a rabbit short-term dry eye model. *J Ocul Pharmacol Ther* 1998;14:99-107.
20. Oram JD, Reiter B. Inhibition of bacteria by lactoferrin and other iron-chelating agents. *Biochim Biophys Acta* 1968;170:351-65.
  21. Majka G, Śpiewak K, Kurpiewska K, Heczko P, Stochel G, Strus M, *et al.* A high-throughput method for the quantification of iron saturation in lactoferrin preparations. *Anal Bioanal Chem* 2013;405:5191-200.
  22. Kruzel ML, Zimecki M, Actor JK. Lactoferrin in a context of inflammation-induced pathology. *Front Immunol* 2017;8:1438. doi: 10.3389/fimmu.2017.01438.
  23. Garreis F, Gottschalt M, Paulsen FP. Antimicrobial peptides as a major part of the innate immune defense at the ocular surface. *Dev Ophthalmol* 2010;45:16-22.
  24. Rusciano D, Pezzino S, Olivieri M, Cristaldi M, Gagliano C, Lupo G, *et al.* Age-related dry eye lactoferrin and lactobionic acid. *Ophthalmic Res* 2018;60:94-9.
  25. Ponzini E, Scotti L, Grandori R, Tavazzi S, Zamboni A. Lactoferrin concentration in human tears and ocular diseases: A meta-analysis. *Invest Ophthalmol Vis Sci* 2020;61:9.
  26. Descamps L, Dehouck MP, Torpier G, Cecchelli R. Receptor-mediated transcytosis of transferrin through blood-brain barrier endothelial cells. *Am J Physiol* 1996;270:H1149-58.
  27. Fillebeen C, Descamps L, Dehouck MP, Fenart L, Benaïssa M, Spik G, *et al.* Receptor-mediated transcytosis of lactoferrin through the blood-brain barrier. *J Biol Chem* 1999;274:7011-7.
  28. Cai X, Conley SM, Nash Z, Fliesler SJ, Cooper MJ, Naash MI. Gene delivery to mitotic and postmitotic photoreceptors via compacted DNA nanoparticles results in an improved phenotype in a mouse model of retinitis pigmentosa. *FASEB J* 2010;24:1178-91.
  29. Ahmed F, Ali MJ, Kondapi AK. Carboplatin loaded protein nanoparticles exhibit improved anti-proliferative activity in retinoblastoma cells. *Int J Biol Macromol* 2014;70:572-82.
  30. Tzekov RT, Locke KG, Hood DC, Birch DG. Cone and rod ERG phototransduction parameters in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2003;44:3993-4000.
  31. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet* 2006;368:1795-809.
  32. Haer-Wigman L, Newman H, Leib R, Bax NM, Baris HN, Rizek L, *et al.* Non-syndromic retinitis pigmentosa due to mutations in the mucopolysaccharidosis type IIIC gene, heparan-alpha-glucosaminide N-acetyltransferase (HGSNAT). *Hum Mol Genet* 2015;24:3742-51.
  33. Khan MI, Kersten FF, Azam M, Collin RW, Hussain A, Shah ST, *et al.* CLRN1 mutations cause nonsyndromic retinitis pigmentosa. *Ophthalmology* 2011;118:1444-8.
  34. Musarella MA, Anson-Cartwright L, Leal SM, Gilbert LD, Worton RG, Fishman GA, *et al.* Multipoint linkage analysis and heterogeneity testing in 20 X-linked retinitis pigmentosa families. *Genomics* 1990;8:286-96.
  35. Rice DS, Huang W, Jones HA, Hansen G, Ye G-L, Xu N, *et al.* Severe retinal degeneration associated with disruption of semaphorin 4A. *Invest Ophthalmol Vis Sci* 2004;45:2767-77.
  36. Kuizenga A, van Haeringen NJ, Kijlstra A. Inhibition of hydroxyl radical formation by human tears. *Invest Ophthalmol Vis Sci* 1987;28:305-13.
  37. Sorrentino FS, Gallenga CE, Bonifazzi C, Perri P. A challenge to the striking genotypic heterogeneity of retinitis pigmentosa: A better understanding of the pathophysiology using the newest genetic strategies. *Eye* 2016;30:1542-8.
  38. Hood DC, Birch DG. Abnormalities of the retinal cone system in retinitis pigmentosa. *Vision Res* 1996;36:1699-709.
  39. Cideciyan AV, Jacobson SG. An alternative phototransduction model for human rod and cone ERG a-waves: Normal parameters and variation with age. *Vision Res* 1996;36:2609-21.
  40. Berson EL. Electrical phenomena in the retina. In: Moses R, editor. *Adler's Physiology of the Eye*. St. Louis: CV Mosby Co.; 1992. p. 466-529.
  41. Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retin Eye Res* 1998;17:175-205.
  42. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000;45:115-34.
  43. Shen JK, Dong A, Hackett SF, Bell WR, Green WR, Campochiaro PA. Oxidative damage in age-related macular degeneration. *Histol Histopathol* 2007;22:1301-8.
  44. Hahn P, Milam AH, Dunaief JL. Maculas affected by age-related macular degeneration contain increased chelate iron in the retinal pigment epithelium and Bruch's membrane. *Arch Ophthalmol* 2003;121:1099-105.
  45. Dunaief JL, Richa C, Franks EP, Schultze RL, Aleman TS, Schenck JF, *et al.* Macular degeneration in a patient with aceruloplasminemia, a disease associated with retinal iron overload. *Ophthalmology* 2005;112:1062-5.
  46. Kalinowski DS, Richardson DR. The evolution of iron chelators for the treatment of iron overload disease and cancer. *Pharmacol Rev* 2005;57:547-83.
  47. Vorland LH. Lactoferrin: A multifunctional glycoprotein. *APMIS* 1999;107:971-81.
  48. Mikulic N, Uyoga MA, Mwasi E, Stoffel NU, Zeder C, Karanja S, Zimmermann MB. Iron absorption is greater from Apo-Lactoferrin and is similar between Holo-Lactoferrin and ferrous sulfate: Stable iron isotope studies in Kenyan infants. *The Journal of Nutrition* 2020;150:3200-7.
  49. Kondapi AK. Targeting cancer with lactoferrin nanoparticles: Recent advances. *Nanomedicine (Lond)* 2020;15:2071-83.
  50. Patra JK, Das G, Fraceto LF, Campos EV, del Pilar Rodriguez-Torres M, Acosta-Torres LS, *et al.* Nano based drug delivery systems: Recent developments and future prospects. *J Nanobiotechnol* 2018;16:71.
  51. Dehouck MP, Méresse S, Delorme P, Torpier G, Fruchart JC. The blood-brain barrier in vitro: co-culture of brain capillary endothelial cells and astrocytes. *Circulation et métabolisme du cerveau*. 1990;7(3):151-62.
  52. Son HJ, Lee SH, Choi SY. Human lactoferrin controls the level of retinoblastoma protein and its activity. *Biochem Cell Biol* 2006;84:345-50.
  53. Ortiz MV, Dunkel IJ. Retinoblastoma. *J Child Neurol* 2016;31:227-36.
  54. Abramson DH, Scheffler AC. Update on retinoblastoma. *Retina* 2004;24:828-48.
  55. Shields CL, Jorge R, Say EA, Magrath G, Alset A, Caywood E, *et al.* Unilateral retinoblastoma managed with intravenous chemotherapy versus intra-arterial chemotherapy. Outcomes based on the international classification of retinoblastoma. *Asia Pac J Ophthalmol (Phila)* 2016;5:97-103.
  56. Ancona-Lezama D, Dalvin LA, Shields CL. Modern treatment of retinoblastoma: A 2020 review. *Indian J Ophthalmol* 2020;68:2356-65.
  57. Schwartzman E, Chantada G, Fandiño A, de Dávila MT, Raslawski E, Manzitti J. Results of a stage-based protocol for the treatment of retinoblastoma. *J Clin Oncol* 1996;14:1532-6.
  58. Chantada GL, Fandiño AC, Gutter MR, Raslawski EC, Dominguez JL, Manzitti J, *et al.* Results of a prospective study for the treatment of unilateral retinoblastoma. *Pediatr Blood Cancer* 2010;55:60-6.
  59. Dryja TP, Rapaport JM, Joyce JM, Petersen RA. Molecular detection of deletions involving band q14 of chromosome 13 in

- retinoblastomas. *Proc Natl Acad Sci U S A* 1986;83:7391-4.
60. Reese AB, Ellsworth RM. The evaluation and current concept of retinoblastoma therapy. *Trans Am Acad Ophthalmol Otolaryngol* 1963;67:164-72.
61. Kanwar JR, Samarasinghe RM, Sehgal R, Kanwar RK. Nano-lactoferrin in diagnostic, imaging, and targeted delivery for cancer and infectious diseases. *J Cancer Sci Ther* 2012;4:31-42.
62. Samuel J, Singh N, Kanwar JR, Krishnakumar S, Kanwar RK. Upregulation of sodium iodide symporter (NIS) protein expression by an innate immunity component: Promising potential for targeting radiosensitive retinoblastoma [published correction appears in *Exp Eye Res*. 2020 Sep; 198:108145]. *Exp Eye Res* 2015;139:108-14.
63. Dong H, Yang Y, Gao C, Sun H, Wang H, Hong C, *et al*. Lactoferrin-containing immunocomplex mediates antitumor effects by resetting tumor-associated macrophages to M1 phenotype. *J Immunother Cancer* 2020;8:e000339.
64. Damiens E, El Yazidi I, Mazurier J, Duthille I, Spik G, Boilly-Marer Y. Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells. *J Cell Biochem* 1999;74:486-98.
65. Moneo V, del Valle Guijarro M, Link W, Carnero A. Overexpression of cyclin D1 inhibits TNF-induced growth arrest. *J Cell Biochem* 2003;89:484-99.
66. Xiao Y, Monitto CL, Minhas KM, Sidransky D. Lactoferrin down-regulates G1 cyclin-dependent kinases during growth arrest of head and neck cancer cells. *Clin Cancer Res* 2004;10:8683-6.
67. Campbell T, Skilton RA, Coombes RC, Shousha S, Graham MD, Luqmani YA. Isolation of a lactoferrin cDNA clone and its expression in human breast cancer. *Br J Cancer* 1992;65:19-26.
68. DeGregori, J. The Rb network. *J. Cell Sci* 2004;117:3411-3.
69. Lee VH, Robinson JR. Topical ocular drug delivery: Recent developments and future challenges. *J Ocul Pharmacol* 1986;2:67-108.
70. Cholkar K, Patel SP, Vadlapudi AD, Mitra AK. Novel strategies for anterior segment ocular drug delivery. *J Ocul Pharmacol Ther* 2013;29:106-23.
71. Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverage R. Ophthalmic drug delivery systems--recent advances. *Prog Retin Eye Res* 1998;17:33-58.
72. Cetinel S, Montemagno C. Nanotechnology for the prevention and treatment of cataract. *Asia Pac J Ophthalmol (Phila)* 2015;4:381-7.
73. Abdelrahman AA, Salem HF, Khallaf RA, Ali AM. Modeling, optimization, and *in vitro* corneal permeation of chitosan-lomefloxacin HCl nanosuspension intended for ophthalmic delivery. *J Pharm Innov* 2015;10:254-68.
74. Kymes SM, Walline JJ, Zadnik K, Sterling J, Gordon MO; Collaborative Longitudinal Evaluation of Keratoconus Study Group. Changes in the quality-of-life of people with keratoconus. *Am J Ophthalmol* 2008;145:611-7.
75. Godefrooij DA, de Wit GA, Uiterwaal CS, Imhof SM, Wisse RP. Age-specific incidence and prevalence of keratoconus: A nationwide registration study. *Am J Ophthalmol* 2017;175:169-72.
76. Lema I, Brea D, Rodríguez-González R, Díez-Feijoo E, Sobrino T. Proteomic analysis of the tear film in patients with keratoconus. *Mol Vis* 2010;16:2055-61.
77. Pattamatta U, Willcox M, Stapleton F, Cole N, Garrett Q. Bovine lactoferrin stimulates human corneal epithelial alkali wound healing *in vitro*. *Invest Ophthalmol Vis Sci* 2009;50:1636-43.
78. Sobrino T, Regueiro U, Malfeito M, Vieites-Prado A, Pérez-Mato M, Campos F, *et al*. Higher expression of toll-like receptors 2 and 4 in blood cells of keratoconus patients. *Sci Rep* 2017;7:12975.
79. Gesto MI, Sanchez JA, Moreiras TS, Perez FC. Biomarkers for diagnosis and prognosis of corneal ectatic disorders. US Patent US15/551,3862018.
80. Durrani OM, Meads CA, Murray PI. Uveitis: A potentially blinding disease. *Ophthalmologica* 2004;218:223-36.
81. Gritz DC, Wong IG. Incidence and prevalence of uveitis in Northern California: The Northern California Epidemiology of uveitis study. *Ophthalmology* 2004;111:491-500.
82. Päivönsalo-Hietanen T, Tuominen J, Vaahtoranta-Lehtonen H, Saari KM. Incidence and prevalence of different uveitis entities in Finland. *Acta Ophthalmol Scand* 1997;75:76-81.
83. Schmidt PJ. Regulation of iron metabolism by hepcidin under conditions of inflammation. *J Biol Chem* 2015;290:18975-83.
84. Kim A, Fung E, Parikh SG, Valore EV, Gabayan V, Nemeth E, *et al*. A mouse model of anemia of inflammation: Complex pathogenesis with partial dependence on hepcidin. *Blood* 2015;123:1129-37.
85. Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood* 2010;116:4754-61.
86. Gardenghi S, Renaud TM, Meloni A, Casu C, Crielaard BJ, Bystrom LM, *et al*. Distinct roles for hepcidin and interleukin-6 in the recovery from anemia in mice injected with heat-killed *Brucella abortus*. *Blood* 2014;123:1137-45.
87. Rageh AA, Ferrington DA, Roehrich H, Yuan C, Terluk MR, Nelson EF, *et al*. Lactoferrin expression in human and murine ocular tissue. *Curr Eye Res* 2016;41:883-9.
88. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). Scientific Opinion on bovine lactoferrin. *EFSA J* 2012;10:1-26.
89. Chen J, Zhou J, Kelly M, Holbein BE, Lehmann C. Iron chelation for the treatment of uveitis. *Med Hypotheses* 2017;103:1-4.