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Review Article

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Relationship of Demodex and Dry Eye Syndrome in Patients with Platelet-Rich Plasma Treatment

Rivera F Nancy[1*], Coloma F Alejandro[2] and San Martin L Kimberly[2]

[1]*Associate Professor, Department of Medical Technology, University of Concepción School of Medicine,* Chile

[2]*Students, Department of Medical Technology, University of Concepción School of Medicine,* Chile

[*]Corresponding author
Rivera F Nancy, Associate Professor, Department of Medical Technology, University of Concepción School of Medicine, Chile

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Abstract

In recent times, multiple eye diseases have been seen associated with an increase in the rate of Demodex infestation as a possible cause, but in the particular case of dry eye syndrome in patients treated with platelet-rich plasma, this increase in mite may be relevant to guide a more adequate treatment focusing on the elimination of the mite in conjunction with the recovery of the ocular ecology. The demodex mite is a commensal parasite that lives in hair follicles, sebaceous glands and meibomian, which in a high rate of infestation can generate alterations in the ocular area. Performing an adequate diagnosis for the detection of the mite and treatment for its eradication can be effective for the recovery of the normal physiology of the tear film that constitutes a cause of dry eye.

Keywords: Eye diseases, Demodex, Dry eye syndrome, PRP

Introduction

Ocular diseases such as blepharitis, chalazion, meibomian gland dysfunction and keratitis correspond to inflammatory and / or dysfunctional diseases which have as a common cause, but which has been overlooked, the increase in the infestation of the Demodex mite, which has been documented in the last time [1-4]. Within eye diseases is a much more complex, dry eye syndrome, which although it has not been directly associated with the mite can be a cause of the alteration of the tear film.

Dry Eye Syndrome

The Dry Eye Syndrome (DES) is a multifactorial disease of tears and the ocular surface where symptoms such as discomfort, visual alteration and instability of the tear film with potential damage on the ocular surface develops [5,6].

The tear film consists of 3 layers (external, middle and internal) which as a whole keep the surface of the eye lubricated. The outer layer corresponds to a layer of lipids which is granted by the meibomian glands, this has the function of avoiding the evaporation and overflow of tears. The middle layer, also called aqueous layer, corresponds to the tears that are secreted by the lacrimal glands whose function is to transport the components of tears, such as oxygen, enzymes and proteins to the ocular surface. And finally, the inner layer, corresponding to a layer of mucins that are secreted by the goblet cells that provide an active taut coating that regulates the surface tension of surface cells hydrophobic eyepieces [7,8]. So knowing the structures that make up the tear film and the glands and cells involved, any modification in these structures will generate an alteration in the tear film itself.

With regard to dry eye, it has been subdivided into two as a classification method [8,9]:

- Dry eye / watery deficiency: specifically corresponds to a reduction in the production of tears.
- Dry / hyper evaporative eye: it is based on an increase in the evaporation of the tear film.

In a study conducted, it was observed that 10% of patients suffering from dry eye syndrome were associated with a deficiency in lacrimal production. In contrast, approximately 80% of the patients were associated with an increase in the evaporation of the tear film due to a dysfunction of the meibomian glands [10].

The dysfunctions associated with the meibomian glands, as previously mentioned, are the most frequent cause of dry eye, but what causes this dysfunction? There are multiple associated causes, among them is one in particular, the demodex ectoparasite infestation [11].

Clinical Aspects

Regarding the relevant clinical aspects of the disease, the patient presents: dryness, visual discomfort, sensation of visual fatigue, pruritus, red eye, photophobia, burning, pain, stinging, irritation, blurred vision that may increase or worsen during the course of the day, in severe cases may present filamentous keratitis, ulceration, trichiasis and keratinization, so it is important to determine the

duration of symptoms, intensity and possible situations that could generate them [12].

Platelet Rich Plasma

Defining platelet-rich plasma is a complex situation because there is no agreement or consensus on it. The only definition consistently defended in the literature defines PRP as a volume of plasma containing a platelet concentration higher than the baseline level (150,000-350,000 / µl) [13].

PRP is based on the preparation and use of plasma enriched with platelets, which is obtained from the patient's own blood. Within this there is great variability in preparation methods, which leads to different products: plasma rich in growth factors (PRGF), platelet-rich plasma and growth factors (PRPGF), platelet-poor plasma (PPP), platelet-rich and leukocyte-rich plasma (LR-PRP), platelet-rich plasma and leukocyte-poor plasma (LP-PRP) [14]. However, PRP is the most studied, obtaining a mechanism of action that explains its effectiveness in different treatments, which allows the in situ release of a set of biologically active proteins that influence and promote a wide range of biological processes, including the recruitment, growth and cell differentiation. These proteins include the epidermal growth factor, the transforming growth factor β, the keratinocyte growth factor, the factor of platelet-derived growth, insulin-like growth factor, neural growth factor. It is known that these factors induce cell migration, attachment, proliferation and differentiation of various cells and tissues [13,15-20].

PRP has emerged as a useful strategy for cellular restoration in different fields of medicine, among which may include the reconstruction of bones and tendons, for the treatment of elbow tendinopathy, and for the regrowth of hair [15,16]. It has also been used successfully in several surgical and clinical treatments, including wound closures, corneal ulcers, chemical burns (topical application), skin rejuvenation, induction of fibroblast proliferation, and remodeling of extracellular matrix collagen [13].

A beneficial effect has also been demonstrated for epithelial recovery in corneous keratomileusis in situ assisted by post-laser 15 corneal burns and in severe dry eye (topical application) [17]. Thus, this technique seems to have great potential for treatment of ophthalmological diseases. Platelet-rich plasma as a treatment for Dry Eye Syndrome.

There is a wide variety of pharmacological and non-pharmacological measures for the management of symptoms associated with dry eye: hygienic measures, anti-inflammatory agents, tear replenishment and tear retention. However, artificial tears continue to be the mainstay of dry eye treatment, although in many cases they do not achieve adequate management of the disease. This is because no artificial tears can reproduce the natural tear exactly [13]. In recent years, several studies have studied the efficacy of PRP in the treatment of SOS.

Merayo-Lloves et al. evaluated the effect of PRGF in cases of refractory ocular surface disease, representing 70% of the studied dry eye of different etiologies. In this retrospective study we included 80 eyes of 41 patients who were treated with PRGF eye drops several times a day from 6 to 24 weeks, according to evolution. There were statistically significant differences in the ocular surface disease index, in the best corrected visual acuity and in the frequency and severity values of the symptoms referred by the patients and measured by visual analog scale before and after the treatment [18].

Another recent study shows that the injection of PRP adjacent to the lacrimal gland in patients with severe dry eye produces a significant increase in tear volume and lacrimal rupture time, also decreasing the lysine staining of the ocular surface of all patients treated, and improving subjective perception [15].

Lopez-Plandolit, et al. treated 16 patients with moderate / severe dry eye refractory to other treatments, including he autologous serum, with eye drops of PRGF, and observed statistically significant differences in the dry eye questionnaire (SDEQ) before and after 3 months of treatment [21].

In the same line, in a study on the effect of PRP eye drops in 18 patients with moderate-severe dry eye, 89% of patients experienced a significant improvement or disappearance of symptoms associated with SOS. An improvement in the quality of the tear film was observed in more than half of the patients, assessed by the height of the tear meniscus and the time of rupture [17].

Demodex

Demodex (from the Greek: demos = fat; dex = carcoma) belongs to the phylum arthropod, class Arachnida, order Acarina, (Superfamily: Demodicodoidea) is a parasite that lives commensally, and its infestation in humans is known as demodicidosis or demodecosis. Several types of Demodex have been described, however there are two that predominate at the time of infestation, these are *Demodex folliculorum and Demodex brevis* [22].

Demodex folliculorum inhabits the palpebral margin, particularly in the infundibular portion of the hair follicle and may be present in a varied number, however, *Demodex brevis* is located in the sebaceous glands and in the meibomian glands; and can be found as a single individual [23,24]. Both are found in all human races, with no sex preference.

It is a frequent and widespread parasite, it presents sexual dimorphism and in its cycle it is possible to observe eggs, larvae, protoninfa nymphs, male and adult female [22]. The latter reaches a size close to 300 mm long by 40 mm wide, with the female being larger than the male, the latter being vermiform, wider at the anterior end [23].

Among the alterations that cause has been implicated as the constant agent of skin dryness, erythematoscamosa rosacea that resembles pityriasis, papular pustular or granulomatous rosacea including localization in isolated inflammatory papules, alopecia scalp and some cases of blepharitis, seborrhea, lung carcinomas, skin atrophy, palpebral skin pigmentation, meibomitis, chalazion; it has also been associated in immunosuppressed patients such as leukemia or HIV infection and cancer chemotherapy [22-24]. However, other studies have shown that the Demodex mite itself does not generate the disease, as it acts as a commensal, but if it has been documented that an increase in the number of this mite can result in an imbalance in the ocular ecology. In this particular situation, it causes an imbalance in the lipid layer of the tear film due to the obstruction of the meibomian gland [11].

Physiopathological Mechanism

In the case of *Demodex folliculorum* (Df) once the mite is adult, it moves with its four pairs of legs, each with two claws that erode

the epithelium. To feed, it projects stilettos puncturing host cells, where it secretes the enzymes of its two salivary glands and initiates the digestion of human epithelial cells until it reaches the dermis, which may contribute to the formation of loose or poorly directed eyelashes [25,26]. If there is any interruption in continuity, Toll-like receptors (TLRs) are activated and the antigens are exposed to the immune system. This is how abrasion of the skin occurs and induces hyperkeratosis and hypersensitivity responses [27].

For its part, *Demodex brevis* (Db) can mechanically block the orifices of the meibomian glands, leading to dysfunction of the meibomian gland with lipid tears deficiency. Histopathological studies show that both species, when they cross the follicular barrier and penetrate the dermis, can precipitate a granulomatous reaction. In the case of Db, it penetrates the meibomian glands and its chitinous exoskeleton can act as a foreign body causing this reaction [25,26]. It has also been observed in the center of Meibomian granulomas, surrounded by epithelioid cells, histocyte fibroblasts, lymphocytes and plasma cells. Therefore, Demodex mites can be a potential cause of recurrent and refractory chalazia [26].

Some authors believe that the parasite passes passively to the dermis after destroying the follicle; however, others suggest that it crosses actively by increasing its number and by the inflammation caused by helper T-cells type TH17, which activate TLR.3 [25,28]. In any case, all studies suggest a type IV immunological reaction [29].

Some researchers have proposed that there is a link between the frequency of presentation of demodecidosis (DD) and leukocyte antigens, since a relationship between individuals expressing HLA Cw2 and Cw4 has been observed with an increase in density of Df, increased infiltration inflammatory and keratinocyte apoptosis [30].

The pathogenic activity of Df in the skin is related to its function in the development of rosacea. Yamasaki et al showed that individuals with rosácea show a significant increase in kallikrein serine protease 5 (KLK5) and abnormal forms of cathelicidins in the skin of the face; also found that two catelecidine peptides (LL-37, FA-29) induce inflammation, erythema and perifollicular and vascular dilation when inoculated on the skin of rats, promoting angiogenesis and inflammation that manifest themselves in the clinic as erythema and papules. LL-37 has little antimicrobial power compared to other cathelicidins found in normal skin, which could contribute to the increase in Df density [29].

Later, in 2011, Yamasaki, et al. observed that the increase in TLR2 promotes the expression of KLK5 through calcium-dependent channels. The authors have suggested that rosacea it represents an immune alteration and that the commensal bacteria of the skin are normal both in this condition and in normal skin [31]. However, this does not apply to Df since the two factors that induce the expression of cathelicidins are skin infection and interruption of the cutaneous barrier, situations that Df causes, at least, theoretically [29].

Studies on the function of body temperature have shown that commensal microorganisms such as Df and Db can become pathogenic, improving their motility and activity at temperatures above 37 °C, however their survival is shorter [32,33].

Some authors state that the mite demodex acts as a vector of bacteria such as Staphylococcus and Streptococcus., Wolbachia sp., and *Bacillus oleronius*. [26,34,35]. Which have antigenic proteins that actívate mononuclear cells in patients with DD, which indicates a fundamental role in the process of parasite sensitization [35].

Diagnosis

There are several techniques to observe and evaluate the density of Demodex.

Eyelash removal

This procedure consists of extracting tabs from the patient for its later visualization in the microscope. The number of tabs to be extracted varies according to the different researchers. This can vary between the extraction of 10 eyelashes per individual, 5 of each eye alternating between lower and upper eyelids [36]. 6 tabs of each eye, both of the upper and lower eyelids [37]. 16 eyelashes, 8 for each eye, of both eyelids [22]. For your observation, these should be placed on a slide which previously contains a drop of immersion oil, peanut or glycerin [22,23]. Being covered by a coverslip to be observed under a microscope with 10x and 40x magnification [22,23,36,37]. To later make the calculation of infestation index given by the formula:

$$\frac{\text{Demodex number}}{\text{Number of tabs}}$$

If it is greater than 0.5, it was interpreted as an over population of mites. The identification of different mites is done on the basis of their morphological characteristics [22,23,36,37].

Skin Biopsy

The procedure consists of applying a drop of cyanoacrylate on the cheek, covering an approximate surface of 1 cm^2, and let it act for one minute until it dries. After removing the adhesive, immersion oil should be applied and a coverslip placed to observe the biopsy under a microscope with different magnifications, looking for Demodex intentionally. The test is considered positive with the finding of 5 Demodex / cm^2 [25].

Real-Time PCR

Real-time PCR (q-PCR) is a technique that allows the diagnosis of the presence of Demodex. Ravera, et al. They developed and standardized this detection method, for which primers designed and directed to detect the specific target sequence 18S rRNA from Demodex canis (Dc) and Demodex injai (Di) were used. For this, 14 dogs with demodicosis were used, whose microscopic examination had been positive (12 for Dc and 2 for Di) The q-PCR was positive for all samples. The amplified fragment was 166 bp as expected, and its sequence coincided with Dc. However, the amplified fragment of the two D. injai samples showed changes in seven of the 166 nucleotides, confirming that the technique is useful for detecting any of the Demodex mites of the dog [38].

Other researchers developed and standardized whose specific target sequence was 18S rRNA from *Demodex folliculorum*. The detection system used was by means of a TaqMan probe with a FAM fluorophore attached to the 5'-end and a signal quencher (Quencher) to the 3-end. In the case Casas et al. They used 50 patients with different types of rosacea, 32 with erythematotelangiectatic rosacea (ETR) and 18 with papulopustular rosacea (PPR). The results obtained showed the presence of Df in 96% of the patients, being positive in 88% of the patients with ETR, and in 100% of the patients, which shows a

PCR efficiency close to 100% [39].

On the other hand, Tenorio, et al. I analyze 46 biopsy samples of nodular type basal cell carcinoma, obtaining as a result the presence of the Df parasite in 50% (23/46) of the samples. The 23 positive samples were confirmed by sequencing, so that the specificity of the technique was considered 100% in the series analyzed [40]. As regards the technique, it can be concluded that the developed quantitative PCR technique presents a sensitivity, specificity and excellent reproducibility, so that it would be ready and available to use as a tool for the study of the hypothetical relationship of parasitization and its density with certain diseases [39,40].

Conclusion

- Although the literature registers a relationship between Demodex and Dry Eye Syndrome, it has not been analyzed in depth, so investigations must be conducted to have tangible evidence of it.
- The mechanism of pathogenicity of *Demodex folliculorum* should be defined more precisely, as well as the mechanism of pathogenicity of *Demodex brevis* should be studied in depth.
- There is sufficient evidence to determine the real-time PCR as a possible laboratory test, however the microscopic visualization of the parasite seems to be enough to be the diagnosis.

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