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Case Report

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The Value of Conjunctival Immunohistochemistry Analysis for the Diagnosis of Ocular Mucous Membrane Pemphigoid

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Abstract

Ocular Mucous Membrane Pemphigoid (OMMP), a subset of Mucous Membrane Pemphigoid (MMP) is an autoimmune-mediated progressive cicatrizing conjunctivitis with the potential for corneal blindness. Direct Immunofluorescence (DIF) is considered the gold standard for the diagnostic confirmation of this condition; nevertheless, in many cases, this immunopathological technique is inconclusive. A 54 years old female presented to our service complaining of progressive diminished visual acuity, foreign body sensation, and photophobia in both eyes. The slit-lamp examination showed bulbar and palpebral conjunctival hyperaemia, as well as bilateral corneal scarring. During clinical follow-up, progressive conjunctival scarring with inferior fornix foreshortening, symblepharon formation, and significant corneal opacity dramatically diminished her visual acuity. A conjunctival biopsy was negative for DIF; therefore, Immunohistochemical analysis (IHCA) for IgG, IgA, and C3 immune reactants linear deposition on the conjunctival Basement-membrane zone (BMZ) was necessary for diagnosis confirmation. The present clinical case highlights the utility of paraffin-embedded conjunctival IHCA as an alternative immunohistopathology tool for the diagnosis of OMMP when DIF is non-conclusive.

Keywords

Ocular mucous membrane pemphigoid; Cicatrizing conjunctivitis; Conjunctival biopsy; Direct immunofluorescence; Immunohisto chemical analysis

Introduction

OMMP is a potentially blinding autoimmune disorder, characterized by a progressive bilateral and asymmetrical sub epithelial fibrosis of the conjunctiva. It is considered a subset of the systemic autoimmune inflammatory disease known as MMP. Patients suffering from OMMP are usually genetically-predisposed women in their sixth decade of life or older [1]. Despite its low incidence (0.7 per 1,000,000), OMMP complications are sight-threatening [2]. A high grade of suspicion is required to diagnose OMMP since the clinical symptoms and signs at the early stages of the disease are unspecific. Although the necessity of pathological confirmation of OMMP has been put in doubt by some authors, a conjunctival biopsy analysed by DIF showing a linear deposition of immune reactants (IgG, IgA, IgM, or complement C3) on the epithelial BMZ is considered a requisite for establishing the diagnosis [3,4].

The performance of DIF analysis depends on the availability of a fresh-frozen tissue biopsy and an expert pathology laboratory technician with specialized equipment, including a micro-cryotome, to produce fine and thin cryosections to preserved tissue morphology, avoiding distortion and excessive background fluorescein staining. Additionally, pure high-affinity fluorinated monoclonal autoantibodies directed against immune-reactants deposited on the BMZ, and a fluorescence microscope are essential [5]. Such technical requirements are not universally available, challenging the adequate handling and processing of conjunctival biopsies to obtain an ideal result and accurate interpretation [6]. This technique may be inconclusive when there are technical limitations or a lack of an adequate laboratory setting to perform a DIF analysis.

IHCA on Formalin-fixed, paraffin-embedded (FFPE) tissue biopsies is an excellent alternative method to confirm the histopathologic diagnosis of OMMP in cases of negative DIF [7,8]. Such confirmation enables us to justify the administration of Immunosuppressive therapy (IMT) to avoid disease progression characterized by a significant conjunctival and corneal scarring, which significantly affect the visual outcome [9].

Case Report

A 54 years old female came to the clinic complaining of diminished visual acuity, foreign body sensation, and photophobia in both eyes. The slit-lamp examination showed bilateral palpebral and bulbar conjunctival hyperemia and a deep leucoma with neovascularization involving the peripheral cornea. The patient had bilateral pterygium resection two years previously. After a few months of follow-up, the ocular inflammatory process worsened in both eyes, with severe damage to left eye progressing to inferior fornix foreshortening and symblepharon formation (Figure 1A), as well as total corneal opacity (Figure 1B). Such changes translated into a dramatic visual acuity diminution. The differential diagnosis included cicatrizing conjunctivitis of autoimmune etiology; therefore, a bulbar conjunctival biopsy of the left eye for DIF analysis was performed, resulting nonconclusive for the diagnosis of OMMP. Due to the

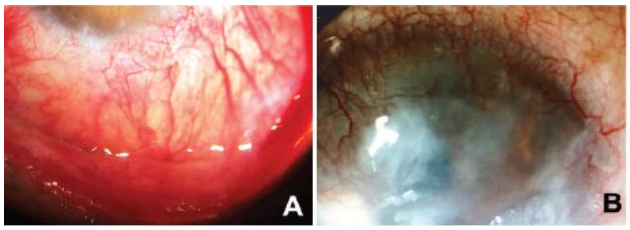

Figure 1: A. Left eye conjunctival hyperemia with subepithelial fibrosis and subtle inferior fornix foreshortening. **B.** Significant corneal stromal scarring with superior, deep neovascularization in the same eye.

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negative DIF result, IHCA was necessary to confirm the diagnosis and justify the administration of IMT. For this purpose, the biopsy tissue was fixed in 10% buffered formalin for the routine histopathologic process. For IHCA, 3-micron thickness sections were made with a microtome to the paraffin blocks and extended on electro-charged slides. Antigenic recovery and immunohistochemical processes followed the sequentially following these steps. Peroxidase blocking is accomplished by applying H_2O_2 for 10 minutes before a primary monoclonal autoantibody directed against IgA, IgG, C3 immune reactants, as well as fibrinogen and albumin negative controls are incubated for 20 minutes. A biotinylated secondary antibody highly specific against the primary one is incubated for another 20 minutes, and finally, the avidin-chromogen complex is applied for 10 minutes. The slides are then stained with Harris hematoxylin, dehydrated in alcohol with subsequent concentrations of 80%, 95%, and 100%, clarified with xylol, and lastly, coverslips are placed. Based on positive staining for IgG, IgA, and C3 antibodies against BMZ, the IHCA confirmed the diagnosis of OMMP (Figure 2A and 2B). Once the diagnosis was confirmed, IMT consisting of oral prednisone (1 mg/kg/day) and Azathioprine (AZT) (100 mg/day) was initiated.

After six months on IMT, absolute control of inflammation was achieved, and visual rehabilitation was considered for this patient according to the corneal scar deepness as analyzed by anterior segment corneal optical coherence tomography (AS-OCT). The right eye was first treated with phototherapeutic keratectomy (PTK) using 0.02% mitomycin-C (MMC) eye drops, improving significantly the corneal scarring that involved the pupillary axis. Two months later, a penetrating keratoplasty (PKP) was performed in the left eye to overcome a dense and deep corneal opacity.

After one year on AZT therapy, the patient presented liver toxicity with double elevation from baseline values of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT); therefore, the drug was switched for oral methotrexate (10mg/week), which remains until today. After four years of treatment, the patient´s best-corrected visual acuity is 20/40-2 in both eyes, with an adequate integration and clarity of the corneal graft in the left eye (Figure 3A). A temporal area of thinning and vascularization remains in the right cornea (Figure 3B). Full ocular lubrication in both eyes, topical loteprednol etabonate 0.5% once a day, and 0.05% cyclosporine-A twice a day for the left corneal graft continues as a current treatment for this patient.

Discussion

The pathogenesis of OMMP begins with dysregulation of T-lymphocyte function in an immunogenetic susceptible host. HLA-DR2, HLADR4, and HLA-DQw7 MHC-II molecules confer susceptibility for the development of the disease [10]. Under a putative external stimulus (eg., virus, topical medications), there is an activation of B-lymphocytes with the subsequent formation of autoantibodies of IgG, IgA, and IgM isotypes directed against adhesion molecules of the conjunctival epithelial BMZ complex, inducing a type II Antibody-dependent hypersensitivity reaction (ADCC). Such complement-fixating autoantibodies are crucial for the pathogenesis and cicatrizing nature of the disease and the establishment of its pathological diagnostic confirmation [9].

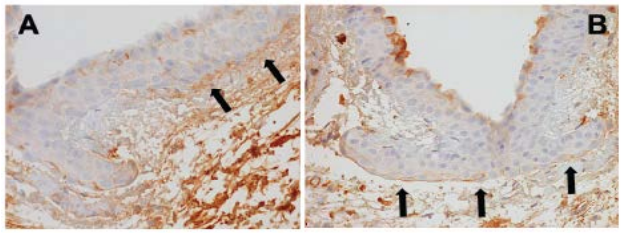

Figure 2: A. Conjunctival immunohistopathology slide showing a fine subepithelial linear staining (red-brick color) corresponding to the IgG antibody on the BMZ (arrows). **B.** Fine linear staining (arrows) along the BMZ corresponding to IgA antibody (ABC-immunoperoxidase technique, 100x).

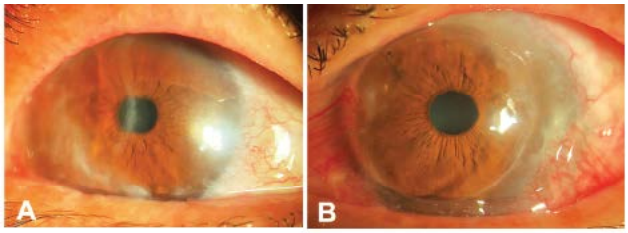

Figure 3: A. Right cornea after phototherapeutic keratotomy (PTK) with intraoperative 0.02% mytomicin-C, showing a clear visual axis and temporal stromal haziness, thinning, and fine vascularization. **B.** Clear corneal graft two years after penetrating keratoplasty in a quiet left eye under immunosuppressive therapy. Significant conjunctival perilimbal scarring (pseudo-pterygia) is present in the nasal and temporal quadrants.

The sensitivity and specificity of formalin fixated paraffin-embedded (FFPE) tissue samples for OMMP have been reported to be 36% - 45% for C3d and C4d in mucosal tissue, and 69% for skin biopsies [6]. A previous report from an experienced laboratory found an initial sensitivity of 52% for DIF in cryosections of 63 conjunctival biopsies from patients with the clinical diagnosis of OMMP, increasing to 83% in negative DIF biopsies processed for IHCA with the ABC technique [7]. A recent report by the same group but with a larger number of biopsies (n=136), achieved an even higher sensitivity (95.6%) for the diagnosis of OMMP with the additional use of the avidin-biotin complex immunoperoxidase technique [8]. Obtaining an appropriate tissue sample and adequate harvesting inducing minimum trauma to the conjunctiva, and careful handling of the tissue should be a priority. However, processing the tissue sample by an experienced laboratory is crucial, considering that mucosal tissue tends to be smaller and more friable than skin specimens.

On the other hand, despite being considered the gold standard for the diagnosis of MMP, it is well-known that up to half of the patients may have intermittent or repeatedly negative DIF analysis [11]. A significant number of biopsy negative results occur in cases of drug-induced OMMP [12]. A recent study shows that the site of biopsy strongly influences the sensitivity of DIF with cutaneous samples showing a markedly higher sensitivity. Nevertheless, this applies only to the onset of extra-ocular manifestations of MMP [13]. Considering the variability, and sometimes, frustrating sensitivity of the conjunctival biopsy, the necessity of pathological confirmation of OMMP has been put in doubt by some authors who propose to initiate systemic immunosuppression even without an immunopathology confirmation [3]. They consider waiting for pathological confirmation can delay the Treatment with the potential sight-threatening complications. Nevertheless, as stated by "The First International Consensus in Mucous Membrane Pemphigoid," clinical and direct immunopathology criteria are essential to confirm the diagnosis considering the potential adverse events of IMT and the continuous evolution of the disease which leads to long periods

of IMT, which may become a legal, medical issue [4]. Additionally, a conjunctival biopsy may reveal other conditions associated with cicatrizing conjunctivitis, including lichen planus, pemphigus vulgaris, paraneoplastic pemphigus, linear IgA disease, epidermolysis bullosa acquisita, drug-induced conjunctival cicatrization, ANCA-associated vasculitides, and sarcoidosis [3, 14].

Although no large cohort randomized clinical trials exist for analyzing the treatment of OMMP, the site of involvement, the severity of the disease, and the speed progression should be considered for making therapeutic decisions [3]. For patients with severe or rapidly progressive ocular disease, the first-line therapy should include prednisone (1mg/kg/day) and cyclophosphamide (1-2 mg/kg/day), which may be administered orally or by intravenous pulses, depending on patient´s gastrointestinal tolerance and availability [15-17]. Complete blood cell counts (leukocytes and platelets), liver function tests, and urine analysis should be performed before beginning therapy and every six weeks while on IMT. Alternatively, azathioprine (1-2 mg/kg/day) may substitute for cyclophosphamide [15,18]. The serum level of thiopurine methyltransferase activity is required in patients receiving azathioprine since it predicts its therapeutic efficacy and side effects [19].

Methotrexate at a dosage of 10–15 mg per week is an alternative in patients with advanced sight-threatening ocular disease showing the highest safety profile [20]. However, like it is the case of any anti-metabolite drug, including azathioprine, therapeutic methotrexate effect may take 4-8 weeks to show clinical improvement; therefore, IV-cyclophosphamide is preferred for rapidly progressive OMMP cases and the induction of disease remission [12,15,17]. Anti-TNFα drugs, intravenous immunoglobulin (IVIg), and rituximab (anti-CD20) remain as second-line options if treatment with the agents mentioned above fails [12]. Our patient achieved disease control with prednisone (1 mg/kg/day) and oral methotrexate at a 10mg per week dosage without any side effects.

Finally, as in this case, once a quiet ocular surface is achieved by absolute control of inflammation, surgical management may be performed. Eyelid surgery, oral mucosa, and amniotic membrane transplantation, superficial keratectomy, lamellar or penetrating keratoplasty, and even keratoprosthesis may be necessary to achieve visual rehabilitation in patients with advanced OMMP.

Conclusion

Despite new trends to dispense immunological diagnosis confirmation, to our consideration, conjunctival biopsy remains a necessary diagnostic tool in the assessment of the OMMP suspect. It helps to rule out other sight-threatening conditions associated with chronic and progressive cicatrizing conjunctivitis and justify the administration of IMT as this is the most efficacious therapeutic alternative to achieve inflammatory control and, more importantly, induce disease remission. The present case highlights the utility of conjunctival IHCA in formalin-fixed, paraffin-embedded tissue as a complementary tool to a negative DIF result to confirm the diagnosis via increasing diagnostic sensitivity of conjunctival biopsy in OMMP patients.

Conflict of Interest

The authors declare no conflicts of interest or financial disclosure.

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