Characteristics of the cornea and the ocular surface in a population of patients with congenital aniridia

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PURPOSE: To describe corneal and ocular surface abnormalities in patients with congenital aniridia.

METHODS: We examined 50 eyes in 25 patients with congenital aniridia. Schirmer’s test I and II, tear film break-up time, vital staining, tear ferning pattern test and impression cytology, Cochet-Bonnet aesthesiometry, specular microscopy and ultrasonic pachymetry were carried out.

RESULTS: Based on Mackman’s classification, we found grade 0 in 12%, grade 1A in 52%, grade 1B in 20%, and grade 2 in 16% cases. Age, history of ocular surgery, dry eye score and aesthesiometry correlated with the degree of aniridia-related keratopathy (ARK). Schirmer’s test I was normal in 86.8%, Schirmer’s test II in 94.4% of the eyes and TFBUT in 83.3% of the cases. Corneal staining was altered in 54.2%, and conjunctival staining was altered in 45.7%. The tear ferning pattern was abnormal in 80%. Conjunctival metaplasia was present in 76.9%, and the presence of conjunctival goblet cells on the cornea correlated weakly with the degree of ARK. Corneal endothelial cell density was normal in 13 eyes of 9 patients (2190.84 cells/mm²). Ultrasonic pachymetry was higher than average in all eyes examined (587 microns).

CONCLUSION: Simple corneal and ocular surface tests are useful in assessing the presence and degree of aniridia-related keratopathy. Impression cytology is a useful tool in diagnosing metaplasia and limbal stem-cell deficiency in patients with congenital aniridia. Cochet-Bonnet aesthesiometry results and tear ferning patterns are reported for the first time in patients with congenital aniridia.

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INTRODUCTION

Congenital aniridia is a rare, bilateral, genetic disorder with variable phenotypic expression. It can result, from different mutations in the PAX6 gene, although this is not the only cause of aniridia. This gene is located in the short arm of chromosome 11 and controls ocular development of the corneal epithelium, iris, lens, ciliary body and retina. Congenital aniridia exists mainly in two forms: 1) familial and 2) sporadic. The familial pattern can be autosomal dominant (85%) or inherited in an autosomal recessive manner (2%) in Gillespie’s syndrome, which is associated with mental retardation and cerebellar ataxia. It is already known that the PAX6 gene is not involved in this syndrome. In the sporadic form, it can be found in WAGR syndrome (Wilms’ tumour, aniridia, genitourinary abnormalities and mental retardation) as a result of alterations in the PAX6 and WT1 genes of chromosome 11p. It can also be found alone without extraocular disorders.

Review of Related Literature

Congenital aniridia is characterized by a partial or total absence of the iris, although it is frequently
seen as a hypoplastic iris stump when examined through gonioscopy\(^5\) (see Figure 1). Other associated abnormalities include dry eye syndrome, meibomian gland dysfunction, corneal disease (aniridia-related keratopathy), glaucoma, lens disorders (cataracts, lens subluxation or luxation and microphakia), pendular nystagmus and strabismus\(^5\)\(^-\)\(^10\). The posterior segment can be equally affected by pathologies such as optic nerve hypoplasia, foveal hypoplasia, and/or retinal dysfunction\(^7\). As a consequence, it results in poor vision.

The degree of severity of corneal involvement has been studied and linked to dysfunction in limbal stem cells, meibomian gland damage and abnormal production of tear film components, with cytological changes in the ocular surface\(^7\)\(^,\)\(^8\)\(^,\)\(^11\)\(^,\)\(^12\). The instability of the ocular surface determines the onset of secondary dry eye and dysfunction of the corneal epithelial barrier, which both lead to conjunctival and corneal metaplastic transformation.

Penetrating keratoplasty has been proven ineffective for the long-term treatment of this disorder because it does not address the stem cell deficiency that is the primary etiologic factor. In the past decade, much has been published about the success of limbal allografts to address the problem of limbal stem cell deficiency. In a study published by the author and colleagues\(^12\) on 24 patients who underwent procedures such as penetrating keratoplasty, limbal cadaveric allograft or HLA-matched living-related limbal allograft for advanced aniridia keratopathy, we were able to demonstrate that long-term visual prognosis was the same independent of whether the patient underwent surgery for the corneal problem. Limbal transplant and penetrating keratoplasty had comparable results through several years of follow-up (mean follow-up time of 14.7 years) due to failure of transplanted allografts.

It is known that several factors like deficiency in tear production, poor tear quality, lack of corneal sensitivity and presence of ocular inflammation are risk factors for graft failure. It is possible that the microenvironment of the ocular surface in a patient with congenital aniridia is abnormal and is what leads to the failure of the said surgical procedures. This has led us to study the characteristics of the cornea and ocular surface in patients with congenital aniridia, in the hope that someday we can find a treatment for aniridia related keratopathy, whether it be medical or surgical.

### METHODS

Fifty eyes in 25 patients were assessed. Members of the Asociación Española de Aniridia were recruited to participate in this descriptive study. All patients/parents or guardians of minor patients gave their consent for participation in the diagnostic study. The study included 6 male (24%) and 19 female (76%) patients, their ages ranging from 3 to 54 years and averaging 20.04 years (SD = ± 15.7). A total of 18 patients (72%) presented with a sporadic inheritance pattern, while the remaining 7 patients (28%) expressed a familial pattern. We found 7 patients (28%) with associated extraocular pathologies. For patients with a sporadic inheritance pattern, 2 (8%) had Wilms’ tumour, 1 (4%) had Hashimoto’s thyroiditis, and 1 (4%) had trigger thumb. For patients with a familial pattern, 1 (4%) had congenital angiomia, and 1 (4%) had type 2 diabetes mellitus. A 3-year-old (4%) patient presented with cardiovascular and genitourinary abnormalities (gonadal dysgenesis), without Wilms’ tumour, and demonstrated a sporadic inheritance pattern. Ten patients (40%) had a history of eye surgery.

All patients underwent complete clinical histories and ocular examinations, including measurement of visual acuity. This was done with and without correction and using eye charts for visually impaired people, based on age and expressed as a LogMAR score\(^13\). Refraction, slit-lamp biomicroscopy of the anterior segment, and specular microscopy (Topcon non-contact specular microscope, SP-1000) were performed, as well as corneal ultrasonic pachymetry (Pocket II Precision Pachymeter, Quantel Medical, Inc.) to obtain central corneal thickness.

Due to time constraints in performing the cornea and ocular surface tests, some tests were performed in only one eye to avoid affecting the outcome of subsequent tests.

To evaluate the ocular surface, the following tests were performed: 1) Schirmer’s test I (without anaesthesia) to measure basal and reflex tear secretion; 2) Schirmer’s test II (with anaesthesia) to measure basal tear secretion, where a value of ≥10 mm/5 min for both tests was considered normal; 3) tear break-up time (TBUT), where a value of ≥10 seconds was defined as normal; 4) corneal staining with 2% sodium fluorescein drop to assess epithelial defects using the Oxford Scheme\(^14\);
5) conjunctival staining with 1% Rose Bengal paper strips using the Van Bijsterveld Scheme\textsuperscript{14,15,16}; 6) tear ferning pattern test to assess the mucous layer (Rolando’s classification grades I to IV \textsuperscript{17, 18}; and 7) conjunctival and corneal impression cytology using Tseng’s classification system\textsuperscript{19}.

Schirmer’s test I, TFBUT and fluorescein (Oxford scheme) and Rose Bengal (van Bijsterveld scheme) staining were used to assess dry eye disease based on the severity levels defined in the Dry Eye Severity Grading Scheme of the Delphi Panel report\textsuperscript{20, 21}. This scheme consists of five levels, where 0 is normal, 1 is mild, and 4 is the most severe (Table 1). The cut-off values for Schirmer’s test I and TFBUT were defined based on the Delphi Panel report. Corneal fluorescein staining has six grades (0–5; Oxford schema) and was multiplied by 0.8 for conversion into five grades (0–4; Delphi Panel report). Rose Bengal staining has 10 grades (0–9; Bijsterveld schema); they were multiplied by 0.44 to convert them into 5 grades (0–4; Delphi Panel report). The four tests with 5 levels (0–4) were joined, and the mean of the four tests was calculated to obtain the dry eye score. We considered dry eye scores between 0 and 0.02 to be normal, 0.021 and 1 as mild, 1.01, 2 moderate, 2.01 and 3 as severe, and 3.01 and 4 as very severe.

Central corneal aesthesiometry (Cochet-Bonnet) was also performed in both eyes of 20 patients (40 eyes). The test started with the aesthesiometry at the maximal length of 60 mm, reducing it gradually until a positive response (blinking) was obtained.

We performed impression cytology (IC) on 26 eyes in 16 patients using 5-x-5-mm nitrocellulose filter papers (Millipore Millicell-CM, 0.4 µm pore size). Samples were obtained from the central cornea and the superotemporal area of the bulbar conjunctiva. A histological examination was performed according to Tseng’s staining protocol\textsuperscript{19}, and microscopic assessment was performed by a single pathologist using Tseng’s classification system\textsuperscript{19}.

The main factors studied were as follows: the degree of aniridia-related keratopathy, age, and history of eye surgery as well as the quantitative and qualitative tests of the ocular surface and cornea discussed above.

Statistical analysis was performed using SPSS, version 13.0, software and the Kruskal-Wallis non-parametric test for correlation analyses. A p value <0.05 was considered statistically significant.

![Figure 2. Aniridia-related keratopathy at different stages shown on direct (left column) and retro illumination (right column). Grade 0: Peripheral and central cornea not affected. Grade 1A: Partial affection of limbus. Grade 1B: Near total affection of the limbus without central opacification. Grade 2: 360 degree affection of the limbus with central corneal opacification.](image)

Table 1. Dry eye score and the different parameters used to assess the cornea and ocular surface. The parameters chosen are based on the Delphi Panel report.

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TFBUT = Tear film break-up time. Table adapted from International Dry Eye Workshop (DEWS, 2007b)
RESULTS

Prevalence of ARK

The prevalence of aniridia-related keratopathy (ARK) according to Mackman’s classification was as follows: grade 0 in 12% (3 of 25 patients), grade 1-A in 52% (13 of 25 patients), grade 1-B in 20% (5 of 25 patients), and grade 2 in 16% (4 of 25 patients). Figure 2 shows the different stages of aniridia-related keratopathy.

ARK and age

The mean ages of patients based on the degree of ARK were as follows: 12.66 (range: 4 to 22; SD: ± 8.06) for grade 0, 17 (range: 5 to 54; SD: ± 14.42) for grade IA, 22.8 (range: 3 to 47; SD: ± 20.57) for grade IB, and 39.5 (range: 33 to 46; SD: ± 5.58) for grade 2. We found a statistically significant correlation between age and aniridia-related keratopathy (Kruskal-Wallis test: p=0.000) (Figure 3).

ARK and history of ocular surgery

In correlating ARK with history of ocular surgery, we found that 88% (22 of 25) of the patients who had previously undergone ocular surgery (penetrating keratoplasty, keratectomy, limbal transplant, trabeculectomy, phacoemulsification with intraocular lens implantation and strabismus surgery) presented with some degree of ARK. We found a statistically significant correlation between ocular surgery and the grade of aniridia-related keratopathy (Kruskal-Wallis test: p=0.014).
Regarding tear production, we obtained the following results: Schirmer’s test I (without anaesthesia) was normal in 33 of 38 eyes (86.8%), with a mean value of 23.03 mm in 5 min (range: 4–30; SD: ± 8.26). Schirmer’s test II (with anaesthesia) was normal in 34 of 36 eyes (94.4%), with a mean value of 22.36 mm in 5 min (range 2–30; SD: ± 7.49) Figure 4 shows the frequency distribution in our study population.

When we evaluated the lipid layer of the tear film, TFBUT was reduced (<10 seconds) in 16.7% (4 of 24 eyes). Figure 5 shows the frequency distribution in our study population.

Corneal fluorescein staining (based on the Oxford scheme) was performed on 48 eyes in 24 patients and was found to be abnormal in 26 of the 48 eyes. We found grade 0 in 22 eyes (45.8%), grade 1 in 17 eyes (35.4%), grade 2 in 5 eyes (10.4%), and grade 4 in 4 eyes (8.3%). None of the patients had grade 3 or grade 5 staining. Figure 6 shows the frequency distribution with respect to oxford scheme. Rose Bengal staining was performed in the 46 eyes of 23 patients and was abnormal in 21 (45.7%). We found grade 0 in 25 eyes (54.3%), grade 1 in 11 eyes (23.9%), grade 2 in 6 eyes (13%), grade 3 in 3 eyes (6.5%), and grade 5 in 1 eye (2.2%). None of the patients had a score between 6 and 9. Figure 7 shows the frequency distribution of the Van Bijsterveld scheme.

We graded dry eye syndrome by calculating the dry eye score as discussed above; we obtained the following results: 10 of 48 eyes (20.83%) had a dry eye score between 0 and 0.02 (normal), 28 of 48 eyes (58.3%) between 0.021 and 1 (mild), 8 of 48 eyes (16.7%) between 1.01 and 2 (moderate), and 2 of 48 eyes (4.17%) between 2.01 and 3 (severe). It was not possible to perform the ocular surface tests in one patient due to young age (4 years old). Figure 8 shows the correlation between frequency distribution of the dry eye score in our study population. We found a statistically significant correlation between the ARK grade and dry eye score (Kruskal-Wallis test: p=0.026). See Figure 9.

The average results of central corneal aesthesiometry (Cochet-Bonnet) performed on 40 eyes in 20 patients with respect to ARK were: grade 0 (median: 32.50 mm; range: 5–60); grade 1A (median: 50 mm; range: 5–60); grade 1B (median: 10 mm; range: 0–40); and grade 2 (median: 10 mm; range: 5–60). As a result, we found a statistically significant correlation between ARK grade and aesthesiometry (Kruskal-Wallis test; p=0.043). Figure 10 shows the frequency distribution of esthesiometry in our study population.

Central corneal thickness was greater than the normal average thickness in all cases (36 eyes of 18 patients), with the following results with respect to ARK grade: grade 0 (median: 627.50 microns; range: 611–644); grade 1A (median: 639.50 microns; range: 611–644); grade 1B (median: 640.50 microns; range: 611–644); grade 2 (median: 640.50 microns; range: 611–644).
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602–697); grade 1B (median: 638 microns; range: 590–749), and grade 2 (median: 672 microns; range: 587–1120). However, we did not find a statistically significant correlation between ARK grade and central corneal thickness (Kruskal-Wallis test; p=0.052). Figure 11 shows the frequency distribution of corneal pachymetry in our study population.

Tear ferning pattern

The tear ferning patterns were as follows: grade I in 20% (4 of 20 eyes), grade II in 50% (10 of 20 eyes), grade III in 20% (4 of 20 eyes), and grade IV in 10% (2 of 20 eyes) of patients. This means that 80% of patients had abnormal tear ferning patterns.

We did not find a significant correlation between the number of conjunctival goblet cells and tear ferning pattern test results (Kruskal-Wallis test; p=0.227) or between ARK and ferning pattern grades (Kruskal-Wallis test; p=0.707). Figure 12 shows the frequency distribution of the ferning patterns in our study population.

Impression cytology

Conjunctival impression cytology was performed on 26 eyes in 16 patients. Based on Tseng's grading system, 6 eyes (23.1%) had grade 0; 2 eyes (7.7%) had grade 1; 5 eyes (19.2%) had grade 2; 10 eyes had grade 3 (38.5%); and 3 eyes (11.5%) had grade 4 of conjunctival metaplasia. We did not find a significant correlation between the degree of conjunctival metaplasia and aniridia-related keratopathy (Kruskal-Wallis test; p=0.244).

We obtained adequate corneal samples from only 21 of 26 eyes (80.77%) because of advanced corneal degenerative changes, such as nodules in the 5 remaining eyes. We evaluated the presence of goblet cells on the corneal surface in order to diagnose limbal stem cell deficiency presenting clinically as conjunctivalization. Goblet cells were present in 3 of 21 corneas studied (14.3%). We found a weakly positive significant correlation between the presence of goblet cells on the cornea and aniridia-related keratopathy (Kruskal-Wallis test; p=0.052).

In 4 conjunctival samples, there were fewer than 50 conjunctival goblet cells per mm². In this group, 2 eyes (50%) had mild keratinization, and 1 eye (25%) had moderate keratinization. One eye (25%) had no conjunctival keratinization. We did not find a statistically significant correlation between conjunctival keratinization and ARK grade (Kruskal-Wallis test; p=0.279).

ARK and endothelial cell count

While technically difficult due to nystagmus, we were able to perform specular microscopy on 13 eyes in 9 patients. Mean endothelial cell density was 2190.84 ± 512.13 cells/mm² (range: 1332–2850 cells/mm²).

DISCUSSION

The PAX6 gene is expressed in the ectoderm during embryogenesis and plays an important role in organizing the development and differentiation of ocular structures, including the cornea, lens, ciliary body, and retina. It is also important in central nervous system development. It is located in the p13 region of chromosome 11, and a mutation here causes the appearance of aniridia. A number of studies found no relationship between various mutations and clinical features; in some cases, patients with the same mutation present with different clinical features and degrees of illness.

Studies of the characteristics of the ocular and corneal surface in congenital aniridia patients have revealed a pathophysiological mechanism set in motion by dysfunction in limbal stem cells. The studies demonstrated a reduction in desmoglein as well as the beta- and alpha-catenin adhesion molecules, leading to epithelial fragility and subsequent, persistent epithelial erosion and defects. The regulation of matrix metalloproteinase gelatinase B (gelB or MMP-9)
depends on the PAX6 gene, and defects in PAX6 lead to fibrin accumulation and infiltration by inflammatory cells; this, in turn, generates stromal opacities and stimulation of neovascular proliferation.17,28.

Prevalence of ARK

In our study, we found that majority of patients with congenital aniridia had some degree of ARK (Mackman’s classification), evidenced by clinical signs such as peripheral thickening and vascularization of the cornea. This high prevalence is consistent with the findings of other authors.10 In contrast, other studies reveal a lower 20% prevalence of ARK, usually associated with dysfunction in limbal stem cells.6,29.

ARK and age

We observed that age positively correlates to the degree of ARK. Other studies also emphasize that the severity of ARK increases with age. In a retrospective study of 124 patients with aniridia, the authors found a positive correlation between the stage of keratopathy and age (p<0.001), even when using a different classification system for aniridic keratopathy based on 5 stages (0 to 5).30 In another retrospective study in 20 patients (36 aniridic eyes), it was observed that the older the patient, the higher the grade of ARK (Mackman’s classification), with a significant statistical correlation (p=0.002). This correlation is expected since limbal stem cell deficiency, the pathophysiological cause of aniridia-related keratopathy, is a progressive disease.

ARK and history of ocular surgery

Our study suggests a strong relationship between patients with a history of ocular surgery and the degree of ARK (p=0.014). Several studies have demonstrated that, due to the great fragility of the corneal epithelium, any kind of trauma, such as eye surgery, can affect homeostatic balance. This can be evidenced through corneal deterioration and the asymmetry of corneal disease, found in eyes that have undergone ocular surgery. This must be taken into account when deciding when to perform surgery in these patients, since risks may outweigh benefits. The same authors found that patients who underwent corneal and ocular surface surgeries such as penetrating keratoplasty and limbal transplant, in the long term (more than five years after surgery) have the same visual prognosis as patients who did not undergo the same surgery. This is explained by the severe fibrotic reaction observed in these cases, in which graft failure, both corneal and limbal, is observed much earlier despite adequate immunosuppression.12 We therefore suggest a less aggressive approach when deciding the surgical treatment of aniridia-related keratopathy.

Schirmer’s test I and II

In assessing the corneal and ocular surfaces, we found that Schirmer’s test I and II were normal in almost 90% of the eyes studied, respectively. Several studies also report normal Schirmer’s test results in patients with congenital aniridia, suggesting that in these patients aqueous deficiency is not the major component of dry eye disease.

TFBUT

We obtained normal results for TFBUT in the majority of our study population. Our results are similar to those reported by Eden et al., where TFBUT was altered in 41% of cases, with no significant correlation between TFBUT and keratopathy (right eye: p=0.186/ left eye: p=0.308). However, other studies found altered TFBUT (<10 seconds) in most patients, with values ranging from 72% to 80.6%. TFBUT testing is an observer-dependent test and may vary depending on the concentration of fluorescein drops, ambient lighting and atmospheric conditions. Perhaps a better way to assess the lipid layer of the tear film is using the tear film interferometer (Tearscope®, Keeler Inc., USA), since it avoids the use of fluorescein drops. Another parameter to study in this respect is meibomian gland function, which takes into account the ease of expression, quality and level of meibomian gland secretion.

Vital staining

We evaluated corneal staining using fluorescein dye (Oxford scheme) to promptly detect the presence of corneal changes, even in their earlier stages (1A and 1B). In our study, corneal fluorescein staining was abnormal in more than half of the eyes studied according to the Oxford scheme. Conjunctival staining with Rose Bengal (van Bijsterveld schema) was also performed to stain areas with poor protection of surface epithelium. In our study, results were abnormal in almost half of the studied eyes. In a retrospective study, Jastaneiah et al. found that fluorescein and Rose Bengal staining were present in 31 of 34 aniridic eyes with 1B and 2 corneal disease. Rivas et al. also found altered Rose Bengal staining in 30 patients (83%) with congenital aniridia. In our study, 45.7% of patients showed positive Rose Bengal staining, while Rivas found abnormal values in 83% of patients. This may be explained by the younger population included in our study, where the mean age was 20.04 (SD ± 15.7). In comparison, the mean age in the Rivas et al. study was 42 (SD ± 15.3). Here, we stress the importance of using Rose Bengal, or lissamine green as an alternative, since it detects...
the presence of devitalized tissue which may be missed when using only fluorescein drops.

**ARK and dry eye severity score**

We evaluated the severity of dry eye using the dry eye score, Schirmer's test, TFBUT and vital staining. We found that the dry eye score increased as ARK worsened. This is in accordance with Jastaneiah et al. who also found a statistically significant correlation between the severity of corneal involvement and the extent of dry eye disease. This is expected, as the lower the quality and quantity of tears, the greater the damage to the cornea and ocular surface. Therefore, treatment of dry eye must be based on these parameters in individual cases. If aqueous deficiency is present, abundant tear substitute must be prescribed. If evaporative dry eye is present, lipid-containing tear substitute and dietary supplements containing essential fatty acids must be prescribed and meibomian gland dysfunction must be treated with lid hygiene.

**ARK and aesthesiometry**

Corneal sensitivity was assessed using the Cochet-Bonnet aesthesiometer showing a significant correlation between ARK and aesthesiometry results. Our results are in accordance with the Eden et al. study in which corneal sensitivity was tested using a thin cotton-tipped applicator in 63 of 221 aniridic eyes (29%). They found a significant positive correlation (p=0.001 for the right eye, and p=0.005 for the left eye) between keratopathy and sensitivity. The decrease in corneal sensitivity could be secondary to neurotrophic phenomena; corneal sensitivity is now being studied in the context of various types of stimuli. To our knowledge, this is the only study to date confirming Eden's results, offering a more objective and quantifiable method of measuring corneal sensitivity.

**ARK and corneal thickness**

Regarding central corneal thickness in congenital aniridia patients, we found higher than normal median values for all grades of ARK, which has been already described in other studies. This contrasts with findings in patients with aetiologically different dry eye syndrome that resulted in reduced corneal thickness. However, there was no statistically significant correlation between ARK and pachymetry, perhaps because of the small number of cases (36 eyes in 18 patients). However, a number of factors should be considered. Firstly, most patients with advanced keratopathy would have thicker corneas due to epithelial conjunctivalization. Secondly, PAX6 mutations cause altered arrangement of collagen fibres resulting from altered metalloproteinases (MMP) that lead to stromal opacities. Since the stroma is the thickest layer of the cornea, this would contribute to increased corneal thickness. The authors are presently performing a parallel study of the histopathological characteristics of aniridia corneal buttons to determine which corneal layer contributes to increased thickness in these patients.

**Tear ferning pattern test**

We carried out the tear ferning pattern test to assess the mucous layer of the tear film and found that it was abnormal in 80% of our patients.

In previous reports, it was shown that many major sources of tear film mucin exist, even in cases of decreased goblet cell density, for example, lacrimal gland and non-goblet epithelial cells from the conjunctiva. As previously discussed in the ARK and dry eye score section, attention must be paid to the deficiency of the tear film layers in individual cases, and appropriate treatment for the corresponding deficiency must be provided, whether involving aqueous, lipid or mucin deficiency. To our knowledge, this is the first study describing the use of the tear ferning pattern test in patients with congenital aniridia.

**Impression cytology**

When considering Tseng’s classification, we found that 73% had conjunctival squamous metaplasia. Jastaneiah et al. found squamous metaplasia in all 23 aniridic specimens (100%), but all had corneal involvement seen clinically according to Mackman’s classification (2 eyes had stage 1A, 21 eyes had stage 1B, and 13 eyes had stage 2). Rivas et al. also found that none of the eyes with congenital aniridia had grade 0 or 1 squamous metaplasia (36 eyes of 18 patients), demonstrating higher degrees of squamous metaplasia in their study. However, Rivas did not include the clinical classification of corneal involvement. That our results demonstrate lower degrees of squamous metaplasia compared to the Jastaneiah and Rivas studies may be explained by our lower mean population age.

Regarding conjunctival goblet cell density, we observed a decrease in the number of goblet cells as squamous metaplasia increased. In our study, we found a decrease in goblet cell density in more than half of the cases which were at stages 0 and 1A according to Mackman’s classification. This is in accordance with Rivas et al. who also found a significant decrease in conjunctival goblet cell density in 20 of 36 aniridic eyes. However, their study did not take into account the clinical features of aniridic keratopathy. On the other hand, Nishida et al. found a statistically significant increase in conjunctival goblet cell density in 16 aniridic
eyes compared with the control group, and all of them had clinical corneal opacification and vascularization in either the peripheral or entire cornea. Jastaneiah et al. also found statistically significant increased conjunctival goblet cell density with mucinous hyperplasia in 23 eyes. However, their study population demonstrated more advanced degrees of ARK, as most of them were at stages 1B and 2 according to Mackman’s classification. Nishida et al. and Jastaneiah et al. may have found increased goblet cell density because their reports include more advanced ARK stages than our study.

Another point of discussion is the lower goblet cell density in the superior bulbar conjunctiva compared with the inferior bulbar area. Nishida took samples from the inferior conjunctival epithelium, while Rivas obtained samples from the superior and inferior bulbar conjunctivas. In our patients, we obtained samples from the superotemporal bulbar conjunctiva. It follows that the anatomical site where the samples were taken from may be the cause of the difference in conjunctival goblet cell densities in several reports.

In our study, we found 2 eyes with decreased conjunctival goblet cell density (less than 50 cells per mm²), but with marked keratinization (one mild case and the other moderate). This confirms that conjunctival squamous metaplasia is a dynamic process and that the major histopathological processes (i.e. loss of goblet cells, cellular stratification and keratinization as discussed by Tseng, observed microscopically to grade metaplasia) may overlap and are not mutually exclusive.

Conjunctival impression cytology demonstrated a weak but significant correlation between the presence of goblet cells in the cornea and ARK. If we consider that limbal stem-cell deficiency contributes to ARK, as discussed above, the probability of finding conjunctival goblet cells on the cornea increases when assessing eyes with more advanced ARK (grades 1B and 2).

It is therefore important to carry out conjunctival and corneal impression cytology to detect changes early on, estimate the severity and progression of the disease, assess treatment efficacy and prevent deterioration in these patients, given that metastasic changes in response to chronic ocular surface damage take place even in the presence of moist epithelia.

**ARK and endothelial cell count**

In our study, the mean endothelial cell count was normal in all eyes where we were able to perform the test, measuring 2190.84 cells/mm² (SD ± 512.13). Our results are similar to those of Weiss et al. In this study specular microscopy photographs in 3 eyes of 9 patients with congenital aniridia were obtained. Peripheral endothelial cell density and morphology were normal; they found cornea guttata and atypical endothelial cells only in older patients and those who underwent previous ocular surgery. Another study reported endothelial cell counts of 3,397/mm² in the right eye and 3,434/mm² in the left eye of a 16-year-old patient with normal structure. They suggest that endothelial dysfunction does not increase corneal thickness in the presence of a mutated PAX6 gene. Our study is the first to present the results of endothelial cell count in a significant number of patients with this rare congenital disorder. This is quite an unexpected finding since, embryologically, the endothelium is closely related to the iris, lens and angle structures, which are, in general, affected in patients with congenital aniridia and manifest as cataracts and glaucoma. As previously discussed, the same authors are carrying out a parallel study of aniridic corneal buttons to correlate our clinical findings with histopathological evidence.

**CONCLUSION**

Congenital aniridia is a rare ocular disease affecting the cornea and ocular surface. In our study, age, history of ocular surgery and dry eye severity correlated positively with the progression of ARK. Corneal sensitivity was decreased, corneal pachymetry was increased, and corneal endothelial cell count was normal in the majority of cases. The tear ferning pattern test showed decreased mucin production, and impression cytology revealed that squamous metaplasia precedes ARK. Consequently, if the grade of ARK correlates to the severity of dry eye disease, it becomes very important to perform impression cytology for early detection of signs and symptoms of dry eye disease in an attempt to prevent the onset or the progression of ARK and delay visual deterioration in this group of patients. ARK is a multifactorial disease. Simple and basic tests to evaluate the ocular surface must be performed to determine the proper treatment for each individual case.

**BIBLIOGRAPHY**

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