Assessment of Ocular Surface in Patients with Alopecia Areata

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Abstract

Introduction: An autoimmune condition called alopecia areata (AA) has been linked to ocular complications. There is not enough information on changes in conjunctival cytology in AA. **Objectives:** to evaluate the value of conjunctival impression cytology (CIC) in diagnosing ocular surface changes in AA patients and link the results with the disease severity. **Patients and Methods:** This case-control research was conducted on 50 participants who were divided into 2 groups: Group 1: included 25 patients with AA; group 2: included 25 age- and sex-matched healthy controls. For assessment of the ocular surface, both groups were subjected to the routine ophthalmological assessment, fluorescein tear breakup time [TBUT], Schirmer test, and CIC. **Results:** The patient group showed CIC grades 0, 1, 2, and 3 in 20.0%,40.0%,24.0%, and 16.0% of patients, respectively. In the control subjects, CIC results showed that all subjects showed grades 0, and 1 in 76.0% and 24.0%, respectively and none of them reported grades 2 and 3. A highly statistically significant difference was found between both groups regarding CIC results with a P-value < 0.000. A statistically significant relation was found between CIC results and SALT score. As regards the results of the TBUT and Schirmer tests, the patient group showed a statistically significantly lower mean score for both tests compared to the control group. **Conclusion:** Examining the ocular surface using impression cytology is a non-invasive procedure. Cell morphology can be utilized to recognize and rate the seriousness of conjunctival changes in patients with AA. Studying the possible underlying mechanisms is required further.

Key Words: Conjunctival Impression Cytology; Dry eye; Tear Breakup Time test; Schirmer test; alopecia areata.

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Introduction:

Alopecia areata (AA) is an autoimmune, recurring inflammatory condition that affects the hair follicles (HFs) and causes non-scarring, asymptomatic hair loss. AA, which is said to affect about 2% of the population overall and affects people of both sexes of any age, can impact anyone [1]. Numerous etiopathogenetic variables, including psychological, genetic, and immunological ones, have been connected to AA [2]. The loss of immune privilege is related to the destruction of HFs in AA. Due to the buildup of HF autoantigen-recognizing autoreactive T helper and T cytotoxic cells inside and around hair bulbs, hair loss and a subsequent autoimmune reaction develop [3-4].

One of the comorbidities linked to AA that has been the subject of numerous investigations is ocular surface disorders. Retinal and lenticular abnormalities are the most noticeable findings [5–6]. However, there are still few investigations on ocular surface findings and tear function affection in AA [7]. A quick and non-invasive method for determining alterations in conjunctival epithelial cells is conjunctival impression cytology (CIC). This technique can assess the goblet and epithelial cells of the conjunctiva's nuclear and cytoplasmic morphological properties [8].

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CIC has been used in several investigations to assess ocular surface conditions such as keratoconjunctivitis sicca, superior limbic keratoconjunctivitis, vernal keratoconjunctivitis, vitamin A insufficiency, atopic illness, and cicatricial pemphigoid [9].

The best that we can tell, conjunctival cytology alterations in AA patients have been documented by a single study. Additionally, no prior studies have looked into the relationship between the CIC results and the severity of AA. Therefore, according to insufficient data, the purpose of this study was to determine how AA affected ocular surface and conjunctival cytology and whether there was a relationship between the severity of the disease and ocular surface affection as well as CIC results.

Patients & Methods:

This case-control research was conducted over a period of 4 months on 50 participants including 2 groups. The patient group included 25 AA patients selected from the dermatology department and outpatient clinic of Al-Zahraa University Hospital, Cairo, Egypt. The control group included 25 age- and sex-matched healthy individuals. All participants received referrals to the ophthalmology division for eye exams. Informed written consent was obtained from all patients or their parents after obtaining the approval of the Research Ethics Committee of our University.

Inclusion criteria: Both male and female patients with any clinical varieties of AA were included.

Exclusion criteria: Included those with hypertension, Sjogren's syndrome, any kind of conjunctivitis or keratitis, a history of refractive and intraocular abnormalities, patients with diabetes mellitus, or other autoimmune illnesses that affect the eyes. Additionally, individuals who had suffered from thermal or chemical damage to the ocular surface, and those who were wearing contact lenses or receiving any topical treatments that would have an impact on the Meibomian gland's or the tear film's activities were not included. Patients utilizing eye drops within the previous three months, receiving systemic corticosteroids, immunosuppressive, diuretic, anti-inflammatory, or anticholinergic medications, or taking any of these drugs were also excluded.

In order to rule out the presence of any other autoimmune disorders alongside AA, patients with the condition underwent thorough history-taking as well as general and dermatological examinations. To identify the clinical categories of the AA patches, a local evaluation of the AA patches' location, number, and size was conducted. AA patches' specific dermoscopic characteristics were assessed using polarised light dermoscopy (Derm Lite, 3 Gen, USA) to confirm the diagnosis. Nails were examined to detect changes associated with AA such as pitting, Beau's lines, trachyonychia, and onychorrhexis. Clinical assessment of AA severity was performed using the Severity of Alopecia Tool score system (SALT) [10].

All subjects underwent ophthalmologic examinations, which included evaluations of their visual acuity, slit-lamp examinations, and ocular surfaces. The tear break-up time (TBUT) and Schirmer tests were done followed by the CIC test performed on the following day. All subjects' right eyes were chosen for study. To measure the tear break-up time (TBUT) test, the examiner used a cobalt-blue filter with a slit-lamp examination. A sterile strip containing sodium fluorescein was placed in the conjunctival inferior sac. The TBUT value was determined by measuring the amount of time between the first dry spot and the last full blink. Three further measurements were taken to arrive at the mean value. The tear film was considered unstable if the value was less than 10 seconds. Schirmer I test was performed after 30 minutes using strips of filter paper (Whatman, UK) without anesthesia. These strips were applied to one-third of the lower eyelid. Five minutes later, the test strip's wetted portion was measured, and the Schirmer I value was calculated in millimeters. One day after performing the previous test, the CIC test was done using filter papers of cellulose acetate Conjunctival Impression Cytology [Millipore filter paper, Sartorius Stedim Biotech, Germany] was cut into small strips [5 mm × 5 mm] that were applied to collect the specimen. Applying light pressure across the lower temporal bulbar conjunctiva, the utilized filter sheets were located three millimeters from the limbus. Filter papers' moistened portions containing epithelial cells were fixed in a solution that contained glacial acetic acid, ethyl alcohol (70%), and formaldehyde (37%), at a 1:1:20 volume ratio for about 10 minutes. After that, periodic Acid-Schiff (PAS) and hematoxylin–eosin (H&E) staining was performed on the fixed samples. A pathologist evaluated the test results and rated them using Nelson's specified

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standards[11].

Statistical analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations, and ranges when parametric and median, and an interquartile range (IQR) when data was found non-parametric. Also, qualitative variables were presented as numbers and percentages. The comparison between groups regarding qualitative data was done by using the Chi-square test and/or Fisher exact test when the expected count in any cell was found to be less than 5. The comparison between two independent groups with quantitative data and parametric distribution was done by using an independent t-test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, P-value < 0.05 was considered significant (S).

RESULTS

The patient group included 15 (60.0%) females and 10 (40.0%) males with a mean age of 25.32 ± 10.12 years (range 9–45), while the control group included 13 (52.0%) females and 12 (48.0%) males with a mean age of 27.48 ± 7.76 years (range 8–40). No statistically significant difference was found regarding the age and sex distribution between both groups (P-value > 0.05) **(Table 1).**

Duration of AA in the patient group ranged from 3-36 months (Mean ± SD=12.76 ± 8.32). The mean SALT score was 17.58 ± 16.39 (range 1.8 – 56.5). Other clinical characteristics of AA patients was demonstrated in **Table 1.**

On ophthalmological assessment using the slit-lamp examination of the selected eyes, no abnormal or significant findings were observed affecting the conjunctiva of both groups. The mean TBUT score in the patient group was 9.16 ± 2.91 (range 6-15) seconds compared to 13.76 ± 1.69 (range 11 – 16) seconds in the control group with a highly statistically significant difference between both groups (P-value = 0.000). The patient group showed a mean Schirmer test score of 8.96 ± 2.17(range 6 – 14) mm compared to 13.56 ± 1.50 (range 11 – 17) mm in the control group with a highly statistically significant difference between both groups (P-value = 0.000). Visual acuity assessment revealed a statistically non-significant difference between both groups **(Table 2).**

The results of CIC in the patient group showed grade 0 in 5 (20.0%), grade 1 in 10(40.0%), grade 2 in 6(24.0%), and grade 3 in 4 (16.0%) patients. In the control group, grade 0 was found in 19 (76.0%), grade I in 6 (24.0%) subjects, and none of them showed grade 2 and grade 3 (0.0%). These results revealed a highly statistically significant difference between both groups (P-value = 0.000) **(Table 2), (Figures 1,2,3, and 4).**

There was a statistically significant relation between CIC results and the duration of disease, type of AA, and SALT score (P-value = 0.002, 0.028, and 0.000), respectively. Also, we observed a highly statistically significant relation between CIC results and TBUT test score, Schirmer test score, and visual acuity. No statistically significant relation was found between CIC results and other clinical and demographic data of the patient group **(Table 3).**

A significant positive correlation between the TBUT test and the Schirmer test was observed (r= 0.876, p-value=0.000). Also, we found a statistically significant negative correlation between the SALT score and both the TBUT test and the Schirmer test scores(p-value=0.000) **(Figures 5,6, and 7).**

Table (1): Demographic and clinical data of the studied groups:

		Patient group No. = 25	Control group No. = 25	Test value	P-value	Sig.
Gender	Female	15 (60.0%)	13 (52.0%)	0.325*	0.569	NS
	Male	10 (40.0%)	12 (48.0%)			
Age	Mean ± SD	25.32 ± 10.12	27.48 ± 7.76	0.847•	0.401	NS
	Range	9 – 45	8 – 40			
Duration (m)	Mean ± SD	12.76 ± 8.32	–	–	–	–
	Range	3 – 36				
Type of alopecia areata	Single patch	12 (48.0%)	–	–	–	–
	Multiple patches	12 (48.0%)	–			
	Alopecia totalis	1 (4.0%)	–			
SALT score	Mean ± SD	17.58 ± 16.39	–	–	–	–
	Range	1.8 – 56.5				
Nail affection	Negative	11 (44.0%)	–	–	–	–
	Positive	14 (56.0%)				
Family history	Negative	15 (60.0%)	–	–	–	–
	Positive	10 (40.0%)				

P-value > 0.05: Non significant; **P-value < 0.05:** Significant; **P-value < 0.01:** Highly significant; ***: Chi-square test**; **•: Independent t-test**; **SALT:** Severity of Alopecia Areata Tool.

Table (2): Comparison between the studied groups regarding the TBUT test, Schirmer test, visual acuity, and conjunctival impression cytology.

		Patients group	Control group	Test value	P-value	Sig.
		No. = 25	No. = 25			
TBUT (second)	Mean ± SD	9.16 ± 2.91	13.76 ± 1.69	6.833•	0.000	HS
	Range	6 – 15	11 – 16			
Schirmer test (mm)	Mean ± SD	8.96 ± 2.17	13.56 ± 1.50	8.716•	0.000	HS
	Range	6 – 14	11 – 17			
Visual acuity	Mean ± SD	0.45 ± 0.17	0.58 ± 0.30	1.985•	0.053	NS
	Range	0.1 – 0.6	0.1 – 1			
Conjunctival impression cytology	Grade 0	5 (20.0%)	19 (76.0%)	19.167*	0.000	HS
	Grade I	10 (40.0%)	6 (24.0%)			
	Grade II	6 (24.0%)	0 (0.0%)			
	Grade III	4 (16.0%)	0 (0.0%)			

P-value > 0.05: Non significant; **P-value < 0.05:** Significant; **P-value < 0.01:** Highly significant
***: Chi-square test; •: Independent t-test** **TBUT:** Tear break-up time

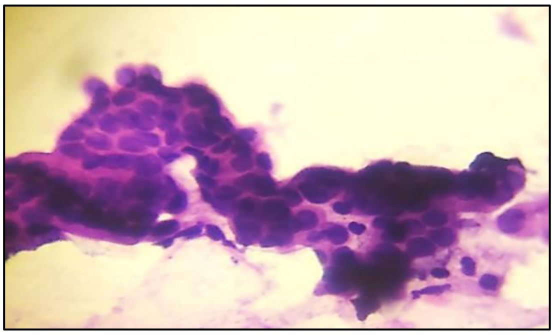

Figure (1): Nelson's grade 0 H&E stained slide (400x) with a normal number and morphology of conjunctival epithelial cells; the epithelial cells are small, round, and with large nuclei.

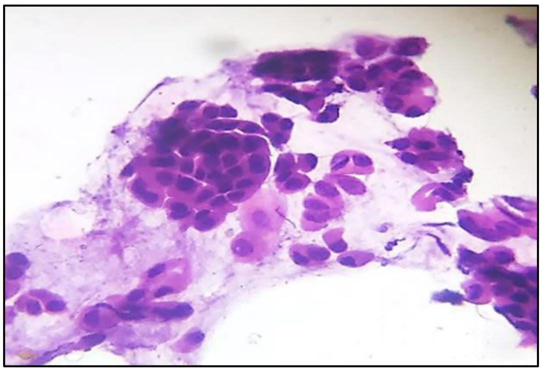

Figure (2): Nelson's grade 1: H&E stained slide (400x) with mildly increased eosinophilic cytoplasm in slightly larger epithelial cells which become more polygonal with smaller nuclei.

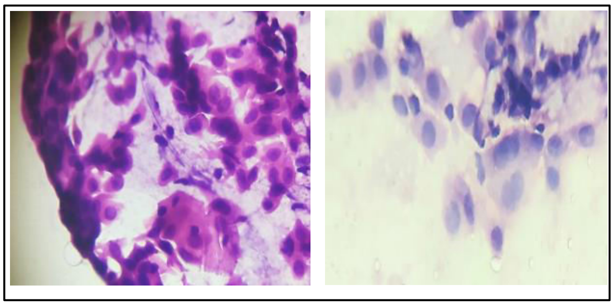

Figure (3): Nelson's grade 2, H&E stained slide (400x) with moderately decreased N/C ratio and reduced goblet cells. The epithelial cells become larger and more polygonal with smaller nuclei than grade 1.

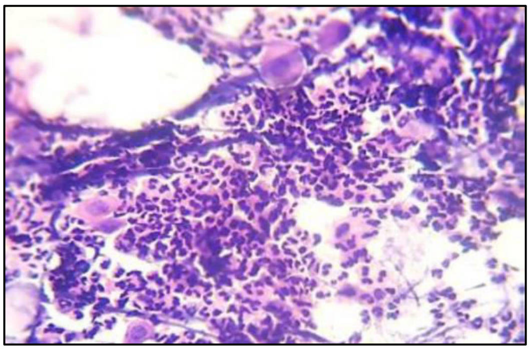

Figure (4): Nelson's grade 3, H&E stained slides (200x) with squamous metaplasia (with markedly decreased N/C ratio with small pyknotic nuclei (arrow) and goblet cell depletion.

Table (3): Relation between conjunctival impression cytology and clinical/demographic data of patient group.

		Impression cytology				Test value	P-value	Sig.
		Grade 0	Grade I	Grade II	Grade III			
		No. = 5	No. = 10	No. = 6	No. = 4			
Gender	Female	4 (80.0%)	7 (70.0%)	3 (50.0%)	1 (25.0%)	3.542*	0.315	NS
	Male	1 (20.0%)	3 (30.0%)	3 (50.0%)	3 (75.0%)			
Age	Mean ± SD	22.40 ± 9.76	21.50 ± 10.01	28.33 ± 5.96	34.00 ± 12.30	1.990•	0.146	NS
	Range	12 – 33	9 – 40	21 – 38	21 – 45			
Duration (m)	Mean ± SD	7.30 ± 3.34	11.60 ± 10.33	15.67 ± 3.88	23.50 ± 9.00	6.762•	0.002	HS
	Range	3 – 12	6-30	10 – 20	16 – 36			
Type of alopecia areata	Single patch	4 (80.0%)	7 (70.0%)	1 (16.7%)	0 (0.0%)	14.132*	0.028	S
	Multiple patches	1 (20.0%)	3 (30.0%)	5 (83.3%)	3 (75.0%)			
	Alopecia totalis	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (25.0%)			
SALT score	Mean ± SD	2.62 ± 0.92	8.39 ± 4.12	30.82 ± 15.77	39.38 ± 6.62	22.477•	0.000	HS
	Range	1.8 – 3.6	4.2 – 17.1	12.2 – 56.5	32.2 – 48.2			
Nail affection	Negative	3 (60.0%)	6 (60.0%)	1 (16.7%)	1 (25.0%)	3.964*	0.265	NS
	Positive	2 (40.0%)	4 (40.0%)	5 (83.3%)	3 (75.0%)			
Family history	Negative	5 (100.0%)	7 (70.0%)	2 (33.3%)	1 (25.0%)	7.569*	0.056	NS
	Positive	0 (0.0%)	3 (30.0%)	4 (66.7%)	3 (75.0%)			
TBUT (second)	Mean ± SD	14.40 ± 0.55	8.90 ± 0.88	7.00 ± 0.00	6.50 ± 0.58	149.431•	0.000	HS
	Range	14 – 15	8 – 10	7 – 7	6 – 7			
Schirmer test (mm)	Mean ± SD	12.80 ± 1.10	8.70 ± 0.48	7.50 ± 0.55	7.00 ± 0.82	69.031•	0.000	HS
	Range	12 – 14	8 – 9	7 – 8	6 – 8			
Visual acuity	Mean ± SD	0.46 ± 0.15	0.57 ± 0.05	0.30 ± 0.18	0.35 ± 0.19	5.838•	0.005	HS
	Range	0.3 – 0.6	0.5 – 0.6	0.1 – 0.5	0.1 – 0.5			

P-value > 0.05: Non significant; **P-value < 0.05:** Significant; **P-value < 0.01:** Highly significant
***: Chi-square test; •: One Way ANOVA test**. **TBUT:** Tear break-up time

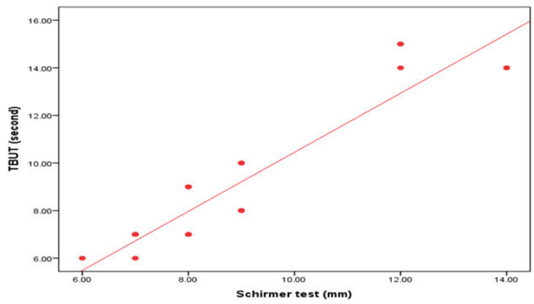

Figure (5): Significant positive correlation between the TBUT test and the Schirmer test.

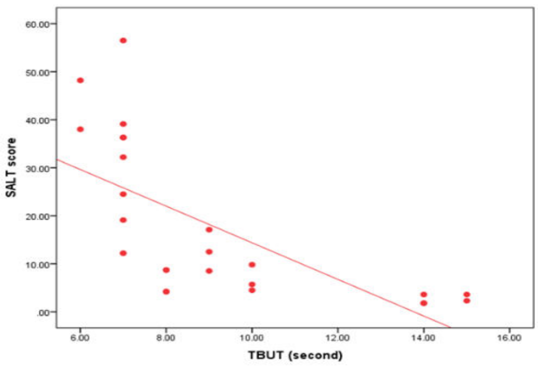

Figure (6): Significant negative correlation between the SALT score and TBUT test.

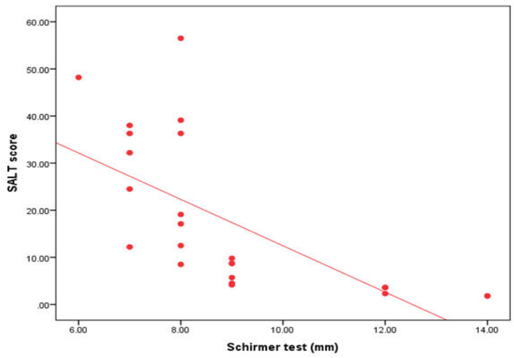

Figure (7): Significant negative correlation between the SALT score and the Schirmer test.

DISCUSSION

The outermost layer of ocular surface cells can be retrieved using the procedure known as impression cytology (IC) via the use of various types of filters. To diagnose dry eye illness, a minimally invasive technique is used to assess the shape of human conjunctival epithelial cells. [12]. It is simple to carry out and gives accurate information about the sampled area with little discomfort to the patient. Consequently, it provides a crucial basis for comprehending ocular surface problems [13]. Despite the paucity of studies on AA-related eye results, it has been noted that patients with AA have higher-than-average levels of CIC alterations in their conjunctival epithelium. They had a higher risk of developing dry eye disease [4].

Our results showed that patients with AA have more frequent ocular dryness than those in the control group. The current study revealed that the mean TBUT score in the AA group was 9.16 ± 2.91 (range 6-15) seconds compared to 13.76 ± 1.69 (range 11 – 16) seconds in the control group with a highly statistically significant difference between both groups (P-value = 0.000). The AA group showed a mean Schirmer test score of 8.96 ± 2.17(range 6 – 14) mm compared to 13.56 ± 1.50 (range 11 – 17) mm in the control group with a highly statistically significant difference between both groups (P-value = 0.000). Our findings suggested that AA patients in our study had problems in both tear production and stability. Unlike **Ergin et al.,** [14] who had reported that no statistically significant difference was observed between the two groups in terms of Schirmer I values, while a significant difference was observed between TBUT values (p = 0.001). Additionally, **Oltulu et al.** [4] observed that patients with AA had statistically lower TBUT scores but not significantly lower Schirmer I values than healthy controls. Instead of tear creation, they proposed a problem with tear stability. However, **Esmer et al.,** [15] used only Schirmer I test among dry eye parameters in AA patients and eyes healthy individuals. They have reported a statistically insignificant difference in the Schirmer values between AA and control groups.

The pathogenesis of both alopecia areata and dry eye syndrome is still unknown. However, it was proposed that autoimmune mechanisms mediated by T cells, environmental factors, and genetic predisposition could all play a part [16].

Regarding CIC results in our study, we reported cytological changes (grades 1+ 2+ 3) in 20(80%) patients compared to 6 (24.0%) subjects with grade 1 only in the control group. Our findings agreed with **Oltulu et al.,** [4] who reported CIC changes in 22/23(95%) AA patients while in the control group, they reported grades 1 and 2 in 11 (44%) and 1 (4%) subjects, respectively. In light of this, it is possible that the inflammatory process associated with AA is responsible for the loss or depletion of goblet cells and changes in squamous metaplasia in the conjunctival epithelium in AA patients. It was claimed that a number of inflammatory conditions, including ulcerative colitis and ankylosing spondylitis, had detrimental consequences on the ocular surface [17], [18].

According to the location, the structure of the conjunctival epithelium can range from a stratified squamous non-keratinizing epithelium (near the lid border) to a stratified columnar epithelium (bulbar). The density of goblet cells, which are unicellular mucus-secreting cells, varies in different regions of the conjunctiva, with the fornices and plica semilunaris having the highest density. The majority of conjunctival mucins are secreted by these cells [19]. The number of goblet cells in the conjunctival epithelium declines in dry eye disease, and the epithelial cells exhibit significant morphologic alterations. Squamous metaplasia is the most noticeable of these modifications. Although the cause of these pathological changes is not yet known, there is evidence that inflammation and apoptosis may be significant factors [20].

In the current study, we found a statistically significant correlation between the CIC results and the SALT score and disease duration. Patients with prolonged AA duration and patients with greater AA severity both had higher conjunctival cytological grading. Based on this research, it was hypothesized that CIC changes, including squamous metaplasia, may represent complications of the immune-mediated inflammatory process in AA rather than a direct cause of the illness. Moreover, CIC grading may be a marker of AA severity. Also, we found a significant negative correlation between both the TBUT test and the Schirmer test scores with the SALT score (p-value=0.000) indicating that eye dryness is exaggerated with increased disease severity. Furthermore, a strong correlation was found between the CIC findings and the outcomes of the TBUT and Schirmer tests. Lower TBUT and Schirmer scores were correlated with higher CIC grades. Similar to **Youssef et al.** [21], In our study, we also found a substantial positive association between the TBUT test and the Schirmer test.

The limitations of this study are the relatively small number of participants and the lack of an assessments of changes to the lenticular and retinal tissues.

In conclusion, research on eye findings in AA patients is insufficient, and the present study is one of the few that included a thorough evaluation of the anterior segment. In contrast to healthy controls, AA patients in our study have a higher frequency of eye dryness in terms of abnormalities in both tear stability and tear production. Furthermore, squamous metaplasia, which may be a consequence of the inflammatory process in AA, is more likely to develop in AA patients due to their increased susceptibility to cytological abnormalities. Additionally, CIC grading can be used to indicate the severity of AA. In AA patients with ocular involvement, early diagnosis and treatment can prevent dry eye symptoms and more serious problems. It is expected that more research, with a bigger patient population and healthy controls, and longer follow-up, be conducted in order to confirm the effects of AA on the ocular surface and dry eye disease. Further research is needed to determine any potential underlying pathogenic pathways linked to ocular surface affection in AA.

Conflict of Interest:

The authors declare that they have no competing interest.

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