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Uveitis

Identification of Subtypes of Herpetic Anterior Uveitis and Characterization of Their Clinical Features and Visual Outcome in a Chinese Population

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Received: November 11, 2024
Accepted: February 11, 2025
Published: April 1, 2025

Keywords: herpetic anterior uveitis; herpes simplex virus; varicella-zoster virus; cytomegalovirus; droplet digital PCR

Citation: Liu H, Guo S, Liu Y, Su G, Yang P. Identification of subtypes of herpetic anterior uveitis and characterization of their clinical features and visual outcome in a Chinese population. Transl Vis Sci Technol. 2025;14(4):2, https://doi.org/10.1167/tvst.14.4.2

Purpose: To identify the subtypes among patients clinically diagnosed as herpetic anterior uveitis (HAU) and characterize their clinical features and visual prognosis.

Methods: Three hundred and seventeen patients were clinically diagnosed as HAU in our department. Aqueous humor (AqH) and serum were collected from 43 of 317 HAU patients during eye surgery. Pathogens were identified using droplet digital polymerase chain reaction and the Goldmann–Witmer coefficient. The AqH levels of 10 inflammatory cytokines were measured. The demographics, clinical features, treatment, and visual prognosis of the subtypes of HAU identified by AqH analysis were analysed.

Results: DNA for herpes simplex virus (HSV), varicella-zoster virus (VZV), and cytomegalovirus (CMV) were identified in 13,18 and 12 eyes, respectively. The AqH levels of interleukin-13, interferon-γ, and tumor necrosis factor-α were significantly higher in VZV-AU as compared with HSV-AU and CMV-AU (all $P < 0.05$). In general, all these three subtypes of HAU had clinical features in common, including mutton-fat keratic precipitates usually toned with pigmentation, iris atrophy, elevated intraocular pressure (IOP), and posterior synechia with pupil pulling appearance unlike that caused by other uveitis. A much higher IOP and poor visual acuity at first visit were more commonly observed in VZV-AU and CMV-AU as compared with HSV-AU (both $P < 0.05$). A poor visual prognosis was noted in VZV-AU as compared with HSV-AU and CMV-AU ($P = 0.010$).

Conclusions: Our study identified three subtypes of HAU and characterized their clinical features. VZV-AU is frequently associated with much higher IOP and a poor visual prognosis.

Translational Relevance: We addressed the similarity and difference regarding clinical features and visual prognosis among three subtypes of HAU and also found droplet digital polymerase chain reaction is a sensitive technique for identifying its subtypes throughout the disease course.

Introduction

Herpetic anterior uveitis (HAU) is the most common etiology of viral uveitis and characterized by unilateral involvement, iris atrophy and increased intraocular pressure (IOP).[1] It has been reported to account for 1.5% of the total cases of uveitis in China and 1% to 10% in Western countries.[2–5] Eight herpes viruses have been found to cause uveitis, among

which herpes simplex virus (HSV), varicella-zoster virus (VZV), and cytomegalovirus (CMV) are the most common pathogens. The diagnosis of HAU is mainly based on the medical history and clinical manifestations. Aqueous humor (AqH) analysis is useful for the identification of viruses. However, anterior chamber paracentesis is not often performed clinically, mainly owing to the adverse effects of the invasive nature of this test. A number of studies have described the clinical characteristics and visual outcomes of HAU.[6–8] The profile of the clinical manifestations among these subtypes of HAU is mainly evaluated using the AqH analysis at acute stage. The whole spectrum of their clinical features overtime as well as visual prognosis has not been well-addressed owing to a lack of sensitive techniques for detecting the virus and long-term follow-up.

Early studies showed that causative agents in AqH of HAU patients can be evaluated by Goldmann–Witmer coefficient (GWC)[9] or polymerase chain reaction (PCR).[6] Quantitative PCR has been used widely for identifying viral nucleic acid. However, it is a relative quantitative technique and has limitations in sensitivity. More recently, droplet digital PCR (ddPCR) has been developed to detect extremely small amounts of nucleic acid in samples and achieve absolute quantification without the use of standard curves.[10] This technique has been used for the measurement of viral nucleic acid in Posner–Schlossman syndrome and yield a better result compared with conventional quantitative PCR.[11] In this study, we used ddPCR and GWC to identify the herpes virus in AqH among patients diagnosed clinically with HAU and evaluate the relevance of these results to clinical manifestations, as well as visual prognosis.

Methods

Patients and Controls Enrolled

A total of 317 patients were diagnosed as HAU with a follow-up time ≥3 months in the First Affiliated Hospital of Chongqing Medical University from June 2018 to December 2022. The clinical diagnostic criteria for HAU were based on the following clinical characteristics[12–16]: (a) unilateral AU, (b) elevated IOP, (c) the presence of stromal keratitis with excluding that caused by other factors such as syphilis, tuberculosis, sarcoidosis, or leprosy, (d) the presence of mutton-fat keratic precipitates (KPs) and iris atrophy, (e) the presence of posterior synechia with or without pupil deformation, and (f) a clear history of herpes keratitis or herpes zoster ophthalmicus. Patients with four or more clinical features were clinically diagnosed as HAU. During a period of 3 to 48 months of follow-up, 62 patients developed complicated cataract (19.6%), and 18 patients (5.7%) developed intractable increased IOP. A total of 43 patients with complicated cataract or intractable increased IOP underwent cataract surgery or antiglaucoma surgery in our department. We simultaneously collected AqH and serum for pathogen detection from these 43 cases undergoing eye surgery (Supplementary Fig. S1). None of them had diabetic retinopathy, macular abnormalities, optic nerve disease, or other retinal diseases. AqH and serum samples were also obtained from 10 patients with Vogt–Koyanagi–Harada, 10 patients with Behcet′s disease, and 10 patients with age-related cataract during cataract surgery serving as controls. The International Study Group's criteria[17] and the criteria proposed by our group[18] were used to diagnose Behcet′s disease and Vogt–Koyanagi–Harada disease, respectively.

The study was approved by the institutional review board of the First Affiliated Hospital of Chongqing Medical University and adhered to the Declaration of Helsinki. All participants signed the informed consent.

AqH and Serum Detection

Samples were collected during surgery and immediately stored in a freezer and kept at −80°C until laboratory assays. ddPCR analysis was used to identify the presence of HSV, VZV, or CMV DNA in AqH samples. The viral DNA was extracted using the QIAamp MinElute Virus Spin Kit (Cat. No. 57704) as instructed by the manufacturer. The final volume of 20 µL ddPCR reaction mixture for QX200 ddPCR contained "2 × ddPCR Supermix for Probes" (Bio-Rad, Hercules, CA) with primers/probe mix (concentrations of 900 and 250 nM, respectively) and templates. The following cycling protocol was run: 95°C × 5 minutes (1 cycle); 95°C × 30 seconds, 57°C × 1 minutes (40 cycles); 98°C × 10 minutes (1 cycle); and 4°C hold. The primers and probes sequences for the DNA detection of HSV, VZV, and CMV are listed in Supplementary Table S1.[11,19]

The concentrations of specific immunoglobulin G against HSV, VZV, CMV, and total immunoglobulin G in serum and AqH were determined by enzyme-linked immunosorbent assay using commercial kits (Ruixin Biotechnology, Changsu, China) and used to calculate the GWC. The intraocular specific antibodies were generally considered to be locally produced when the GWC value of greater than 4.[20] Positive laboratory results were defined as ddPCR positive, a GWC of greater than 4, or both.

Additionally, 10 cytokines including interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-13, monocyte chemotactic protein 1, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α in the AqH were measured in 22 samples using the Quantibody Human Inflammatory Array 1 (RayBiotech, Peachtree Corners, GA) to evaluate their association with subtypes of HAU as well as with clinical manifestations.

Clinical and Laboratory Examinations

Complete ocular examinations were performed at each visit, including best-corrected visual acuity (BCVA) measurement, tonometry, direct ophthalmoscopy, and slit-lamp biomicroscopy. Fundus fluorescence angiography, visual field, ultrasound biomicroscopy, optical coherence tomography, corneal specular microscopy, anterior segment photography, and B-scan ultrasound examination were performed if needed or possible. In addition, all patients underwent laboratory tests, such as routine blood tests, fasting plasma glucose, and kidney and liver function.

Therapy

All patients with HAU with active anterior segment inflammation were treated with topical and systemic corticosteroids in combination with topical antivirals, followed by gradual tapering if the intraocular inflammation was controlled. The activity of anterior segment inflammation was assessed according to the SUN criteria.[21]

Patients with complicated cataract underwent cataract surgery with implantation of intraocular lens if the anterior segment inflammation was completely controlled. Intractable increased IOP was defined as IOP of 24 mm Hg or greater with or without visual field or optic disc changes after 3 months of medications. Patients with intractable increased IOP underwent antiglaucoma surgeries, including trabeculectomy and glaucoma valve implantation, if they failed to response to the medical treatment.

Data and Statistical Analyses

The following information, including demographics, unilateral or bilateral involvement, BCVA, IOP, presence or history of keratitis, KPs, anterior segment inflammation, posterior synechia, iris atrophy, fundus examination, ocular complications, surgical treatment, and visual outcome, was analyzed among the definite three subgroups of HAU diagnosed with laboratory examinations.

All data were analyzed statistically with SPSS software version 25.0 or GraphPad Prism software version 7.0. P values of less than 0.05 were considered as significant. Mean $\pm$ standard deviation or medians were used to describe quantitative variables, while relative frequencies were used to describe categorical variables. Quantitative data were analyzed using the paired t test, Kruskal–Wallis test, analysis of variance, and Mann–Whitney U test, and categorical data were analyzed using the Fisher exact test and χ^2 test. Finally, the Bonferroni correction was used to control the type I error rate in multiple comparisons. After Bonferroni adjustment, the α level was set to 0.017.

The BCVA was transformed to logarithm of the minimum angle of resolution (logMAR) for calculation purposes. Final logMAR BCVA was defined as the patient's visual outcome at 6 months after surgery. Counting fingers, hand motions, light perception, and no light perception were converted to 2.6 logMAR, 2.9 logMAR, 3.1 logMAR, and 3.4 logMAR, respectively.[22]

Results

A total of 317 patients (128 males and 189 females) were diagnosed as HAU, accounting for 2.2% of the 14,218 cases of uveitis referred to our department during this study period. The mean age of patients with HAU was 48 years, and the follow-up time ranged from 3 to 48 months. AqH samples from 43 patients were evaluated for pathogens, and all of them were diagnosed as having herpes virus infection either by ddPCR alone or by combination of ddPCR with GWC. The past medical history, treatment history, and clinical features of 274 patients with a clinical diagnosis of HAU and 43 patients with a definite diagnosis of HAU are shown in Table 1. In general, they are similar except for the initial visual acuity and IOP between these two groups.

All 43 AqH samples were tested by ddPCR for HSV, VZV, and CMV and 22 samples were also analyzed using GWC. Among these 43 HAU patients, 13 were diagnosed with HSV-AU (30%), 18 with VZV-AU (42%), and 12 with CMV-AU (28%) based on the ddPCR results. The GWC-positive results were completely identical to ddPCR-positive results in 22 cases. There was no detectable DNA for these three types of herpes virus in the AqH samples from patients with Vogt–Koyanagi–Harada disease, Behcet's disease, and cataract patients, and all of the patients in the control groups had GWC value of less than 4.

Table 1. Comparison of Past Medical and Treatment History and Clinical Characteristics of Patients With a Clinical Diagnosis of HAU and Those With a Definite Diagnosis of HAU Using AqH Analysis

Items	Patients With a Clinical Diagnosis of HAU ($n = 274$ Eyes)	Patients With a Definite Diagnosis of HAU ($n = 43$ Eyes)	*P* Value
Mean age at first visit (range), years	47 (5–88)	51 (10–82)	0.167*
Male gender, *n* (%)	109/274 (40%)	19/43 (44%)	0.584†
Past medical history before referral to our department			
Presence or history of herpes zoster ophthalmicus, *n* (%)	34/274 (12%)	6/43 (14%)	0.777†
History of ocular pain, *n* (%)	170/274 (62%)	30/43 (70%)	0.329†
History of redness, *n* (%)	216/274 (79%)	32/43 (74%)	0.514†
History of photophobia, *n* (%)	138/274 (50%)	22/43 (51%)	0.923†
History of decreased vision, *n* (%)	221/274 (81%)	32/43 (74%)	0.343†
Previous treatment before referral to our department			
Patients who have received treatment, *n* (%)	224/274 (82%)	36/43 (84%)	0.755†
Topical antiglaucoma agents, *n* (%)	205/274 (75%)	33/43 (77%)	0.786†
Topical and/or systemic corticosteroids, *n* (%)	219/274 (80%)	35/43 (81%)	0.823†
Topical antivirals, *n* (%)	216/274 (78%)	34/43 (79%)	0.972†
Systemic antivirals, *n* (%)	82/274 (30%)	12/43 (28%)	0.787†
Clinical characteristics at the first visit to our department			
Initial logMAR BCVA, median (range)	0.3 (0–3.4)	0.6 (0.1–2.9)	<0.001*
Initial IOP, median (range), mm Hg	19 (5–60)	33 (16–60)	<0.001*
Mutton-fat KPs toned with pigmentation, *n* (%)	196/274 (72%)	29/43 (67%)	0.583†
Posterior synechia, *n* (%)	219/274 (80%)	35/43 (81%)	0.823†
Iris atrophy, *n* (%)	204/274 (75%)	34/43 (79%)	0.515†
Pupil deformation, *n* (%)	164/274 (60%)	27/43 (63%)	0.714†

*P values were performed by Mann–Whitney U test.
†P values were performed by χ^2 test.

Demographic and Baseline Clinical Features

Table 2 summarizes the demographic and clinical characteristics at baseline in these three subgroups of HAU. For patients with HSV-AU, VZV-AU, or CMV-AU, the mean age at first visit to our department was 48, 50, and 55 years, respectively. The ratios of male to female were 1:1.2, 1:1.3, and 1:1.4, respectively. There were no differences concerning the age at first visit and sex among these three subgroups.

The time interval between disease onset and being referred to our department in the patients with a definite diagnosis of HAU was significantly different, with the longest interval in HSV-AU patients (range, 1–36 months), followed by CMV-AU (range, 0.2–8.0 months) and VZV-AU patients (range, 0.23–13.00 months) ($P = 0.002$). The presence or a history of herpes zoster ophthalmicus was observed in 33% VZV-AU patients. Neither the patients with HSV-AU nor those with CMV-AU had this sign. There was no significant difference concerning the incidence of presence or a history of keratitis among these three subgroups.

As for ocular symptoms in the patients with a definite diagnosis of HAU, more than 80% of VZV-AU and CMV-AU patients complained about ocular pain, whereas only 15% of HSV-AU patients had this symptom. There were no differences concerning redness and photophobia among these three subgroups. Unilateral involvement was observed in all these patients with a definite diagnosis of HAU, although bilateral involvement was also noted in 15 out of 274 patients with a clinical diagnosis of HAU. The median logMAR BCVA at the first visit for HSV-AU, VZV-AU, and CMV-AU eyes was 0.4, 0.7, and 1.0, respectively, and poor visual acuity was noted in VZV-AU patients or CMV-AU patients as compared with HSV-AU patients ($P = 0.006$ and $P = 0.005$). The increased IOP (>24 mm Hg) at the first visit was observed in more than 75% of the patients in all three subgroups, and the mean IOP in HSV-AU, VZV-AU, and CMV-AU patients was 24.2 ± 4.0, 40.6 ± 10.9, and 36.0 ± 12.2 mm Hg, respectively, showing a statistical difference among these three groups ($P < 0.001$). Ciliary or mixed injection was noted in more

Table 2. Demographic and Baseline Clinical Features Among These Three Subgroups of HAU

Items	HSV-AU ($n = 13$ Eyes)	VZV-AU ($n = 18$ Eyes)	CMV-AU ($n = 12$ Eyes)	*P* Value			
				HSV Vs VZV Vs CMV*	HSV Vs VZV**	HSV Vs CMV†	VZV Vs CMV†
Mean age at first visit (range), years	48 (38–62)	50 (10–77)	55 (40–82)	0.353‡	>0.999	0.520	0.761
Male gender, *n* (%)	6/13 (46%)	8/18 (44%)	5/12 (42%)	0.974§	>0.999	>0.999	>0.999
Time interval, median (range), months	12 (1–36)	3.0 (0.2–13)	4.0 (0.2–8.0)	0.002‖	0.002	0.002	0.771
Presence or history of herpes zoster ophthalmicus, *n* (%)	0/13 (0%)	6/18 (33%)	0/12 (0%)	0.010§	0.028	NA	0.057
Presence or history of keratitis, *n* (%)	5/13 (39%)	1/18 (6%)	2/12 (17%)	0.079§	0.059	0.378	0.548
Ocular pain, *n* (%)	2/13 (15%)	15/18 (83%)	10/12 (83%)	<0.001§	<0.001	0.001	>0.999
Redness, *n* (%)	13/13 (100%)	16/18 (89%)	11/12 (92%)	0.611§	0.497	0.480	>0.999
Photophobia, *n* (%)	9/13 (70%)	12/18 (67%)	9/12 (75%)	0.917§	>0.999	>0.999	0.704
Initial logMAR BCVA, median (range)	0.4 (0.1–1.0)	0.7 (0.4–2.9)	1.0 (0.3–2.9)	0.006‖	0.006	0.005	0.768
Initial IOP >24 mm Hg, *n* (%)	10/13 (77%)	16/18 (89%)	10/12 (83%)	0.870§	0.625	>0.999	>0.999
Initial IOP, mean ± SD (range), mm Hg	24.2 ± 4.0 (17–30)	40.6 ± 10.9 (18–60)	36.0 ± 12.2 (16–58)	<0.001‡	<0.001	0.013	0.646
Ciliary or mixed injection, *n* (%)	8/13 (62%)	11/18 (61%)	2/12 (17%)	0.032§	>0.999	0.041	0.026
Corneal lesions, *n* (%)	4/13 (31%)	6/18 (33%)	7/12 (58%)	0.289§	>0.999	0.238	0.264
Mutton-fat KPs toned with pigmentation, *n* (%)	9/13 (69%)	12/18 (67%)	8/12 (67%)	>0.999§	>0.999	>0.999	>0.999
Anterior chamber cells ≥2+, *n* (%)	2/13 (15%)	15/18 (83%)	2/12 (17%)	<0.001§	<0.001	>0.999	0.001
Anterior chamber flare ≥2+, *n* (%)	1/13 (8%)	7/18 (39%)	2/12 (17%)	0.109§	0.095	0.593	0.249
Posterior synechia, *n* (%)	11/13 (85%)	15/18 (83%)	9/12 (75%)	0.785§	>0.999	0.645	0.660
Iris stromal atrophy, *n* (%)	10/13 (77%)	15/18 (83%)	9/12 (75%)	0.804§	0.676	>0.999	0.660
Pupil deformation with pulling appearance, *n* (%)	8/13 (62%)	12/18 (67%)	7/12 (58%)	0.924§	>0.999	>0.999	0.712

NA, not applicable; SD, standard deviation.

*$P < 0.05$.

†$P < 0.017$.

‡*P* values compared among three groups using analysis of variance.

§*P* values compared among three groups using Fisher exact test or χ^2 test, as appropriate.

‖*P* values compared among three groups using Kruskal–Wallis test.

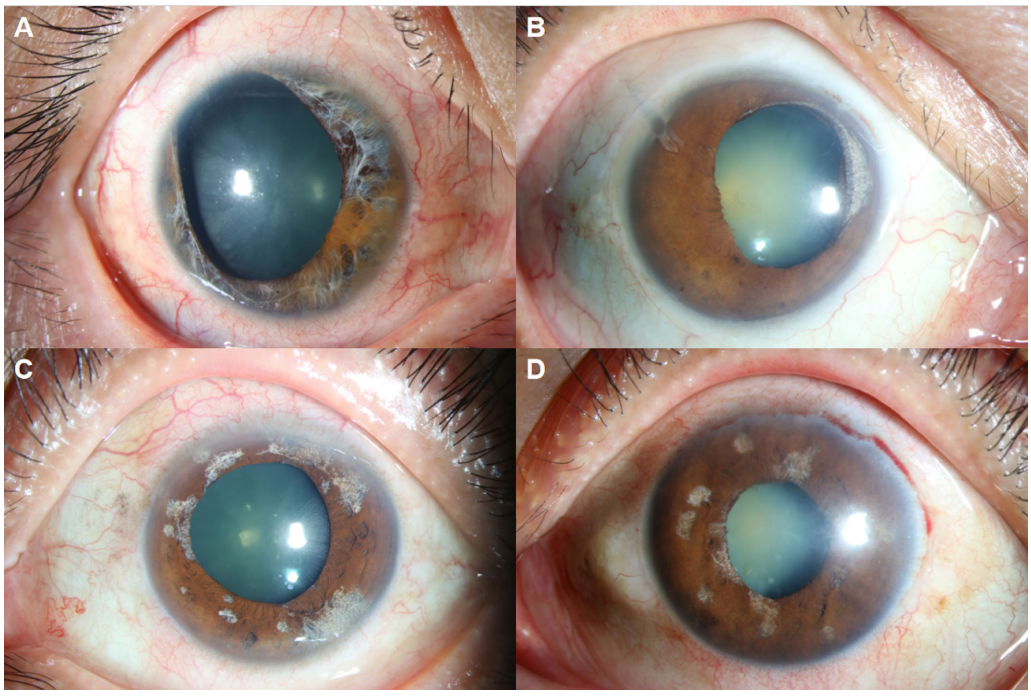

Figure 1. Diffuse or single patchy iris atrophy was observed in a HSV-AU patient (**A**) and a VZV-AU patient (**B**). Multifocal round, oval, or irregular iris atrophy was observed in CMV-AU patients (**C** and **D**).

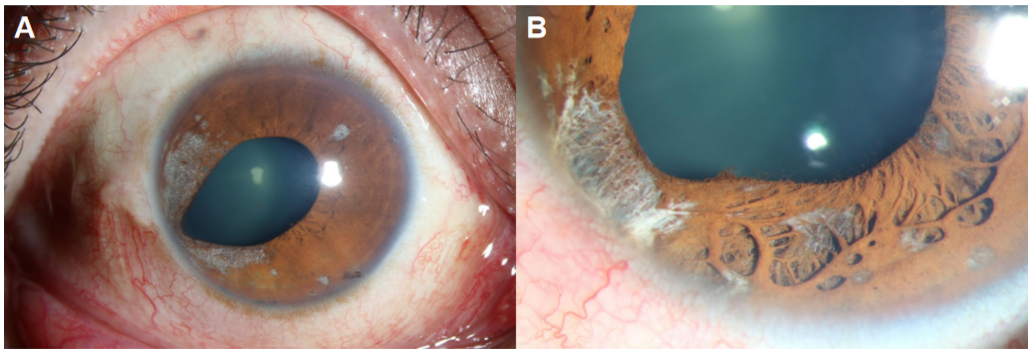

Figure 2. Pupil deformation in HAU patients with a pulling appearance unlike that caused by other uveitis entities (**A** and **B**).

than 60% of HSV-AU and VZV-AU patients, whereas it was observed only in 17% of CMV-AU patients. Corneal lesions manifested mainly as edema or opacity in all these subgroups without difference in frequency among them. KPs were observed in more than 60% of the affected eyes at the first visit and characterized by mutton-fat KPs almost universally toned with pigmentation. Anterior chamber cells were noted in all the patients in these 3 subgroups at first visit, and a severe inflammation (anterior chamber cells $\geq$2+) was more frequently noted in VZV-AU patients than in HSV-AU or CMV-AU patients ($P < 0.001$ and $P = 0.001$), although no difference was observed concerning the anterior chamber flare among these three subgroups. Iris stromal atrophy was observed in more than 75% of the patients in all three subgroups. It was single or multiple in number, and round, oval, or irregular in appearance. HSV-AU and VZV-AU patients usually presented as single or multiple patchy, or diffuse iris atrophy, whereas CMV-AU patients mostly displayed multifocal round, oval, or irregular iris atrophy (Fig. 1, Supplementary Table S2). There were no differences regarding posterior synechia and pupil deformation among these subgroups (Table 2). The deformed pupil mostly showed a pulling appearance unlike that caused by other nonherpetic uveitis entities (Fig. 2).

Auxiliary Examinations

Fundus fluorescence angiography was nonselectively performed in 33 patients with a definite diagnosis

of HAU, of which two VZV-AU patients had cystoid macular edema and one CMV-AU patient had retinal vasculitis. A thin retinal nerve fiber layer was noted in 13 of 42 patients examined by optical coherence tomography, and all of them had intractable increased IOP. Corneal specular microscopy was nonselectively performed in 31 patients, and the median corneal endothelial cell density in HSV-AU, VZV-AU, and CMV-AU patients was 2203.3, 2058.2, and 1536.9 cells/mm^2, respectively, showing a significant difference among these subgroups ($P = 0.001$) (Supplementary Table S3). Additionally, the median corneal endothelial cell density in the patients with age-related cataract was 2989.4 cells/mm^2, which is significantly higher than that in these three HAU subtypes (all $P < 0.01$). Visual field defect was noted in 15 of 40 HAU patients, and a higher percentage of visual field defect was observed in VZV-AU patients as compared with the other two types of HAU ($P = 0.005$).

AqH Analysis

All AqH samples from HAU patients were analyzed by ddPCR, and herpes virus DNA was detectable in these patients. The mean AqH viral load for HSV-AU, VZV-AU, and CMV-AU patients was 1.7×10^3 copies/mL (range, 0.2×10^3–7.5×10^3 copies/mL), 2.4×10^3 copies/mL (range, 0.3×10^3–1.0×10^4 copies/mL), and 1.1×10^3 copies/mL (range, 0.2×10^3–2.6×10^3 copies/mL), respectively. The mean viral load was significantly higher in patients with intractable increased IOP than those without this sign at sampling ($P = 0.004$). Among the 22 samples evaluated by GWC, 5 were diagnosed with HSV-AU, 11 with VZV-AU, and 6 with CMV-AU. The mean GWC values for the HSV-AU, VZV-AU, and CMV-AU groups were 6.1 (range, 4.1–7.8), 10.9 (range, 4.1–33.4), and 4.8 (range, 4.1–7.8), respectively. These results were completely identical to those detected by ddPCR.

AqH samples obtained from 8 HSV-AU, 6 VZV-AU, and 8 CMV-AU patients were also evaluated for inflammation-associated cytokines. As shown in Supplementary Figure S2, the levels of IL-13, IFN-γ, and TNF-α were significantly higher in VZV-AU patients as compared with HSV-AU or CMV-AU patients. There were no differences concerning the levels of IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, or monocyte chemotactic protein 1 among these three subgroups.

Treatment

All patients were treated with antiviral and anti-inflammatory eye drops and systemic corticosteroids, and those with elevated IOP were treated with antiglaucoma medications. Most patients had complete control of the anterior segment inflammation at 3 months after treatment. Complicated cataract was observed in 62 of 317 patients with HAU, of which 37 patients underwent cataract surgery in other hospitals and 25 patients underwent cataract surgery in our department. Intractable increased IOP was noted in 18 of 317 patients with HAU and antiglaucoma surgery, including trabeculectomy and glaucoma valve implantation, was performed in these patients. All these patients achieved a normal IOP after surgery. The complication and surgical therapy among these three subgroups of HAU are shown in Supplementary Table S4.

Prognosis

The final visual acuity of 274 patients with a clinical diagnosis of HAU was better than that of 43 patients with a definite diagnosis of HAU ($P = 0.043$). The final median logMAR BCVA for patients with HSV-AU, VZV-AU, and CMV-AU was 0.0, 0.7, and 0.1, respectively (Table 3). Improved visual acuity was noted in HSV-AU and CMV-AU patients ($P = 0.001$ and $P = 0.002$), but not in VZV-AU patients after treatment. All the patients in these three subgroups achieved a better visual acuity following cataract surgery ($P < 0.001$). An improved visual field and visual acuity were

Table 3. Visual Outcome Among These Three Subgroups of HAU

	LogMAR BCVA, Median (Range)			
	HSV-AU ($n = 13$ Eyes)	VZV-AU ($n = 18$ Eyes)	CMV-AU ($n = 12$ Eyes)	*P* Value
First visit	0.4 (0.1–1.0)	0.7 (0.4–2.9)	1.0 (0.3–2.9)	0.006*
Final visit	0.0 (0.0–0.3)	0.7 (0.0–2.9)	0.1 (0.0–0.5)	0.010*
P Value	0.001†	0.281†	0.002‡	

*P values compared among three groups using the Kruskal–Wallis test.
†P values were performed by paired *t*-test.
‡P values were performed by paired samples Wilcoxon signed rank test.

noted in HSV-AU (form 0.6 ± 0.4 logMAR to 0.2 ± 0.1 logMAR) and CMV-AU patients (from 0.7 ± 0.3 logMAR to 0.0 ± 0.0 logMAR), but not in VZV-AU patients (from 1.4 ± 1.0 logMAR to 1.3 ± 1.0 logMAR) after antiglaucoma surgery.

Discussion

In this study, we characterized the clinical manifestations of 274 patients with a clinical diagnosis of HAU and 43 patients with a definite diagnosis of HAU. The results showed that these three subgroups of HAU have clinical features in common, including unilateral involvement, increased IOP, mutton-fat KPs usually toned with pigmentation, iris atrophy, and posterior synechia with a deformed pupil. VZV-AU was usually associated with a higher frequency of severe anterior segment inflammation, intractable increased IOP, and poor visual prognosis as compared with the other two types of HAU.

HAU has long been recognized as an independent type of uveitis. It is diagnosed principally based on clinical manifestations and named clinically as a presumed diagnosis in the absence of laboratory examination.[23] A definite diagnosis has been made by detection of viral DNA in AqH or evaluation of GWC. It has been shown that viral DNA detection is useful in the diagnosis of HAU within 2 months of the onset of disease, and GWC can be used for its diagnosis in the whole course.[24] In this study, we used a sensitive PCR technique, ddPCR, to detect the viral DNA in AqH obtained from patients with a long history of clinically diagnosed as HAU. Interestingly, our results showed that viral DNA was all detectable in these patients. More important, the viral DNA detected was completely identical to GWC result. The superiority of ddPCR in detecting target DNA for viral diseases at extremely low concentrations compared with conventional quantitative PCR has been demonstrated in previous studies.[25,26] Our study showed that the detection limit of ddPCR was 10^2 copies/mL, in line with a previous report,[27] significantly lower than that reported by De Groot-Mijnes et al.[24] In addition, we found that viral load was correlated with IOP rather than disease duration. Patients with intractable increased IOP had much higher viral loads, consistent with that reported in the literature.[11] Our results suggest, on one hand, that the parameters chosen in this study are pertinent to the diagnosis of HAU clinically. The clinical diagnosis of HAU was demonstrated in all the patients with AqH testing. On the other hand, ddPCR seems to be a sensitive technique for the definite diagnosis of HAU, even in the patients with a long history.

It is well-known that HAU has distinct clinical manifestations, including mutton-fat KPs toned with pigmentation, iris atrophy and increased IOP. These clinical features generally allow us to differentiate HAU from other AU entities. However, the differences among the three subtypes of HAU during the whole course of disease have not been well-addressed clinically. In this study, we compared the clinical manifestations among patients with a definite diagnosis of HSV-AU, VZV-AU, or CMV-AU. As for iris atrophy, previous reports showed that HSV-AU and VZV-AU patients mostly presented with small patchy, segmental or sectorial iris atrophy, whereas CMV-AU patients usually displayed diffuse iris atrophy at first visit or at the acute stage.[6,28,29] Our study showed a similar result. Additionally, we found that CMV-AU patients generally presented as multifocal round, oval, or irregular iris atrophy. As regards to IOP, Terada et al.[6] reported that the IOP was significantly higher in CMV-AU patients at the acute stage as compared with HSV-AU and VZV-AU patients. In this study, we showed a similar result regarding the increased IOP in CMV-AU patients. However, we also found that the IOP was much higher in VZV-AU patients as compared with HSV-AU patients. The reasons as to why there is a difference concerning increased IOP among three subtypes of HAU between our results and those of a previous study are not clear. Clinical differences have been found in other diseases among various race populations.[30–32] The difference concerning elevated IOP among these three subtypes of HAU between our study and others may be attributed to a racial origin. More studies are needed to clarify this issue. It has been shown that corneal endothelial cell loss is a common sign of these three subtypes of HAU.[6] In this study, we also revealed a decreased density of corneal endothelial cells in all three subtypes of the disease. Interestingly, a much lower corneal endothelial cell density was noted in CMV-AU patients as compared with HSV-AU and VZV-AU patients. It is not yet exactly known how the viral infection leads to corneal endothelial cell loss and why there is a difference concerning this abnormality among these three subtypes of HAU. More studies are expected to clarify these issues.

Systemic and topical corticosteroids mostly combined with systemic antiviral agents have been used for the treatment of HAU.[6,33–35] In this study, we used a relatively low dose of systemic corticosteroids in combination with corticosteroids and antiviral eye drops for the treatment of this disease and showed a complete control of the anterior segment inflammation in most patients 3 months after treatment. Unexpect-

edly, an elevated IOP persisted in some patients, although there was no anterior segment inflammation. A greater percentage of intractable increased IOP was observed in VZV-AU patients as compared with HSV-AU and CMV-AU patients. The reason as to why there is much higher IOP in VZV-AU patients is not known. The higher levels of IL-13, IFN-γ and TNF-α detected in the present study may partially explain the difference of IOP among these three subtypes of HAU. However, it is not known why there is a persistent release of these cytokines in VZV-AU patients. Further studies are needed to address this issue. It is worthwhile to point out that the elevated IOP was all controlled after antiglaucoma surgery. However, persistently elevated IOP leaded to a worse visual prognosis in VZV-AU patients, possibly owing to its damage to optic nerve as evidenced by severely impaired visual field. Therefore, it should be kept in mind that controlling the IOP is essential to the recovery of visual acuity in HAU patients, especially in those with VZV-AU.

There were some limitations to our study. First, it was a retrospective study and a definite diagnosis was made only in a minority of the patients using AqH analysis. The clinical features of these 43 patients with definite HAU diagnosis did not reflect the overall characteristics of different types of HAU patients. These results reported here are expected to be clarified using prospective studies on the patients with definite HAU diagnosis. Second, owing to the limited volume of AqH, we only evaluated the three most common subtypes of herpes virus; thus, the possibility of co-infection with other viral subtypes cannot be excluded completely. Third, this study was performed only in Chinese Han patients and the results need to be validated in other populations. Fourth, as the full data regarding the patients underwent cataract surgery in other hospitals were not available, we only analyzed the data of the patients underwent surgery in our department. Studies of the multiple centers are needed to accurately evaluate the effects of complicated cataract relevant surgeries on the patients' visual outcome.

Conclusions

Our study showed that HAU can be diagnosed solely based on clinical manifestations and ddPCR is a sensitive technique for identifying its subtypes during the whole course of the disease. More important, our study addresses the similarity and difference regarding clinical features and visual prognosis among patients with HSV-AU, VZV-AU, and CMV-AU.

Acknowledgments

The authors thank all the patients and controls who participated in this study.

Supported by the National Natural Science Foundation Key Program (82230032), National Natural Science Foundation Key Program (81930023), Key Project of Chongqing Science and Technology Bureau (CSTC2021jscx-gksb-N0010), Chongqing Outstanding Scientists Project (2019), Chongqing Chief Medical Scientist Project (2018), and Chongqing Science and Technology Bureau Mountaineering Project (cyyy-xkdfjh-jcyj-202301, cyyy-xkdfjh-lcyj-202303, cyyy-xkdfjh-cgzh- 202302).

Author Contributions: PY and HL conceived and designed the study. HL, SG and YL carried out experiments. HL, SG and GS collected and analysed the data of the study. HL wrote the manuscript. PY interpreted the data and revised the manuscript. PY is the guarantor.

Statement of Ethics: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of the First Affiliated Hospital of Chongqing Medical University (Approval ID:2018-048) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Data Availability Statements: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Disclosure: **H. Liu**, None; **S. Guo**, None; **Y. Liu**, None; **G. Su**, None; **P. Yang**, None

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