Application of aqueous humor polymerase chain reaction for virus in Posner-Schlossman syndrome

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Abstract

Background

To study the effectiveness and safety of aqueous humor PCR viral detection in the diagnosis of PSS.

Methods

20 patients (case group) with PSS who suffered from recurrences and poor intraocular pressure (IOP) control by use of drugs underwent anterior chamber puncture. The concentration of different viruses in aqueous humor were detected by PCR. 20 patients (control group) with POAG were given the same treatment and we compared the difference of positive rate of the viruses between two groups. Finally, the effectiveness and safety of aqueous humor PCR viral detection in the diagnosis of PSS were observed. Besides, the advantage of PCR viral detection technology in treatment was evaluated.

Results

No complications and long-term sequelae related to anterior chamber puncture were found. The total positive rate of aqueous humor viral detection was 30% (6/20) in case group ,and was found none in control group (0/20) ($P = 0.027$). Then, we divided the case group into two groups. Six virus positive patients were given local and systemic antiviral treatment for 3 months and then interrupted the treatment. Fourteen patients left were given no antiviral treatment. All PSS patients were observed for 9 months from the first time of treatment. There is significant difference between two groups in recurrence rate ($P = 0.013$).

Conclusions

Viral infection is a risk factor of PSS. The detection of virus in aqueous humor by PCR is effective and safe for diagnosis of PSS, and helpful for targeted treatment on virus-positive patients.

Background

Posner-Schlossman syndrome (PSS) or glaucomatocyclitic crisis was first identified by Posner and Schlossman in 1948 [1]. It is a special type of inflammatory glaucoma accompanied by uveitis. It is characterized by unilateral, recurrences of mild, non-granulomatous anterior uveitis with elevated IOP. Other features include mild corneal edema, sparse keratic precipitates and a large pupil. During acute attack, anterior chamber drainage angles remain open. PSS trends to affect males ranging from 20 to 50 years old [2, 3]. Though the majority of these patients are found with normal visual fields and optic discs, some of them usually suffer from blurred vision due to PSS. Moreover, with the increasing attacks

and IOP, a small part of these patients suffer from irreversible optic nerve damage [4, 5]. Thus, it is important to diagnose and treat PSS at early stage.

Several factors have been proved to be related to the development of PSS, including infection, allergic conditions, vascular endothelial dysfunction and autoimmune drive [6–8]. Notably, it has been supported by a variety of studies that PSS is related to viral infection such as Cytomegalovirus (CMV), Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV) [9, 10]. As an elusive disease, whether viral infection is the major risk of PSS still needs further study.

Polymerase chain reaction (PCR) has been widely used in the diagnosis of infectious etiology in aqueous humor [11]. In this study, aqueous humor PCR was used to detect positive rate of CMV, HSV, VZV and rubella virus (RV). Finally, it was proven that PCR analysis of aqueous humor specimens was efficient and safe for viral detection in PSS.

Methods

Patients and Samples

20 patients with PSS, who suffered from recurrences and poor IOP controlled by drugs between April 2018 to January 2019 at the Affiliated Eye Hospital of Nanjing Medical University, were recruited in this study. The inclusion criteria in PSS group were (1) unilateral, (2) recurrent episodes of mild, non-granulomatous cyclitis, (3) symptoms of mild discomfort, halos, and slight blurring of vision, (4) findings of elevated IOP, open angles, hoar and suet-shaped KP, no or minimal cells and flare, and mydriasis in the affected eye, (5) IOP > 21 mm Hg after the treatment of drugs, (6) more than one recurrence. All the inspections and diagnosis of these patients were done by the same glaucoma specialist. The exclusion criteria were (1) with retinal disease, non-glaucoma optic neuropathy, anterior uveitis or corneal abnormalities, (2) suspected chronic glaucoma, (3) angle-closure glaucoma excluded by gonioscopy examination, (4) received antiviral treatment (5) best-corrected vision < 20/50, absolute spherical lens value ≥ 5 D, absolute column lens value ≥ 3 D.

20 age-matched patients with POAG at the corresponding period were selected as control group in this study. The homolateral eyes of PSS patients in control group were set as control eyes. The inclusion criteria in control group were (1) open anterior segment angles; (2) reproducible glaucomatous visual field loss on reliable tests; (3) IOP > 21 mm Hg. The exclusion criteria were (1) with identifiable secondary cause for optic nerve diseases, (2) best-corrected vision < 20/50, absolute spherical lens value ≥ 5 D, absolute column lens value ≥ 3 D.

All participants underwent a series of ophthalmologic examinations, including vision measurement, best-corrected vision measurement, specular microscopy, refraction test, IOP measurement (Goldmann applanation tonometry, Haag-Streit, Koeniz, Switzerland), slit-lamp biomicroscopy (Haag-Streit, Koeniz, Switzerland) and gonioscopy (Haag-Streit, Koeniz, Switzerland).

Methods

1. How to perform anterior chamber puncture

All operations were performed by the use of microscope in operating rooms. The whole surgical procedure was sterile. Post-operative eyes were rinsed with sterile saline before puncture. We performed puncture in corneal limbus with a 15º stab knife. 100 μl of aqueous humor was extracted with a rinse needle. All operations were performed by the same person. After operation, all patients received levofloxacin eyedrops three times a day.

2. DNA extraction and amplification

DNA extraction of aqueous humor was done by the use of EZ1 Advanced XL automatic nucleic acid purification system (Qiagen, Hilden, Germany). The aqueous humor specimen was taken from the affected eyes of 20 PSS patients and homolateral eyes of 20 POAG patients. A pair of primers was designed according to the gene sequence of the tested virus. Quantitative PCR (qPCR) of CMV, HSV and VZV was performed within 24 hours after sample collection. All the qPCR detection was performed according to the manufacturer's instructions. All samples were sent to Beijing GiantMed Diagnostics Inspection Office for quantitative detection.

3. RNA extraction and amplification

RNA extraction of aqueous humor was done by the use of TRI reagent (Sigma, St. Louis, USA). This step must be performed immediately. The cDNA synthesis was carried out in the thermocycler. qPCR was performed by use of cDNA samples for amplification of studied gene RV. All the qPCR detection was performed according to the manufacturer's instructions. All samples were sent to Beijing GiantMed Diagnostics Inspection Office for quantitative detection.

This study was carried out in accordance with the principles of *Declaration of Helsinki*. All participants were provided with written informed consent.

In this study, all PSS patients were divided into two groups. Patients with negative viral infection were set as the observation group while patients with positive viral infection were set as the treatment group. Patients in treatment group were given local and systemic antiviral treatment for 3 months and then the treatment was discontinued.

Data were analyzed by SPSS software (version 22.0, SPSS Inc., Chicago, IL). All demographic data were expressed as mean ± SD. The mean differences of different groups were analyzed by Student's *t* test, Levene's test, paired-samples T test and Chi-Square test. P value of less than 0.05 was considered as indicating significant statistical difference.

Results

In all, 40 patients were enrolled in this study. Table 1 lists the comparison of case group and control group in demographic data. Patients of the two groups were mainly male (75%). The average age of PSS patients was 43 ± 13.77 (range: 26–69). The average age of POAG patients was 44.7 ± 13.58 (range: 22–69). There was no significant difference in age between the two groups ($P = 0.573$). The mean IOP was 30.05 ± 9.96 mm Hg in case group, and there was no significant difference when compared with control group (29.5 ± 7.97 mm Hg) ($P = 0.849$). There was no significant difference both in corrected vision and in mean endothelial cell density between the two groups ($P > 0.05$).

To determine the relationship between PSS and viral infection, we performed anterior chamber puncture to extract 100 μl of aqueous humor. All POAG patients underwent the same process. We extracted DNA and RNA from the aqueous humor specimen of 40 patients and amplified DNA and RNA of the viruses mentioned above from the samples. Table 2 lists the number of affected patients with different viruses in two groups. In this study, 6 PSS patients were found with positive viral infection (30%). Among the 6 patients, 2 were VZV positive and 4 were CMV positive. All POAG patients were found with negative viral infection. There was significant difference in viral infection between PSS patients and POAG patients ($P = 0.027$; Table 3).

During follow-up visit, there was a significant reduction of IOP in the treatment group after antiviral treatment ($P = 0.007$). However, we did not find the difference of IOP at the same follow-up time points($P = 0.051$; Table 4).

The mean IOP was 16.33 ± 3.08 mmHg in treatment group after therapy, and there was significant difference when compared with observation group (27.00 ± 6.70 mm Hg) ($P = 0.002$; Table 5).There was no recurrence in treatment group during follow-up visit for the next 6 months. In observation group, there were 6 patients who suffered from recurrence at least once. There were significant differences in the number of recurrence rate between treatment group and observation group ($P = 0.013$; Table 6). What's more, no complication related anterior chamber puncture was found in all the subjects included in this study.

Discussion

PSS which is recognized as inflammation-related glaucoma is a type of secondary glaucoma. In original article, Posner and Schlossman believed that PSS was benign [4]. However, researchers have noticed loss of vision in some PSS patients recently [12, 13]. In recent years, PSS has gradually drawn clinicians' attention. Main causes that lead to PSS are considered to be allergy, vascular endothelial dysfunction, viral infection and autoimmunization [8]. For example, previous studies have shown that PSS might be associated with allergy. The condition of patients with PSS improved when they received anti-allergic treatment [10, 14]. A small prospective study has shown that PSS was significantly related to lower flow-mediated vasodilation (FMD). It was implied that peripheral vascular endothelial dysfunction is involved in the progress of PSS [7, 15, 16]. Some studies also suggested that some PSS patients were with positive autoimmune markers. Therefore, PSS was thought to be related to autoimmunization [17, 18].

Recently, a variety of studies have supported that viral infection is the leading cause [19, 20]. In our study, it was believed that viral infection was a risk of pathogenesis of PSS. And it was found that CMV and VZV were related to PSS, which was correspond with many previous studies [21, 22]. However, patients in this study were found with negative HSV infection. This result was different from other studies [23]. No enough evidence has been found to prove the effect of rubella virus (RV) in PSS both in our study and previous studies [24]. Combined with previous studies, we have proposed the idea that viral infection could initiate the immune response, which results in PSS patients' abnormal immunization and vascular regulation. Of course, this viewpoint needs to be further verified.

Diagnosis of PSS is mainly shown through clinical manifestations. Recently, PCR has been applied in the diagnosis of PSS [11]. It has been proven to be very useful because only a very small sample is required. Paracentesis of anterior chamber has been applied to obtain aqueous humor for the analysis of viruses. In this study, we found that the total percentage of viral infection was 30% in PSS patients by PCR analysis. 10% PSS cases were found with positive VZV infection while 20% PSS patients were found with positive CMV infection. However, we didn't find viral infection in control group. The difference between the two groups was statistically significant. These results supported that viral infection might be the main risk of PSS while POAG was not associated with viral infection. The majority of other studies viewed CMV as the major source of infection [25, 26]. In this study, CMV infection rate was much lower than that reported in a previous study [25]. Reasons for this difference might be related to the number of cases, regional disparity, different courses of disease and genetic factors of patients. Thus, it is possible that the true CMV infection rate may be diverse in different regions and races. Subsequently, all PSS patients were divided into two groups. The treatment group (positive viral infection patients) was treated with topical and systemic anti-viral therapy for 3 months and then the treatment was discontinued. The observation group (negative viral infection patients) didn't receive anti-viral therapy. All PSS patients were provided with follow-up visit for 9 months. Patients in the treatment group were observed with controlled IOP and with no recurrence during follow-up visit. However, 6 patients in the observation group suffered from recurrence at least once during follow-up visit. This result suggested that anti-viral therapy might be beneficial to the treatment on PSS patients with positive viral infection. Surprisingly, four of the six patients who suffered from recurrence in observation group were finally treated with topical and systemic anti-viral therapy, achieving good results which we will report separately. In these cases, we thought, the viral detection might be false negative. In other words, if we add the type of virus for detection, we may have different results. In this study, no complication was found after anterior chamber puncture. Viral detection of aqueous humor by PCR identified the etiology of PSS of 30% patients and it was found that this technique was safe and beneficial to specific therapy.

There are some limitations of this detection method. The whole operational process was simple but the positive rate was low. Moreover, the number of cases in this study was low, and more cases need to be followed up. Certainly, we need to conduct in-depth studies of these cases. Furthermore, more types of viruses need to be detected.

In short, viral infection is a risk factor of PSS. PCR detection of aqueous humor from anterior chamber puncture is safe and it can identify the etiology of some PSS patients. Once the cause of positive viral infection for PSS is clear, it is beneficial to clinical treatment, especially for patients with recurrence and poor IOP control by drugs.

Conclusion

In our study, We confirmed that there was a certain correlation between virus infection and PSS. So, detection of virus concentration in the aqueous humor of patients with PSS by PCR can clarify the etiology of some patients. In clinical practice, we give targeted antiviral treatment to the patients who are positive for the detection of aqueous humor virus infection by PCR, which can significantly improve the prognosis of the patients.

Abbreviations

IOP:intraocular pressure; PSS: Posner-Schlossman syndrome; POAG:primary open angle glaucoma;PCR: Polymerase chain reaction;CMV :Cytomegalovirus;HSV：Herpes Simplex Virus;VZV:Varicella Zoster Virus; RV:rubella virus

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the affiliated eye hospital of NanJing Medical University(NO 2018002), and conformed to the tenets of the Declaration of Helsinki. Each patients signed a consent form.

Consent for publication

We confirm that all patients in our study provided written informed consent for the medical information to be published. A copy of the written consent is available for review by the editor of this journal.

Availability of data and materials

The data of the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Guofan Cao designed the project, and wrote the manuscript. Shuya Tao performed experiments and wrote the manuscript. Qin Jiang designed and supervised the project. Li Tang performed statistical analysis. Xiyan Ding collected data. Xiumiao Li performed experiments. All authors read and approved the final version of the manuscript.

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Tables

TABLE 1. Demographic data of all enrolled subjects

	Case group (n=20)	Control group (n=20)	P-value
Gender (male:female)	15:5	15:5	1.0
Age (mean±SD) (years)	43±13.77	44.7±13.58	0.573
IOP (mean±SD) (mmHg)	30.05±9.96	29.5±7.97	0.849
Corrected visual acuity in the diseased eye (mean±SD) (logMAR)	0.58±0.31	0.39±0.22	0.066
Endothelial cell density of the diseased eye (mean±SD) (cell/mm^2)	2321.35±549.82	2560.70±436.87	0.130

The mean differences of different groups were analyzed by Student's *t* test. A P value of less than 0.05 was considered as indicating a statistically significant difference.

TABLE 2. Affected patient numbers of different viruses in case group and control group

	Case group (n=20)	Control group (n=20)
CMV	4	0
HSV	0	0
VZV	2	0
RV	0	0

TABLE 3. The difference of viral infection between two groups

		Case numbers		Total	*P*
		Negative	Positive		
Case group	PSS	14	6	20	0.027
Control group	POAG	20	0	20	
Total		34	6	40	

The differences of two groups were analyzed by Chi-Square test. A P value of less than 0.05 was considered as indicating a statistically significant difference.

TABLE 4. The difference of IOP before and after treatment during follow-up visit in treatment group and observation group

	IOP (mmHg) of the affected eye in PSS patients before treatment (mean±SD)	IOP (mmHg) of the affected eye in PSS patients after treatment (mean±SD)	P-value
observation group (n=14)	29.14±9.79	27.00±6.70	0.051
treatment group (n=6)	32.17±10.96	16.33±3.08	0.007

The differences of IOP before and after treatment were analyzed by or paired-samples T test. A P value of less than 0.05 was considered as indicating a statistically significant difference.

TABLE 5. The difference of IOP between treatment group and observation group before and after treatment during follow-up visit

	Observation group (n=14)	Treatment group (n=6)	P-value
IOP (mmHg) of the affected eye in PSS patients before antiviral treatment (mean±SD)	29.14±9.79	32.17±10.96	0.55
IOP (mmHg) of the affected eye in PSS patients after antiviral treatment (mean±SD)	27.00±6.70	16.33±3.08	0.002

The differences of two groups were analyzed by Levene’s test. A P value of less than 0.05 was considered as indicating a statistically significant difference.

TABLE 6. The difference of number of relapses between treatment group and observation group during follow-up visit

	Number of relapses	People counting	P-value
Treatment group	0	6	0.013
Observation group	0	8	
	1	3	
	2	3	

The differences of two groups were analyzed by Levene’s test. A P value of less than 0.05 was considered as indicating a statistically significant difference.