Diagnostic Accuracy of Vitreous Cytology in Patients with Vitreoretinal Lymphoma

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ABSTRACT

Diagnostic Accuracy of Vitreous Cytology in Patients with Vitreoretinal Lymphoma

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Purpose: To determine the diagnostic value of vitreous cytology in patients with vitreoretinal lymphoma (VRL) and to evaluate its diagnostic accuracy compared with that of other diagnostic tests

Methods: Retrospective observational design. From Jan 2005 to May 2021, the medical records of patients with VRL who underwent diagnostic vitrectomy and were followed-up for at least 3 months were analyzed.

Results: Data from 44 eyes (44 patients) were analyzed. Twenty-eight (63.6%) patients had primary VRL, 10 (22.7%) were VRL from the brain, and 6 (13.6%) had VRL from another organ. The presence of vitreous cells or opacity was the most common ophthalmic finding (97.7%), followed by sub-retinal pigment epithelial infiltration (56.8%) and retinal hemorrhage (20.5%). VRL diagnostic tests and associated rates were as follows: interleukin(IL)-10 levels of > 50 pg/mL (91.2%), IL-10 to IL-6 ratio (85.3%), immunoglobulin heavy chain and immunoglobulin kappa light chain clonality assays (65.7% and 68.6%, respectively), and vitreous cytology (31.8%). Steroid pre-treatment was performed in 29.5% of cases. The diagnostic rate of vitreous cytology was significantly lower in the steroid pretreatment group than in the non-steroid pretreatment group (p=0.035). No between-group differences in the rates of other diagnostic

tests were noted.

Conclusions: The VRL detection rate of vitreous cytology was lower than that of other tests, in particular, in patients who underwent steroid pretreatment. These findings suggest that even if vitreous cytology findings are negative, other test and characteristic fundus findings should be evaluated to confirm VRL; treatment should be initiated to prevent central nervous system involvement.

Key words: vitreoretinal lymphoma; vitreous cytology; diagnostic vitrectomy; masquerade syndrome

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I. INTRODUCTION

Vitreoretinal lymphoma (VRL) is a intraocular malignancy, which may present in a manner consistent with that of severe intermediate or posterior uveitis [1,2]. Histologically, most cases of VRL are high grade B-cell lymphoma[3]. As VRL often involves the central nervous system, early diagnosis and active treatment are required. The incidence of VRL is approximately 0.047 per 100,000 people[4], affecting adults aged 30-80 years [5-7]; immunosuppression is a risk factor for VRL [8,9].

The most reliable diagnostic test for VRL is vitreous cytology. A typical histologic feature of lymphoma cells is the relative lack of cytoplasm, a prominent nucleus, and coarse chromatin pattern [10,11]. However, the number of vitreous samples that can be obtained from vitreous tapping is limited; moreover, even when diagnostic vitrectomy is performed, VRL remains difficult to confirm histologically due to possible tumor cell loss or direct insult to tumor cells by a vitrectomy cutter [11,12]. To compensate for these shortcomings, alternative methods have been introduced such as measuring the amount of interleukin (IL)-6 and IL-10 in the vitreous cavity [13], immunoglobulin gene rearrangements for clonality assessment [14], and detecting oncogenic myeloid differentiation primary response

gene 88 mutation [15].

Nevertheless, when steroids are used for the treatment of posterior uveitis in cases suspected of VRL, lymphoma cell lysis may occur, making accurate diagnosis difficult. In such cases, it is recommended to wait for a certain period of time between initiating steroid treatment to induce vitreous infiltration and performing diagnostic vitrectomy[16] However, this approach is associated with delayed diagnosis and treatment. This study aimed to compare the sensitivity of various diagnostic tests used to confirm VRL to propose an alternative to vitreous cytology in patients undergoing steroid pretreatment for posterior uveitis.

II. MATERIALS AND METHODS

This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Gangnam Severance Hospital (IRB approval number: 3-2021-0006). The informed consent requirement was waived by the Gangnam Severance Hospital Institutional Review Board (IRB approval number: 3-2021-0006).

1. Study design and inclusion/exclusion criteria

This study was a retrospective, observational study, based on medical records of patients who visited tertiary medical institutions between May 2005 and May 2021. Patients were eligible for this study if they had VRL, confirmed by either vitreous cytology or characteristic funduscopic features/vitreous opacity with favorable response to intravitreal (IVit) methotrexate (MTX) treatment; underwent diagnostic vitrectomy for vitreous cytology; and were followed-up for at least 3 months. Patients were excluded from the present study if they had another ophthalmic disease affecting vision such as diabetic retinopathy, age-

related macular degeneration, or glaucoma, among others.

2. Diagnostic procedures and other findings to be analyzed in this study and statistical processing method

Data on the patients' demographic, medico-surgical, and treatment characteristics were extracted. Corrected visual acuity, intraocular pressure, slit lamp findings, funduscopic examination findings, and results of various imaging tests were collected. Patients underwent conventional 23-gauge or 25-gauge diagnostic vitrectomy. After collecting both undiluted and diluted vitreous samples, patients underwent vitreous cytology tests and measurements of IL-6 and IL-10 levels; immunoglobulin heavy chain (IGH) and immunoglobulin kappa light chain (IGK) clonality assays were performed. Findings from biopsies of other organs affected by lymphoma, if available, were examined. SPSS version 25.0 (IBM Inc., Armonk, NY, US) was used for statistical analyses. Descriptive statistics were reported, and subgroup analysis was performed, using the Chi-square or Fisher exact tests to determine the effect of steroid pretreatment on diagnostic accuracy.

III. RESULTS

1. Demographic characteristics of patients with vitreoretinal lymphoma

A total of 44 patients were included in this study. Among them, 31 (70.5%) showed bilateral VRL during the follow-up period. The mean age of the patients was 62.2 ± 11.5 years, and 56.8% (n = 25) of patients were female. Thirteen (29.5%) patients had a history of hypertension, and 10 (22.7%) had diabetes. There were no immunocompromised patients. Forty (90.9%) patients

received IVit MTX; the average number of injections was 15.3 ± 7.6. Twenty-six patients with VRL had central nervous system involvement within the follow-up period. The treatment patterns were as follows: 57.7% of patients received IVit MTX combined with systemic chemotherapy, and 42.3% received IVit MTX combined with systemic chemotherapy and regional radiation therapy. Patients were followed up for an average of 35.8 ± 32.8 months, and 31.8% (n = 14) of patients died of the disease during the follow-up period (Table 1).

Table 1. Demographic characteristics of patients with vitreoretinal lymphoma

	Count / Mean ± Standard deviation
Patients	44
Laterality (Right/Left)	22 / 22
Age (years)	62.2 ± 11.5
Sex (M/F)	19 / 25
Past history	
Hypertension	13 (29.5%)
Diabetes mellitus	10 (22.7%)
Mean follow-up periods (months)	35.8 ± 32.8
Treatment patterns for VRL	
IVit. MTX (count, %)	40 (90.9%, 15.3 ± 7.6)
None	4 (9.1%)
Treatment patterns for CNS lymphoma	26 (59.1%)
IVit. MTX + Systemic CTx	**15 (57.7%)**
IVit. MTX + Systemic CTx +	**11 (42.3%)**

Brain/Eye RTx	
Expire	14 (31.8%)

M: male; F: female; IVit.:Intravitreal; MTX: methotrexate; CTx: chemotherapy; RTx: radiation therapy

2. Ocular findings at diagnosis

The average initial best-corrected visual acuity of the patients was 0.7 ± 0.9 logMAR (0.2 ± 0.1 Snellen equivalent visual acuity), and the intraocular pressure was 13.3 ± 3.9 mmHg. Inflammatory cells were observed in the anterior chamber in 43.2% of patients (grade 1.4 ± 1.2, according to the Standardization of Uveitis Nomenclature). Vitreous cells or haziness were observed in 97.7% of patients, whereas retinal pigment epithelium infiltration was present in 56.8% (n = 25); retinal hemorrhage was present in 20.5% (n = 9) of patients (Table 2, Figure 1).

Table 2. Ocular findings at diagnosis

	Count / Mean ± Standard deviation
LogMAR BCVA (Snellen equivalent)	0.7 ± 0.9 (0.2 ± 0.1)
IOP (mmHg)	13.3 ± 3.9
Anterior segment findings	
Keratic precipitates	2 (4.5%)

Corneal edema	1 (2.3%)
Cells / SUN grading	19 (43.2%) / 1.4 ± 1.2
Posterior segment findings	
Vitreous cells or haziness	43 (97.7%)
SubRPE infiltration	25 (56.8%)
Retinal hemorrhage	9 (20.5%)

logMAR: logarithm of the minimum angle of resolution; BCVA: best-corrected visual acuity; IOP: intraocular pressure; SUN: standardization of uveitis nomenclature; RPE: retinal pigment epithelium

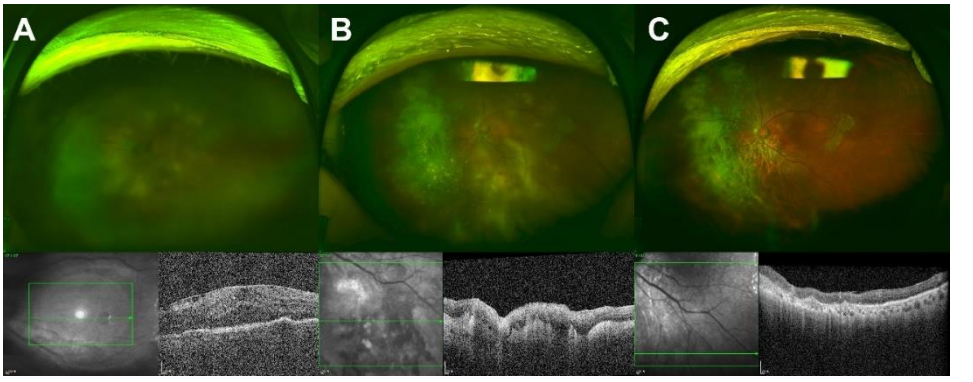

Figure 1. Representative ocular findings before and after diagnostic vitrectomy.

A 75-year-old woman visited the outpatient clinic with a complaint of decreased visual acuity in her left eye that began 3 months prior. She was receiving oral prednisolone for chronic uveitis. At the time of the first examination, the visual acuity was 20/200 and the intraocular pressure was 11 mmHg. Inflammatory cells were present in the anterior chamber and vitreous cavity on the slit lamp examination. (A) Funduscopic examination reveals

vitreous opacity with multifocal subretinal yellowish granular infiltration. Optical coherence tomography (OCT) images show the epiretinal membrane, dense infiltration of sub-retinal pigment epithelium (subRPE), lumpy-bumpy choroid, subretinal fluid, and intraretinal fluid. Diagnostic vitrectomy was performed. (B) After diagnostic vitrectomy, yellowish subretinal infiltration is clearly visible, and subRPE infiltration and lumpy-bumpy choroid are well visible on OCT images. In vitreous cytology, accurate diagnosis was not possible due to cellular paucity, although it was confirmed as positive in IGK gene clonality assay and diagnosed as VRL. (C) The lesions have improved after four injections of intravitreal methotrexate.

3. Origin and involvement patterns of vitreoretinal lymphoma

Overall, 63.6% of VRL cases were histologically diagnosed as diffuse large B-cell lymphoma; in 36.4% of cases, histological diagnosis was not possible. Primary VRL originating from the eye accounted for 63.6% (n = 28) of cases, primary central nervous system lymphoma originating from the brain accounted for 22.7% (n = 10), and lymphoma originating from other organs accounted for 13.6% (n = 6). The rate of brain involvement of primary VRL during the follow-up period was 42.9% (n = 12, 17.9 ± 12.3 months). Central nervous system involvement was detected by routine checkup magnetic resonance imaging scan in 75.0% (n = 9) of patients, newly developed neurologic symptoms in 16.7% (n = 2), and visual field defects in 8.3% (n = 1) (Table 3).

Table 3. Origin and involvement patterns of vitreoretinal lymphoma

	Count / Mean ± Standard deviation
Primary origin of lymphoma	
Eye	28 (63.6%)
CNS involvement during follow-up	12 (42.9%)
Routine checkup MRI	9 (75.0%)
Neurologic symptoms	2 (16.7%)
Visual field defect	1 (8.3%)
Brain	10 (22.7%)
Other	6 (13.6%)
Head and neck	3 (50.0%)
Testis	2 (33.3%)
Breast	1 (16.7%)

4. Comparing diagnostic values of tests

Diagnostic accuracy comparisons between confirmatory tests for VRL were performed. The VRL detection rate of vitreous cytology was 31.8% (n = 14); among patients with negative findings, 40% (n = 12) showed cellular paucity. IL analysis was performed in 77.3% of patients; the detection rate of the IL-10:IL-6 ratio of > 1 was 85.3% (n = 29). The detection rate of the IL-10 level of > 50 pg/mL was 91.2% (n = 31). Of 79.5% of patients who underwent the IGH/IGK gene clonality assays, the detection rate of the former test was 65.7% (n = 23) and that of the latter test was 68.6% (n = 24). The detection rate of either the IGH or IGK gene clonality test was 85.7% (n = 30) (Table 4).

Table 4. Comparisons of test diagnostic values

	Count / Mean ± Standard deviation
Vitreous cytology	44 (100.0%)
Positive	14 (31.8%)
Negative	30 (68.2%)
Unsatisfactory specimen	12 (40.0%)
Interleukin analysis	34 (77.3%)
IL-10:IL-6 > 1	29 (85.3%)
IL-10 > 50 pg/mL	31 (91.2%)
IGH/IGK gene clonality assay	35 (79.5%)
IGH positive	23 (65.7%)
IGK positive	24 (68.6%)
IGH or IGK positive	30 (85.7%)

IL: interleukin; IGH: immunoglobulin heavy chain; IGK: immunoglobulin kappa light chain

5. Effect of steroid pre-treatment on diagnostic accuracy

Additional analyses were performed to determine whether the use of steroids before diagnostic vitrectomy affected diagnostic test findings. A total of 29.5% (n = 13) patients received steroids before undergoing diagnostic vitrectomy. The rate of positive vitreous cytology findings in the steroid pretreatment group was 7.7% (n = 1), which was lower than the rate of 41.9% (n = 13) in the non-steroid pretreatment group (P = 0.035). No significant difference was observed in the

findings of IL analysis and IGH/IGK assays between the two groups (Table 5).

Table 5. Effect of steroid pre-treatment on diagnostic tests for vitreoretinal lymphoma

	Steroid pretreatment (N=13)	No steroid pretreatment (N=31)	P-value
	Count / Mean ± SD	Count / Mean ± SD	
Vitreous cytology	13 (100.0%)	31 (100.0%)	
Positive	1 (7.7%)	13 (41.9%)	0.035†
Negative	12 (45.5%)	18 (40.0%)	
Interleukin analysis	10 (76.9%)	24 (77.4%)	
IL-10:IL-6 > 1	9 (90.0%)	20 (83.3%)	0.999†
IL-10 > 50 pg/mL	10 (100.0%)	21 (87.5%)	0.539†
IGH/IGK gene clonality assay	12 (92.3%)	23 (74.2%)	
IGH positive	9 (75.0%)	14 (60.9%)	0.476†
IGK positive	8 (66.7%)	16 (69.6%)	0.999†
IGH or IGK positive	10 (83.3%)	20 (87.0%)	0.999†

IL: interleukin; IGH: immunoglobulin heavy chain; IGK: immunoglobulin kappa light chain

†Fisher's exact test

IV. DISCUSSION

In the present study, primary VRL was more common than VRL originating from the central nervous system or other organs. In addition, most (42.9%) patients with primary VRL developed brain involvement during the follow-up. Moreover, the diagnostic rate of IL analysis was highest, followed by that of IGH/IGK gene clonality assays; the detection rate of vitreous cytology was low, in particular, in the steroid pretreatment group.Identifying lymphoma cells in a vitreous specimen is key to the diagnosis of VRL. However, the diagnostic rate of vitreous cytology is 45-55%[11,17]. The reason for this low detection rate may be that lymphoma cells are not detected in the sample obtained through vitreous tapping or diagnostic vitrectomy or that a vitreous specimen is contaminated by other cellular structures such as reactive T lymphocytes, necrotic cells, debris, and fibrin [7]. In this study, only 31.8% of patients diagnosed with VRL had lymphoma cells identified by vitreous cytology. Therefore, although vitreous cytology is the first-line diagnostic method for confirming VRL, a negative finding cannot definitively exclude it.

Corticosteroids are important in the treatment of uveitis; in fact, IVit injection of steroids is common in clinical practice [18]. However, up to 2.5% of patients referred for uveitis treatment may exhibit neoplastic masquerade [19] ; initiating corticosteroid therapy before obtaining accurate diagnosis can worsen the causative disease. In older adults, when baseline visual acuity is severely deteriorated and posterior segment involvement is severe, neoplastic masquerade should be discriminated. However, pretreatment with corticosteroids increases the likelihood of negative vitreous cytology findings due to the lymphocytic effect of steroids and tumor cell lysis during vitreous biopsy[16]. In this study, diagnostic sensitivity of vitreous cytology was lower in the steroid pretreatment group than in the non-steroid pretreatment group. However, the results of IL

analysis or immunoglobulin gene rearrangement for clonality assessment did not show any significant difference between the groups, suggesting that cytokine assay or polymerase chain reaction analysis for immunoglobulin gene sequence may be diagnostically sensitive even in very small-volume samples.

This study has some limitations. First, this study was retrospective. Second, some patients whose histological diagnosis of VRL was not confirmed were included in this study; as a result, lymphoma types other than B-cell lymphoma may be included in this study. In addition, the number of patients who received pretreatment with steroids was relatively small. However, this study is meaningful because it has revealed the diagnostic accuracy of IL analysis and immunoglobulin gene rearrangement for clonality assessment.

V. CONCLUSION

The present study suggests that vitreous cytology findings should be interpreted carefully in patients with steroid pretreatment because of the high false negative rate. In such cases, measuring the levels of IL-6 and IL-10 and immunoglobulin gene rearrangement for clonality assessment may help diagnose VRL.

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ABSTRACT(IN KOREAN)

유리체망막 림프종 환자에서 유리체 세포검사의 진단적 가치 분석

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이 동 현

목적: 유리체망막 림프종 환자를 확진할 때 사용하는 유리체 세포검사의 진단적 가치를 확인하고, 다른 진단 검사들과 비교하여 유용성을 확인하고자 하였다.

방법: 2005년 1월부터 2021년 5월까지 본원에 내원한 유리체망막 림프종 환자들 중 진단적 유리체절제술을 시행받고 3개월 이상 관찰한 환자들의 기록을 후향적으로 분석하였다. 시력에 영향을 줄 수 있는 다른 안과적 질환을 동반한 경우는 제외하였다.

결과: 총 44명의 44안을 분석하였다 28명(63.6%)은 원발성 유리체망막 림프종이었으며, 10명(22.7%)은 뇌에서 기원한 림프종, 6명(13.6%)은 그 외의 장기에서 유래하였다. 대상안의 97.7%에서 유리체강의 세포 혹은 유리체혼탁 소견을 보였으며, 56.8%에서 망막색소상피하 침윤, 20.5%에서 망막출혈을 보였다. 각종 검사들의 진단 성적은 다음과 같다: 인터루킨(Interleukin, IL)-10이 50pg/mL을 초과하는 경우는 91.2%였으며, IL-10:IL-6 비율이 1을 초과하는 경우는 85.3%, IGH/IGK 유전자 재배열

검사 결과 진단률은 각각 65.7% 및 68.6%였다. 유리체 세포검사의 진단률은 31.8%였다. 전체 대상안의 29.5%는 스테로이드 전처치를 받았으며, 스테로이드 전처치군과 그렇지 않은 군 간에 진단검사들의 진단률을 비교하였을 때 스테로이드 전처치 군에서 유리체 세포검사의 진단률이 유의하게 낮았으나 (p=0.035) 다른 검사들은 유의한 차이를 보이지 않았다.

결론: 유리체 세포검사는 유리체망막 림프종의 확진에 필요한 검사이지만 진단력이 다른 검사들에 비해 낮게 나왔으며, 특히 스테로이드 전처치를 받은 환자들에서 저조한 성적을 보였다. 따라서, 유리체망막 림프종을 시사하는 특징적인 소견들이 있을 경우 유리체 세포검사 상 음성이라고 하더라도 다른 진단검사들의 도움을 받아 진단 및 치료를 시작해야 한다.

핵심되는 말 : 유리체망막 림프종; 유리체 세포검사; 진단적 유리체절제술; 가면증후군