Preliminary Efficacy of Autologous Plasmin Injection in Pediatric Vitrectomy

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Abstract

***Purpose*:** To evaluate the efficacy of autologous plasmin in pediatric vitrectomy and to report the anatomic outcomes of autologous plasmin assisted pediatric vitrectomy

***Methods*:** In an interventional prospective study, Thirty-four pediatric patients with vitreoretinal disorders who needed vitrectomy during 2008 to 2010, included in the study. The patients were divided into two groups: the retinopathy of prematurity (ROP) and the other vitreoretinal disorders (non-ROP) groups. The patients underwent plasmin assisted vitrectomy. The anatomic outcome was studied among patients with ROP and rhegmatogenous retinal detachment (RRD). Grading of the induced posterior vitreous detachment (PVD) and intraoperative complications were reported by the surgeon. Follow-up examinations revealed anatomic outcomes and complications. The PVD was graded as 0-4 or questionable and anatomic outcomes was reported as total attachment, partial attachment, and detachment.

***Results*:** The mean age of patients was 21.82±31.94 months. Forty-four percent were male. Among the ROP group 82.3% had questionable PVD, 5.9% grade 2, 5.9% grade 3, and 5.9% grade 4; and among the non-ROP group: 12%, 12%, 23%, and 53% had grade 1, 2, 3, and 4 PVD, respectively ($p<0.001$). After 6 months follow-up, in ROP cases, 29.5% had total attachment, 47% partial attachment, and 23.5% detachment. Among RRD cases 100% had total attachment. No complications were seen during surgery.

***Conclusion*:** Intravitreal plasmin injection may facilitate the complete PVD induction with few complications and acceptable anatomic outcomes in pediatric vitreoretinal disorders.

***Keywords*:** Plasmin, Pediatric Vitreoretinal Disorder, Posterior Vitreous Detachment, Retinopathy of Prematurity, Vitrectomy

Iranian Journal of Ophthalmology 2013;25(1):53-58 © 2013 by the Iranian Society of Ophthalmology

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Received: August 21, 2012
Accepted: April 10, 2013

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Source of support: This study was not supported by any of the pharmaceutical companies, or by any grants.

Introduction

Mechanical posterior vitreous detachment (PVD) induction in vitreoretinal surgery may lead to complications including retinal break and optic nerve damage.[1-3] Despite recent improvements in surgical techniques, mechanical PVD is yet very difficult specially among children because of stronger attachment. More complications such as retinal breaks, retinal detachments, nerve fiber damage, and vitreouschisis can occur.[3-5] So it is tended to use enzyme vitrectomy to induce complete and safe PVD.

Among the enzymes, plasmin is more interested due to fewer complications and no morphological retinal surface changes.[6] Plasmin is a serine protease that effects on retinal surface laminin and fibronectin resulting in PVD.[7,8] During the last decade, different studies have been performed on the efficacy of plasmin on PVD that showed plasmin effectiveness in animal and human models.[1-3,8-16] Based on these studies, using plasmin alone, or with surgical techniques would facilitate the PVD induction with fewer complications and better anatomic outcomes which is investigated in some diseases such as diabetic retinopathy,[10,13] traumatic macular hole,[8,14,17] cystoid macular edema (CME),[8] and retinopathy of prematurity (ROP).[15,16]

Despite the importance of inducing PVD in children, there is no report from Iran on this issue. So in the current study we aimed to investigate the efficacy and safety of intravitreal autologous plasmin on a number of pediatric patients suffering from different retinal diseases.

Methods

In a prospective interventional case series 34 pediatric patients who needed vitrectomy were included.

The inclusion criteria were all the children younger than 9 years old, needing vitrectomy i.e. ROP stage 4 and 5, Coats' disease, familial exudative vitreoretinopathy (FEVR), rhegmatogenous retinal detachment (RRD), vitreous hemorrhage (VH), persistent hyperplastic primary vitreous (PHPV), sub-hyaloid hemorrhage (SHH), and persistent idiopathic CME. Exclusion criteria were previous vitrectomy surgery, uveitis, vasculitis, and lossed to follow-up. All patients were included in the study after obtaining written informed consent from patient's parents or guardians and were operated by two surgeons. Though we did not expect to have PVD in pediatric patients, they were evaluated clinically preoperatively in cases of clear media and in the cases of VH or RRD when it was not possible to see the fundus, B-scan was performed.

All patients underwent serial physical examination on 1st, 3rd, 7th postoperative days, and monthly for 9 months after the operation. These serial examinations were performed by another single ophthalmologist for all patients to reduce the bias.

Age, gender, diagnosis, previous treatment, PVD grading, intra and postoperative complications, and anatomic outcome were recorded. The main outcome measure was anatomic success.

The autologous plasmin enzyme (APE) was prepared in the operating room one hour before the operation. A sample of 3 cc citrated blood of each patient was drawn and centrifuged for 15 minutes at 4,500 rpm in a vacuum tube aiming for separating the cells from plasma. The 1.5 cc purified plasma then was added to7,500 U streptokinase which was incubated for 15 minutes in 37°C in the same time. The obtained product was again incubated for 15 minutes in 37°C. After anterior chamber (AC) tap the produced APE (0.2 cc) was injected to each patient's vitreous under sterile condition using a 30 gauge needle, 20 minutes before the operation. The remaining solution of each patient was sent to laboratory in order to test the sterility and was observed for 72 hours.[13,14,18]

In the case of no or partial PVD, it was induced by active suction with the 23 gauge vitrectomy probe and its rate was recorded. The grading of PVD was determined by the surgeon subjectively using the defined PVD grading:

- Grade 1: Absent PVD and achieving PVD difficultly (more than 300 mmHg suction rate)
- Grade 2: Absent PVD and achieving PVD easily (less than 300 mmHg suction rate)
- Grade 3: Partial PVD spontaneously
- Grade 4: Total PVD spontaneously

In condition of narrow funnel, such as most of patients with stage 5 ROP, vitreous

detachment during surgery was undeterminable and PVD was considered as questionable. In these patients we studied anatomic outcome and possible complications.

All patients were operated with 23 gauge vitrectomy. We evaluated plasmin's efficacy in PVD induction with intraoperative and postoperative complications. Response to treatment was determined as retinal reattachment in patients with ROP and RRD which had detachment before surgery. This was categorized as total attachment, partial attachment, and no attachment.[16]

- Total: Complete reattachment to the retina
- Partial: Reattachment at least in macula
- Off: Detachment

In the case of silicone filled eye anatomic success was evaluated three months after silicone oil removal. The probable intraoperative complications such as hemorrhage, new retinal break, and fibrin formation were recorded; and the postoperative complications were carefully observed i.e. hemorrhage, inflammation, cataract, and endophthalmitis and detachment in patients with retinal attachment before surgery.

Because of variations in disease pathogenesis, we considered two groups of patient: ROP and non-ROP groups.

Analyzing the data: χ^2 and independent samples t tests were used by means of SPSS Software version 15 (SPSS, Chicago, Ill). $p<0.05$ was considered as significant.

Results

We had 34 cases with the average age of 21.82±31.94 (range of 2-108 months). Fifteen cases (44%) were male and 19 cases (56%) were female. The patients' profiles are shown in table 1. We had 17 patients in ROP group 12 cases (70.6%) were female and 5 cases (29.4%) were male, and in non-ROP group 10 of 17 cases (59%) were male and seven of 17 cases (41%) were female. Twelve of 34 cases (35%) had history of previous treatments such as laser therapy, lensectomy, avastin injection, and buckling; and 22 of 34 cases (65%) didn't have such history.

Preoperation PVD was seen in no patient which was assessed clinically or by echography. Among these, 6 out of 14 patients with stage 5 ROP had history of receiving laser therapy and avastin injection, 1 of 2 patients with Coats' had received laser therapy, 1 of 3 patients with VH had received avastin, 2 of 7 patients with RRD had underwent lensectomy and buckling, 1 of 3 patients with stage 4 ROP had received laser therapy and avastin injection, and the patient with SHH had history of lensectomy. One patient with FEVR, one patient with PHPV hadn't received any treatment previously and two patients with CME had received 2-3 intravitreal triamcinolone (idiopathic persistent CME).

In ROP group, PVD grading was as follow: one of 17 cases (5.9%) had PVD grade 2, one of 17 cases (5.9%) had PVD grade 3, and one of 17 cases (5.9%) had PVD grade 4. In our study, among stage 5 ROP patients PVD grading by surgeon seemed difficult due to vitreoretinal morphological changes and membrane formation instead of formed gelatinous vitreous, was reported as questionable; while in surgeon's opinion, the separation achieved easier and faster than mechanical vitrectomies. So 14 of 17 (82.3%) cases were reported as questionable and the surgeon could not asses the PVD grading. In non-ROP group, 2 of 17 cases (12%) had PVD grade 1, 2 of 17 cases (12%) had PVD grade 2, 4 of 17 cases (23%) had PVD grade 3, and 9 of 17 cases (53%) had complete PVD as graded 4 ($p<0.001$). The details of these data are shown in table 2.

During follow-up visits these results were seen; in ROP, cases (29.5%) had total attachment, 8 cases (47%) had partial attachment, and 4 cases (23.5%) remained detached. And in RRD group all 7 cases (100%) had total attachment. This information is shown in table 3.

Only 12% of non-ROP cases needed suction rate more than 300 mmHg.

We did not see any complications during surgery caused by intravitreal plasmin injection. The patients did not have any complications after surgery such as cataract, hemorrhage and endophthalmitis. One patient who underwent vitrectomy due to VH developed partial detachment that we considered as plasmin-vitrectomy complication.

Table 1. Patients' profiles in details

Number	Gender	Age (mo)	Diagnosis	Previous treatment	Eye	PVD	Response
1	Female	2	ROP5	Laser+Avastin	OS	Q	Partial
2	Female	2	ROP5	Laser+Avastin	OD	Q	Off
3	Female	2	ROP5	Laser+Avastin	OS	Q	Off
4	Female	2	ROP5	Laser+Avastin	OD	Q	Partial
5	Male	6	ROP5		OS	Q	Partial
6	Male	12	ROP5		OD	Q	Total
7	Female	5	ROP5		OD	Q	Partial
8	Female	9	ROP5	Laser+Avastin	OS	Q	Total
9	Female	12	ROP5		OD	Q	Partial
10	Male	4	ROP5		OS	Q	Partial
11	Female	4	ROP5	Laser	OS	Q	Off
12	Female	10	ROP5		OS	Q	Total
13	Female	10	ROP5		OD	Q	Total
14	Female	2	ROP5		OS	Q	Off
15	Male	3	ROP4		OD	3	Partial
16	Male	6	ROP4	Laser+Avastin	OD	4	Total
17	Female	2	ROP4		OS	2	Partial
18	Male	72	CME		OD	4	Total
19	Male	72	CME		OS	4	Total
20	Male	108	COATS'	Laser	OD	4	Total
21	Female	72	FEVR		OS	1	Total
22	Male	6	RRD		OS	4	Total
23	Female	2	RRD		OS	3	Total
24	Male	3	RRD	Lensectomy	OS	4	Total
25	Female	6	RRD		OS	4	Total
26	Male	6	RRD	Buckling	OS	4	Total
27	Female	2	VH		OD	4	Total
28	Male	9	VH	Avastin	OS	4	Total
29	Male	84	VH		OD	3	Partial
30	Female	9	SHH	Lensectomy	OS	1	Total
31	Female	12	PHPV		OD	3	Total
32	Female	84	RRD		OS	2	Total
33	Male	18	RRD		OD	3	Total
34	Male	84	Coats'		OS	2	Total

PVD: Posterior vitreous detachment, ROP: Retinopathy of prematurity, CME: Cystoid macular edema, FEVR: Familial exudative vitreoretinopathy, RRD: Rhegmatogenous retinal detachment, VH: Vitreous hemorrhage, SHH: Sub-hyaloid hemorrhage, PHPV: Persistent hyperplastic primary vitreous, OS: Left eye, OD: Right eye, Q: Questionable

Table 2. Comparison of Posterior vitreous detachment induction between patients of retinopathy of prematurity and non-ROP groups

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Questionable	Total and p-value
ROP	-	-	1 (5.9%)	1 (5.9%)	1 (5.9%)	14 (82.3%)	17p>0.05
Non-ROP	-	2 (12%)	2 (12%)	4 (23%)	9 (53%)	-	17p<0.001
Total	-	2 (6%)	3 (9%)	5 (15%)	10 (29%)	14 (41%)	34

ROP: Retinopathy of prematurity

Table 3. Comparison of treatment response among patients with retinopathy of prematurity and rhegmatogenous retinal detachment

	Total Attachment	Partial Attachment	Off	Total
ROP	5 (29.5%)	8 (47%)	4 (23.5%)	17
RRD	7 (100%)	-	-	7
Total	12 (50%)	8 (33%)	4 (17%)	24

ROP: Retinopathy of prematurity, RRD: Rhegmatogenous retinal detachment

Discussion

Induction of PVD is one of the most important strategies in vitrectomy surgery.[1-3] Despite of surgical improvements, mechanically PVD induction has many intraoperative or postoperative complications due to retinal manipulations or incomplete separation.[16] So, many studies have been designed to create safe and complete PVD pharmacologically.[8,10,13-17] Among enzymes which have been studied, plasmin was the most effective.[6-8] Previously it has been reported that plasmin could induce PVD due to its proteolysis role. Plasmin does hydrolysis laminin and fibronectin at the retinal surface.[7,8] This separation has fewer complications, better anatomic outcome and lesser time of the surgery.[11,13-15]

Considering the above mentioned benefits, we designed the current study due to the lack of experience on this issue in Iran.

Although we could not evaluate the PVD induction in all ROP cases, in our experience patients, in surgeons' opinion, vitrectomy surgery was facilitated by using plasmin, and anatomic outcome was much better in comparison of our previous patients in our hands (not reported).

In non-ROP cases, 53% of them had spontaneous PVD (grade 4) and did not need any other processes, 23% of them showed grade 3 PVD, and only 12% of them had grade 1 PVD, as we know the usual suction rate in PVD induction with 23 gauge system is much higher, almost 600 mmHg. In cases with incomplete separation, the eyes required lower pressure suction in order to complete the induction.

The success rate as determined as total attachment in our study was 29.5% among cases with ROP while this rate was reported 100% and 68.8% in studies of Tsukahara and Wu, respectively.[15,16] These differences may be due to earlier interventions, and/or the multifactorial nature of the disorder. Some cases in Wu study had a history of previous vitrectomy in stage 4 ROP which can improve the results,[16] while the patients with history of vitrectomy were excluded from our study. Although surgical outcome was improved in ROP patients by using plasmin, the final outcome was yet disappointing, that shows the need of considering other factors such as earlier diagnosis and interventions.

In RRD cases, we had 100% total attachment which is comparable with Heimann study that had a success rate of 70.7% of retinal reattachment with one operation among 512 patients.[19] It shows that plasmin assisted vitrectomy has positive role in inducing complete and easier PVD and causes better anatomic outcome. In some studies, the success rate of pediatric RRD surgeries with buckling and vitrectomy has been reported to be 82-87%.[20,21] Because of high success rate in our RRD cases, it seems that plasmin may help in better anatomic outcome.

As it was expected we did not see any intraoperative complications related to PVD induction, and also we did not see any postoperative complications such as hemorrhage, inflammation, and endophthalmitis. Only one patient developed RRD suffering from VH with attached retina before surgery which seems to be a complication of vitrectomy procedure, so it seems that plasmin usage is safe and effective.

It would be better to inject 0.1 ml triamcinolone in addition to APE before Vitrectomy and then evaluating the rate of PVD objectively or by B-scan, but it needs injection of at least 0.3 ml fluids into the vitreous that is not safe to inject this amount even if we perform AC tap. We had some limitations in our study; the PVD grading was dependent on surgeon's opinion and is subjective and we did not evaluate PVD presurgery by ultrasonography; we did not measure functional outcome because of patients' age range, they did not have enough cooperation; time consuming for plasmin preparation, the low number of patients and the lack of control group were other shortages.

Considering above limitations, recently few studies have reported the use of microplasmin for inducing PVD.[22,23] Microplasmin is a recombinant molecule of human plasmin which is more available and storable.[22] It has been shown that it could facilitate PVD induction in comparison with placebo group but it is not compared with plasmin yet.[23] We suggest to compare plasmin and microplasmin in a double blinded case-control study to clarify microplasmin safety and effectiveness.

Conclusion

Our study showed that intravitreal plasmin injection before vitrectomy may be safe and effective and we had acceptable anatomical outcome with limited complications.

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