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SAFETY AND EFFICACY OF PHARMACOLOGICAL VITREOLYSIS BY INTRAVITREAL INJECTION OF HYALURONIDASE

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ABSTRACT: AIM: To study the safety and efficacy of pharmacological vitreolysis by intravitreal injection by hyaluronidase. **METHODS:** a prospective comparative interventional case series of 20 eyes of 20 patients who underwent intravitreal injection of Hyaluronidase 100 IU in one eye in a tertiary eye care centre by a single surgeon between June 2012 to June 2014 at Sarojini Devi Eye Hospital. 20 eyes of 20 patients were taken with other eye being taken as control **RESULTS:** Hyaluronidase injection into the vitreous cavity can induce Posterior Vitreous Detachment (PVD) within 15 days without causing any side effects and complications. **CONCLUSION:** Hyaluronidase is safe and effective in the vitreous cavity

KEYWORDS: Pharmacological vitreolysis by intravitreal injection of Hyaluronidase.

INTRODUCTION: Pharmacologic Vitreolysis: Vitreoretinal interface is involved in a wide range of vitreoretinal disorders and separation of posterior vitreous face from retinal surface is an essential part of vitrectomy surgery.

Pharmacologic vitreolysis is a new therapy which could greatly facilitate vitreoretinal surgery today and offer possibility of preventing serious vitreoretinal disorders in future. These vitreolytic agents may ultimately eliminate the need for surgery altogether by inducing prophylactic posterior vitreous detachment so as to prevent anomalous posterior vitreous detachment in diabetic patients and in fellow eye of patients with retinal detachments and macular holes. The intent is to use pharmacologic agents {both enzymatic and nonenzymatic} to liquify gel vitreous and induce complete dehiscence of vitreoretinal adhesion.

OBJECTIVES OF PHARMACOLOGIC VITREOLYSIS: Goal of pharmacologic vitreolysis is to cleave vitreoretinal junction thereby inducing posterior vitreous detachment and to liquefy vitreous gel.¹

- 1) Mechanical vitrectomy may be incomplete, both at posterior pole and retinal periphery. Remnants of cortical vitreous may be left behind at internal limiting membrane of retina leading to vitreoretinal traction and proliferation of cells.
- 2) Offers complete posterior vitreous detachment without mechanical manipulation at vitreoretinal interface such as internal limiting membrane peeling, thus minimizing risk of iatrogenic damage.
- 3) An intravitreal injection resulting in complete posterior vitreous detachment is less traumatic than vitrectomy and might be beneficial as prophylactic treatment regime in retinal disease characterized by fibrocellular and fibrovascular proliferation of vitreoretinal interface to prevent advanced stages of disease.²

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- 4) Cleaving cortical hyaloid completely from retina changes molecular flux across vitreoretinal interface and improves oxygen supply to retina thereby alleviating retinal hypoxia and overexpression of vasoactive substances.
- 5) In 25 guage vitrectomy surgery, it would be advantageous to pharmacologically breakdown vitreous macromolecules so as to decrease vitreous macroviscosity and facilitate surgical removal, such a pharmacologic adjunct would make surgical approach safer, faster and facilitating surgery as in patient and office setting.²

Pharmacologic vitreolysis involves use of diverse range of enzymatic & nonenzymatic agents being studied as an adjunct before or during vitrectomy to facilitate induction of posterior vitreous detachment.³

Posterior Vitreous Detachment (PVD) & Anomalous PVD and Its Consequences: Liquefaction originates in premacular vitreous cortex, focal collections of liquefaction or liquid lacunae subsequently form throughout vitreous increasing in number during adolescence and adulthood, this process in coalescence of extensive areas of synchysis. As liquefaction progresses, collagen fibrillar meshwork undergoes collapse which leads to aggregation of collagen into parallel bundles that form thick fibres seen clinically as synergetic debris which becomes progressively dense and tortuous with age.⁴

Studies demonstrate that majority of patients below 60 years have intact vitreoretinal interface despite presence of extensive liquefaction. After 60 years of age there is much stronger correlation between posterior vitreous detachment (PVD) incidence and extent of liquefaction indicating presence of some factor which enhances effect of liquefaction and collapse on PVD induction, this factor is likely a weakened adhesive force between retina and vitreous.

Variation in the strength of this force likely determine whether given PVD is accompanied by pathological sequale. Age related PVD progresses normally except where especially firm vitreoretinal adhesions are encountered in the macula, optic disc margin or at focal sites in retinal periphery. In such eyes, dynamic (Saccade) vitreous traction is exerted upon retina at residual adhesion sites leading to various complications including macular hole, vitreomacular traction, tractional diabetic macular edema, vitreopapillary traction syndrome, vitreous hemorrhage and retinal tears.

Anomalous posterior vitreous detachment is a condition in which extent of gel liquefaction and collapse exceed of gel liquefaction and collapse exceed attenuation of vitreoretinal adhesion i.e, vitreous gel liquefaction occurs without sufficient dehiscence at vitreoretinal interface precluding clean separation of post vitreous cortex away from retina known as anomalous posterior vitreous detachment.

Consequences of Anomalous Posterior Vitreo Detachment: May vary depending on;

- Where gel is most liquefied.
- Where posterior vitreous cortex is firmly adherent to retina.
- If in peripheral retina (eg in areas of lattice)then retinal tears and retinal detachment is Consequence of anomalous posterior vitreous detachment.⁴
- If attached at optic disc and along retinal vessels, anomalous posterior vitreous detachment plays important role in proliferative diabetic retinopathy.⁵
- If vitreous adhesion to macula is unusually strong, then variety of vitreomaculopathies result from anomalous posterior vitreous detachment.

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- In macula, anomalous posterior detachment varies depending upon whether attached Vitreous is full thickness or whether there is a split in posterior vitreous cortex (vitreschisis).⁶

Hyaluronidase: In the present indication, Hyalase is aimed to cleave hyaluronic acid into smaller fragments to create liquefaction of vitreous. It also allows diffusion and movement of cells with in vitreous to phagocytose the clot. In addition xenobiotic Hyalase in vitragan is intended to produce a limited acute inflammatory reaction to increase recruitment of phagocytic cells and to further increase rate of clearance of resolved clot. Hyaluronidase was developed as a spreading or diffusing substance to increase permeability of connective tissue through hydrolysis.⁷ Hyaluronan plays critical role in maintaining gel like character of vitreous. Hyalase possesses considerable potential as a liquefactive agent. Hyalase mediated vitreous liquefaction has been demonstrated both invitro and invivo and more recently in phase 3 clinical trials studying its potential in clearance of vitreous hemorrhage.⁷ Investigators hypothesized that induction of posterior vitreous detachment by hyalase follows liquefaction of central vitreous with collapse and subsequent loss of support for cortical vitreous fibres, rendering it susceptible to separation by mechanical forces such as eye movements.⁸

Uses: Used to promote absorption of blood and fluid in traumatic or postoperative edema or haematoma.⁹

Data support role of lysosomal hyaluronidases in metastases and role in tumor suppression.¹⁰

Role in Pathogenesis: Bacteria such as streptococci & staphylococcal pathogens use hyaluronidase as a virulence factor to destroy polysaccharide which holds animal cells together, making it easier for pathogen to spread through tissues of host organism.¹¹

MATERIAL AND METHODS: This study was a prospective comparative interventional case series of 20 eyes of 20 patients who underwent intravitreal injection of Hyaluronidase 100 IU in one eye in a tertiary eye care centre by a single surgeon between June 2012 to June 2014.

20 eyes of 20 patients were taken with other eye being taken as control.

Patients were explained about the diagnosis, various treatment options & possible Complications & prognosis of the condition before enrolment.

Evaluation included complete comprehensive ocular examination at baseline and at each follow up visits i.e.,

1. S/L examination of anterior & posterior segment (Using 90 D Lens).
2. BCVA by snellens chart.
3. Intraocular pressure measurement with Goldmann applanation tonometer.
4. Indirect ophthalmoscopy.
5. Fundus photography.
6. FFA (When required).
7. B-Scan.
8. OCT (When required).

RBS, Syringing & B.P were checked. Snellens V.A was converted to logarithm of minimum angle of resolution (Log mar scale) for analysis. Patients were investigated routinely for surgical procedure (Intravitreal injection).

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Follow Up: Patients were examined before injection Hyaluronidase and repeated on 1st, 3rd, 5th, 7th and 15th day post-injection.

The data thus collected was subjected to statistical analysis. The analysis was performed using graphpad instant windows software. The level of statistical significance was set at $P < 0.05$.

Selection and Exclusion Criteria:

Selection Criteria: Clear vitreous and opaque vitreous with Diabetic retinopathy without Retinal detachment cases.

All cases of Diabetic retinopathy with clear or opaque vitreous where no PVD is present.

Exclusion Criteria:

1. Clear vitreous or opaque vitreous with existing PVD.
2. Retinal detachment.
3. Intraocular tumors or intraocular foreign bodies.
4. H/O Ocular trauma and eyes with previous eye surgery or intravitreal injection.

OBSERVATION AND RESULTS: Total no. of patients were 20. other eye of same patient are taken as control.

The follow up period was maximum for 15 days.

Variables including gender, age, BCVA, Bscan, IOP were recorded at baseline. BCVA, IOP, Induction of posterior vitreous detachment (PVD) through B-Scan examination & Fundus photographs were taken at each follow up ie before injection Hyaluronidase & on 1st, 3rd, 5th, 7th, 15th POD. Presenting complaints, comorbid risk factors were recorded according to case proforma included in the study.

GENDER DISTRIBUTION:

GENDER	NO.OF PATIENTS (%)
Male	8 (40)
Female	12 (60)

AGE DISTRIBUTION:

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Population under study were between age groups 50-72, out of which majority, 8(40%) were between 50-59 years, next 9(45%) were between 60-69 years & 3(15%) patients were between 70-79 years of age.

GRAPHS DEPICTING IOP CHANGES DURING STUDY PERIOD:

All 20 patients under study had normal IOP before injection Hyaluronidase. 8 patients had IOP 10 mm Hg, 6 patients had IOP 11 mm Hg, 4 patients had IOP 12 mm Hg & 2 patients had IOP 13 mm Hg.

On 1st POD, 15 patients had developed increase in IOP compared to that before inj.Hyalase. 5 patients had IOP 16 mm Hg, 3 patients had IOP 17 & 18 mm Hg, 4 patients had IOP of 19 mm Hg and remaining 5 patients had IOP values as that before inj. Hyalase ie, 2 patients had IOP of 11 mm Hg & 3 patients had IOP 12 mm Hg.

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On 3rd POD, 10 patients had increase of IOP compared to that before inj. Hyalase ie, 2 patients had IOP of 16 & 17 mm Hg, 3 patients had IOP 18 mm Hg, 3 patients had IOP 19 mm Hg and 10 patients had IOP values similar to that before inj. Hyalase.

All patients had IOP values similar to that before inj. hyalase except for 4 patients showing a slight 1mm rise of Hg.

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On 7th & 15th POD, all patients had IOP values similar to that before inj. Hyalase ie, 8 patients had IOP of 10mm Hg, 6 patients had IOP of 11, 12mm Hg.

GRAPHS DEPICTING VISUAL ACUITY CHANGES DURING STUDY PERIOD:

BCVA of Patients and No. of Patients before inj. Hyalase	Percentage of patients
(6/9) 1	5
(6/12) 1	5
(6/18) 2	10
(6/36) 1	5
(6/60) 4	20
(5/60) 1	5
(4/60) 4	20
(3/60) 4	20
(2/60) 2	10

BCVA Changes of Patients before Inj. Hyalase

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BCVA of patients remained same before inj.Hyalase and on 1st POD.

On 3rd POD, BCVA of 2 patients was decreased by 1 Snellens line.

On 5th POD, 3 more patients i.e., total 5 patients developed 1 Snellens lines decrease of visual acuity

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On 7th POD, 5 more patients i.e., total 10 patients developed 1 Snellens lines decrease of visual acuity.

On 15th POD, 5 more patients i.e., total 10 patients developed 1 Snellens lines decrease of visual acuity.

BCVA was recorded before injection Hyalase, & on 1st, 3rd, 5th, 7th & 15th POD. On 1st POD and before injection Hyalase, BCVA values were same. On 3rd POD, 2 patients developed 1 snellens lines decrease of visual acuity, on 5th POD 3 more patients i.e., total 5 patients developed 1 snellens lines decrease of visual acuity, on 7th POD 5 more patients i.e., total 10 patients developed 1 snellens lines decrease of visual acuity.

GRAPH DEPICTING PVD CHANGES DURING STUDY PERIOD:

	BEFORE INJ.HYALASE	1 ST POD	3 RD POD	5 TH POD	7 TH POD	15 TH POD
NO PVD	20	20	12	0	0	0
PARTIAL PVD	0	0	8	20	13	3
TOTAL PVD	0	0	0	0	7	17

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Out of 20 patients under study, on 1st POD and before inj.Hyalase all 20 patients had no PVD, ON 3rd POD 12 patients had no PVD and 8 patients had partial PVD, on 5th POD all 20 patients had partial PVD, on 7th POD 13 patients had partial PVD and 7 patients had total PVD. at the end ie., on 15th POD, 17 patients had total PVD and remaining 3 patients had partial PVD.

DISCUSSION: Creating a complete and atraumatic vitreoretinal separation and attaining an ultrastructurally smooth and vitreous-free ILM surface is an essential step for successful vitreoretinal surgery. This may also improve retinal oxygenation and decrease the risk of PVR.

Many retinal diseases involve an anomalous vitreoretinal interface. The mechanical relationship between the vitreous and the retina is mediated by the posterior vitreous cortex and the internal limiting membrane. The posterior vitreous cortex consists of densely packed collagen fibrils arranged tangentially upon the ILM. The adhesion of the posterior vitreous cortex is a dynamic process with topographic and age-related variations. There are no apparent gross anatomic correlates to explain the occurrence of vitreoretinal adhesion, which is therefore believed to be mediated by a molecular glue consisting of glycoproteins such as fibronectin, laminin and chondroitin, as well as integrins and other glycoconjugates. This molecular glue is an important target.

As with other extracellular matrices, the vitreous contains matrix metalloproteinases, which are regulated by tissue inhibitors of MMP (TIMP). MMP and TIMP play both a physiologic and pathologic role in the vitreous. These enzymes contribute to the proteolytic state that clears the vitreous of potential opacification and maintains optical clarity. In disease states, MMP have been implicated in proliferative vitreoretinopathy, retinopathy of prematurity and diabetic retinopathy. The above understanding of vitreous molecular structure is the basis for current attempts at pharmacologic vitreolysis. Although multiple enzymes and other agents have been examined in animal models, only hyaluronidase, plasmin and microplasmin have had significant clinical experience reported.

The vitreous has long been recognized as an integral factor in a wide spectrum of retinal diseases including retinal detachment, macular hole formation, vitreomacular traction syndromes, diabetic retinopathy, proliferative vitreoretinopathy and most recently, neovascular age-related macular degeneration. The pathogenic mechanisms by which the vitreous affects the retina are not completely understood, but include abnormalities in vitreoretinal adhesion manifesting in tractional phenomena, vitreous opacification and direct effects on retinal oxygenation and growth factor and cytotoxine production and distribution within the eye. Currently, the pathologic manifestations of vitreopathies are managed primarily with vitrectomy. However, recent developments suggest a new era of manipulation of the vitreous is imminent.

Jerry Sebag, MD, coined the term "pharmacologic vitreolysis" to describe the therapeutic manipulation of molecular structure of the vitreous to achieve posterior vitreous detachment and vitreous liquefaction.

More recently, Michael Trese, MD, has used the term "vitreodynamics" in recognition of changes in vitreous oxygenation and growth factor levels that result from pharmacologically induced changes in vitreous molecular structure.

The majority of developmental work performed in pharmacologic vitreolysis and vitreodynamics involves enzymes including hyaluronidase, chondroitinase, tissue plasminogen activator, dispase, urokinase and plasmin.

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Vitreolytic agents have been categorized on the basis of their biologic effect into those which induce vitreous liquefaction ("liquefactants") and those which induce dehiscence at the vitreoretinal interface ("interfactants").

While other vitreolytic agents act by separating the vitreoretinal interface, hyaluronidase exerts its effect by dissolving the glycosaminoglycan network of the vitreous gel which is predominantly composed of hyaluronan. Collagen fibrils of the vitreous are very resistant to proteolytic degradation and therefore not a good target for pharmacological vitreolysis, but enzymatic degradation of the hyaluronan helps vitreous degradation and thus may facilitate dispersal of vitreous hemorrhage. Some studies have shown that intravitreal injection of hyaluronidase could increase the rate of vitreous removal during vitrectomy.

Highly purified bovine hyaluronidase (Vitrace) is the only vitreolytic agent that has passed phase III trials. Hyaluronidase basically acts on the glycoside bonds of hyaluronan and other mucopolysaccharides in the vitreous and causes vitreous liquefaction. There is no evidence to support a direct effect of hyaluronidase on the vitreoretinal interface. Vitrace was evaluated in two phase III trials for management of vitreous hemorrhage.

Gottlieb et al. investigated effects of intravitreal hyaluronidase on rabbit eye and concluded that it induced partial vitreolysis. A subsequent study by Harooni et al. showed that hyaluronidase induced posterior vitreous detachment but this finding was not substantiated by other investigators. A possible explanation for these differences is that preparations of hyalase are often contaminated with significant amounts of proteolytic enzymes and that it was nonspecific proteolysis which induced PVD in Harooni study. A highly purified ovine hyalase (Vitrace) has been developed for clinical use. 2 phase III randomized controlled trials have shown that a single intravitreal injection of vitrase is safe and demonstrated efficacy in aiding clearance of vitreous hemorrhage.

A total of 1,125 patients were randomized in a 1: 1: 1 ratio to intravitreal injection of saline and 55 IU or 75 IU of hyaluronidase. The primary end point was clearance of vitreous hemorrhage. There was a statistically significant improvement in the 55 IU group at 1, 2 and 3 months as compared to the saline group. Intravitreal injection of hyaluronidase was well tolerated without any significant complication.

Although the study investigators concluded that intravitreal hyaluronidase was useful in the management of vitreous hemorrhage, this agent has not yet received FDA approval for this indication. Furthermore, other studies have shown that although hyaluronidase may help in vitreous liquefaction and vitreous hemorrhage clearance, it does not induce vitreoretinal separation. Sutureless vitrectomy using the 23 or 25 gauge systems has gained increasing popularity in recent years. One of the main concerns in small gauge vitrectomy is the decreased rate of vitreous removal by smaller vitrectomy probes.

20 patients with NPDR were selected for study. Variables including gender, age, BCVA, Bscan, IOP were recorded at baseline. BCVA, IOP, Induction of posterior vitreous detachment (PVD) through B-Scan examination & Fundus photographs were taken at each follow up i.e. before injection Hyaluronidase & on 1st, 3rd, 5th, 7th, 15th POD.

This study comprised both males & females with males accounting about 8 in no. out of 20 i.e. 40% of population under study were males & 12 in no. out of 20 i.e. 60% of population under study were females.

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CONCLUSION AND SUMMARY:

- The results of our study shows that Hyaluronidase injection into the vitreous cavity can induce Posterior Vitreous Detachment (PVD) within 15 days without causing any side effects and complications.
- It is safe and Effective in the vitreous cavity.
- Compare to recent drugs, which are used to induce PVD, it is more economical and easily available in India.
- However, a study with large sample size is required to determine the safety and efficacy for wider acceptance of this procedure.

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