Original Article

Intravitreal Injections of Bevacizumab Plus Methotrexate versus Bevacizumab Alone for the Treatment of Diabetic Macular Edema: A Randomized, Sham-Controlled Trial

Farhad Fazel[1], Behrooz Oliya[1], Majid Mirmohammadkhani[2,3], Mohammadreza Fazel[1], Ghasem Yadegarfar[4], Mohsen Pourazizi[1,5]

[1]Department of Ophthalmology, Isfahan Eye Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Social Determinants of Health Research Center, Semnan University of Medical Sciences, Semnan, Iran, [3]Department of Epidemiology and Biostatistics, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran, [4]Department of Epidemiology and Biostatistics, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran, [5]Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Purpose: To evaluate the efficacy of intravitreal bevacizumab (IVB) combined with intravitreal methotrexate (IVM) in the treatment of diabetic macular edema (DME).

Methods: In this prospective, interventional contralateral eye study, patients with bilateral DME were randomly allocated to receive three monthly injections of IVB (1.25 mg/0.05 mL) plus IVM (400 μg; 0.16 cc) or IVB alone. The outcome measure was changes in the best corrected visual acuity (BCVA), central macular thickness (CMT), and central macular volume (CMV).

Results: Thirty-six treatment-naive eyes of 18 patients with a mean age of 62.38 ± 6.2 years were included in the study. BCVA logMAR changed from 0.95 ± 0.53 at baseline to 0.75 ± 0.53 in the combination group and from 0.72 ± 0.57 to 0.49 ± 0.50 in the IVB alone group at 1 month after the 3rd injection. BCVA improvement in both groups was not statistically significant compared with the baseline value ($P > 0.99$). Compared with the baseline values, mean CMT and CMV were reduced in both groups; however, these changes did not reach a significant level. The differences of CMT changes between the groups were not statistically significant at month 1 ($P = 0.82$), month 2 ($P = 0.21$), and month 3 ($P = 0.10$). Furthermore, the differences of CMV changes between the groups were not statistically significant at month 1 ($P = 0.76$), month 2 ($P = 0.82$), and month 3 ($P = 0.11$).

Conclusions: This pilot study demonstrated no significant therapeutic effects for IVB combined with IVM compared to IVB alone in treatment-naive DME patients over a 3-month course.

Keywords: Anti-vascular endothelial growth factor, Bevacizumab, Diabetic macular edema, Diabetic retinopathy, Methotrexate

Address for correspondence: Behrooz Oliya, Department of Ophthalmology, Isfahan Eye Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: behrooz_oliya1th@yahoo.com
Submitted: 03-Nov-2019; **Revised:** 18-Jan-2020; **Accepted:** 26-Jan-2020; **Published:** 30-Apr-2020

Introduction

Diabetic macular edema (DME), an accumulation of fluid within the macula due to the failure of the blood-retinal barrier, is one of the most common causes of decreased vision in diabetic retinopathy.[1,2]

A large variety of therapeutic approaches, including laser photocoagulation, anti-vascular endothelial growth factor (anti-VEGF) therapy, steroid, and surgical therapy are available to treat DME. A new era of DME therapy has started with more focus on pathophysiology and the mechanism of the development of DME.[1,3] Regarding this trend, the majority of clinical studies have focused on intravitreal injection protocols. Anti-VEGFs are expensive and not always effective

Access this article online

Quick Response Code:

Website: www.jcurrophthalmol.org

DOI: 10.4103/JOCO.JOCO_101_20

How to cite this article: Fazel F, Oliya B, Mirmohammadkhani M, Fazel M, Yadegarfar G, Pourazizi M. Intravitreal injections of bevacizumab plus methotrexate versus bevacizumab alone for the treatment of diabetic macular edema: A randomized, sham-controlled trial. J Curr Ophthalmol 2020;32:164-9.

and may have systemic events. Therefore, other alternatives are needed. Furthermore, frequent and high exposure to intravitreal anti-VEGF therapy (e.g., monthly injections) in patients with DME has been reported to be associated with detectable levels of VGEF in the systemic circulation[4] and may lead to an increased risk of thrombosis due to suppressed systemic VEGF levels and decreased nitric oxide (NO) and prostaglandin-I2 (PGI2) production.[5]

Considering the strong role of inflammation in the pathogenesis of DME, the intravitreal injection of anti-inflammatory drugs can be considered a promising option in naive or unresponsive eyes.[3,5-7] Recently, Falavarjani *et al*.[8] used intravitreal methotrexate (IVM) for the treatment of persistent DME. The results of their study showed that IVM injection is significantly effective in treating persistent DME due to its anti-inflammatory effects. Based on the mechanism of action of methotrexate (MTX) and the results of the previous study,[8] it can be hypothesized that IVM also has a role in naive eyes with DME in patients with systemic risk factors for anti-VEGF agents, including patients at a high risk of atherothrombotic events, patients with a recent stroke or multiple strokes,[5] or those who are not willing or unable to pay for the expensive anti-VEGF agents.

MTX may also be used in combination with anti-VEGF agents to enhance their therapeutic effect.[8] Since the benefits of IVM have not been approved in the treatment of DME, this study was designed using a combination of intravitreal bevacizumab (IVB) and IVM.

The aim of this study was to evaluate the efficacy and safety of the IVB, an anti-VEGF agent, combined with the intravitreal injection of MTX, as a therapeutic option for naive eyes with DME. The present study is the first prospective, interventional pilot study to evaluate the efficacy of MTX on DME in patients with no previous treatments.

Methods

This prospective, randomized, contralateral eye pilot study was conducted on treatment-naive eyes in patients with diabetes mellitus (DM) diagnosed to have DME at Feiz Hospital, a referral ophthalmology center affiliated with Isfahan University of Medical Sciences in Isfahan, Iran, from April 2017 to April 2018.

The study was approved by the Medical Ethics Committee of Isfahan University of Medical Sciences and adheres to the tenets of the Declaration of Helsinki. The study procedures, including the risks and benefits of intravitreal injections, were explained to all the participants before beginning the study. All the participants signed written informed consent forms for inclusion in the study.

The study was registered at the Iranian Registry of Clinical Trials under the registration code IRCT2017090736088N1.

DM patients ≥18 years of age diagnosed with clinically significant DME entered into the study [Figure 1]. DME

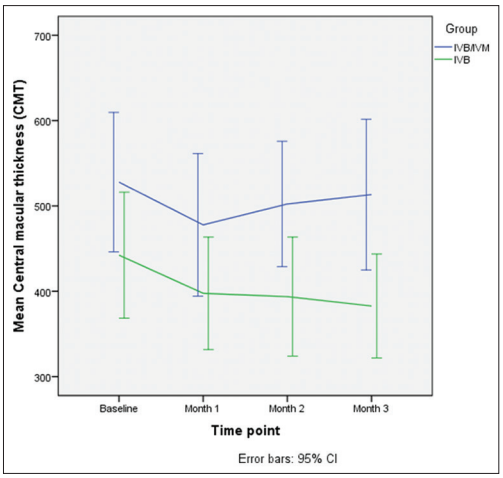

Figure 1: Overall trend of the central macular thickness by intervention and control groups

was diagnosed by retina specialists as defined by the Early Treatment Diabetic Retinopathy Study.[9]

Patients with a best corrected visual acuity (BCVA) <20/50 and a center involving DME with the central macular thickness (CMT) >300μ were included in the study. Optical coherence tomography (OCT) was done by spectral-domain-OCT Heidelberg engineering (Heidelberg, Germany) to evaluate CMT.

The exclusion criteria consisted of pregnancy, breastfeeding, history of allergy to the study medications, a visual acuity loss that was unlikely to improve following the resolution of macular edema (e.g., foveal atrophy, corneal dystrophy, etc.), other causes of macular edema (e.g., uveitis or other ocular inflammatory diseases, neovascular glaucoma, epiretinal membrane, etc.), intraocular surgery with the prior 3 months, recent significant change in diabetic medications, life-threatening comorbidities such as cancer under therapy, vitreous hemorrhage (active) in the study eye, media opacities, herpetic disease of the cornea, corneal dystrophy with significant corneal edema, eyes treated for glaucoma, a DME recently treated with panretinal or grid laser photocoagulation and HbA1c >8.5. The withdrawal criteria consisted of not attending the follow-up visits, receiving other topical or systemic immunosuppressive or inflammatory agents during the study and intolerable side-effects.

Patients with bilateral DME were randomly allocated to receive three courses injections of either IVB plus IVM (with a 15-day interval) as the combination group or IVB alone as the IVB group, using random allocation software.[10]

All the patients were administered 1.25 mg/0.05 mL of IVB (Avastin; Genentech, Inc., South San Francisco, CA, USA). The injections were administered at baseline and one and 2 months later with 27G needles through the superotemporal quadrant in sterile conditions.

Fifteen days after each injection of bevacizumab, 400 µg (0.16 mL) of MTX (Ebetrex, Ebewe Pharma Ges.m.b.H. Nfg., Unterach, Austria) was administered in the combination group.

In the sham group, a needleless applicator was pressed against the conjunctiva for all the IVM injections.

The primary outcome measure was changes in the BCVA logMAR, and the secondary outcome measures included changes in the CMT and central macular volume (CMV) as reported in the OCT prints.

In the baseline examinations, the patients underwent ophthalmologic examinations, including BCVA measurement using a Snellen chart, intraocular pressure (IOP) measurement, anterior segment slit-lamp and fundus examination, and CMT and CMV measurement by OCT. These examinations were repeated 1 month after each injection. The time point of measurements before the first injection was a maximum of 10 days.

All of the patients also underwent an examination 1, 3, and 7 days after the injection, mainly for potential injection-related complications, such as ocular hypertension, anterior chamber reaction, lens opacity, and traumatic cataract.

As a matter of normality problem, BCVA was transformed into logMAR for the statistical analysis. Mean, standard deviation (SD), and frequency were used to present data. To compare variables of interest (CMT, CMV, BCVA, and IOP) at each time point of measurement (baseline, month 1, month 2, and month 3) and their changes from baseline between the two eye groups (IVB/IVM and IVB), we used the paired samples *t*-test. Adjusting for the dependencies between the two eyes of a participant, and considering the time point as a repeated factor, omnibus comparisons of the two eye groups were made based on separated linear mixed models for each variable. In addition, changes within each group were either evaluated by linear mixed models, and multiple comparisons were performed using the Bonferroni method. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 23 (SPSS Inc.,Chicago, IL, USA). *P* values < 0.05 were considered statistically significant.

Results

Thirty-six treatment-naive eyes of 18 patients completed the study. The mean (SD) age of the participants was 62.38 (6.2) years (range, 55–78). There were no significant baseline differences between the two groups ($P > 0.05$).

Table 1 demonstrates the trend of CMT changes in both groups. Compared with the baseline values, mean CMT was reduced in both groups; however, these changes did not reach a significant level in within-group analyses (combination group, $P = 0.83$; and control group, $P = 0.60$). CMT changed from 527.8 ± 164.3 at baseline to 513.3 ± 177.67 in the combination group and from 442.3 ± 148.53 to 382.7 ± 122.54 in the IVB alone group, at 1 month after the 3rd injection. CMT reduction in both groups was not statistically significant compared with the baseline value ($P > 0.99$) [Table 1]. The differences of CMT changes between the groups were not significant at month 1 ($P = 0.82$), month 2 ($P = 0.21$), and month 3 ($P = 0.10$) [Table 1].

During the study, CMV changed from 0.41 ± 0.131 at baseline to 0.40 ± 0.138 in the combination group and from 0.35 ± 0.111 to 0.30 ± 0.094 in the IVB alone group, at 1 month after the 3rd injection. At month 3, the mean CMV reduction in both groups was not statistically significant compared with the baseline value ($P > 0.99$) [Table 2].

Compared with the baseline values, mean BCVA improved at 1, 2, and 3 months in both groups; however, these improvements did not reach a significant level in within-group analyses (combination group, $P = 0.69$; and control group, $P = 0.65$). LogMAR BCVA changed from 0.95 ± 0.53 at baseline to 0.75 ± 0.53 in the combination group and from 0.72 ± 0.57 to 0.49 ± 0.50 in the IVB alone group, at 1 month after the 3rd injection. BCVA improvement in both groups was not statistically significant compared with the baseline value ($P > 0.99$) [Table 3]. The differences of logMAR BCVA changes between the groups were not significant at month 1 ($P = 0.39$), month 2 ($P = 0.96$), and month 3 ($P = 0.63$) [Table 3]. Figures 1-3 present an overall trend in terms of CMT, CMV, and logMAR BCVA in each group.

No major ocular complications or systemic side-effects related to IVM were noted. None of the patients needed to withdraw from the study because of severe or intolerable adverse effects. There was no statistically significant difference in IOP during the study within (combination group: $P > 0.99$ vs. IVB alone group: $P = 0.81$) or between (3rd month: $P = 0.27$) the groups.

Discussion

The present study demonstrated no significant therapeutic effects for IVB combined with IVM compared to IVB

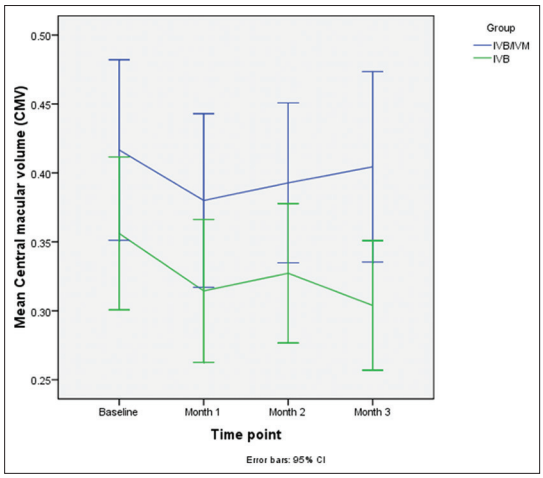

Figure 2: Overall trend of the central macular volume by intervention and control groups

Table 1: Central macular thickness after intravitreal injection in the eye groups (intravitreal bevacizumab [IVB]/ intravitreal methotrexate and IVB)

Time point	Eye groups			95% CI		*P**
	IVB/IVM	IVB	Difference	Lower	Upper	
Baseline						
Mean±SD	527.8±164.3	442.3±148.53	85.5	−4.5	175.6	0.061
Month 1						
Mean±SD	477.7±168.06	397.6±132.30	80.1	10.7	149.5	0.002
Change (baseline-1)						
Mean±SD	50.1±87.47	44.7±64.38	5.3	−43.9	54.7	0.821
P-whitin†	>0.99	>0.99				
Month 2						
Mean±SD	502.3±147.79	393.7±140.39	108.6	44.8	172.3	0.002
Change (baseline-2)						
Mean±SD	25.5±70.10	48.6±77.69	−23.0	−60.7	14.6	0.214
P-whitin†	>0.99	>0.99				
Month 3						
Mean±SD	513.3±177.67	382.7±122.54	130.6	55.7	205.5	0.002
Change (baseline-3)						
Mean±SD	14.5±100.42	59.6±82.45	−45.0	−101.2	11.1	0.109
P-whitin†	>0.99	>0.99				
P‡	0.833	0.609				

*Based on paired samples *t*-test, †Based on linear mixed model, multiple comparison considered by Bonferroni method, ‡Based on linear mixed model in each eye group, omnibus test within time points of measurements. CI: Confidence interval, IVB: Intravitreal bevacizumab, IVM: Intravitreal methotrexate, SD: Standard deviation

Table 2: Central macular volume after intravitreal injection in the eye groups (intravitreal bevacizumab [IVB]/intravitreal methotrexate and IVB)

Time point	Eye groups			95% CI		*P**
	IVB/IVM	IVB	Difference	Lower	Upper	
Baseline						
Mean±SD	0.41±0.131	0.35±0.111	0.06	−0.01	0.12	0.078
Month 1						
Mean±SD	0.38±0.126	0.31±0.104	0.06	0.01	0.11	0.013
Change (baseline-1)						
Mean±SD	0.03±0.068	0.04±0.050	−0.005	−0.04	0.03	0.769
P-whitin†	>0.99	>0.99				
Month 2						
Mean±SD	0.39±0.116	0.32±0.101	0.06	0.01	0.11	0.013
Change (baseline-2)						
Mean±SD	0.02±0.057	0.03±0.067	−0.004	−0.03	0.03	0.821
P-whitin†	>0.99	>0.99				
Month 3						
Mean±SD	0.40±0.138	0.30±0.094	0.06	0.01	0.11	0.012
Change (baseline-3)						
Mean±SD	0.01±0.079	0.05±0.064	−0.04	−0.09	0.01	0.114
P-whitin†	>0.99	0.835				
P‡	0.861	0.490				

*Based on paired samples *t*-test, †Based on linear mixed model, multiple comparison considered by Bonferroni method, ‡Based on linear mixed model in each eye group, omnibus test within time points of measurements. CI: Confidence interval, IVB: Intravitreal bevacizumab, IVM: Intravitreal methotrexate, SD: Standard deviation

alone in treatment-naive DME patients in terms of BCVA, CMT, and CMV in a 3-month, randomized pilot study.

Although the exact mechanism of DME is unclear, inflammation and inflammatory cytokines play an important role. The elevated intraocular levels of proinflammatory mediators, including

Table 3: LogMAR best corrected visual acuity after intravitreal injection in the eye groups (intravitreal bevacizumab [IVB]/intravitreal methotrexate and IVB)

Time point	Eye groups			95% CI		*P**
	IVB/IVM	IVB	Difference	Lower	Upper	
Baseline						
Mean±SD	0.95±0.531	0.72±0.574	0.23	0.003	0.46	0.047
Month 1						
Mean±SD	0.81±0.581	0.54±0.481	0.26	0.06	0.47	0.015
Change (baseline-1)						
Mean±SD	0.13±0.133	0.17±0.136	−0.04	−0.12	0.05	0.399
P-whitin†	>0.99	>0.99				
Month 2						
Mean±SD	0.79±0.533	0.55±0.445	0.23	0.03	0.44	0.029
Change (baseline-2)						
Mean±SD	0.16±0.088	0.16±0.199	−0.00	−0.09	0.09	0.969
P-whitin†	>0.99	>0.99				
Month 3						
Mean±SD	0.75±0.538	0.49±0.506	0.25	0.05	0.45	0.018
Change (baseline-3)						
Mean±SD	0.20±0.128	0.22±0.132	−0.01	−0.11	0.07	0.637
P-whitin†	>0.99	>0.99				
P‡	0.694	0.650				

*Based on paired samples *t*-test, †Based on linear mixed model, multiple comparison considered by Bonferroni method, ‡Based on linear mixed model in each eye group, omnibus test within time points of measurements. CI: Confidence interval, IVB: Intravitreal bevacizumab, IVM: Intravitreal methotrexate, SD: Standard deviation

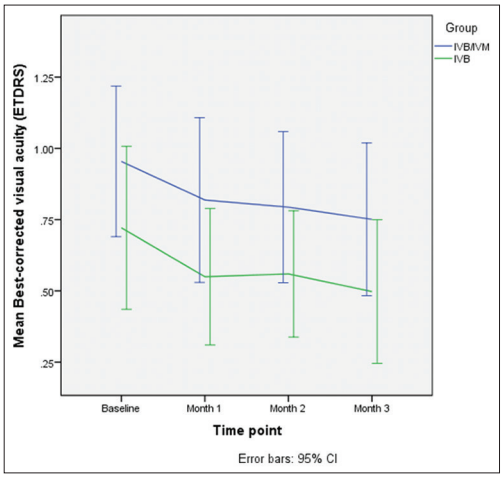

Figure 3: Overall trend of logMAR best corrected visual acuity by intervention and control groups

tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, monocyte chemotactic protein 1, IL-8, VEGF, platelet-derived growth factor, etc., support this finding.[7,11]

Several studies have demonstrated the pharmacological effects of MTX in suppressing the activity of TNF and some ILs. The safety of use of MTX has also been demonstrated in some studies. The intravitreal injection of MTX as an anti-inflammatory and anti-proliferative drug has been studied in selected ophthalmologic conditions, such as uveitis, primary central nervous system lymphoma, non-Hodgkin's lymphoma, and age-related macular degeneration, and has been found to control the inflammatory process without significant complications.[12-15] Frenkel *et al*. showed that vitreoretinal lymphoma can be controlled effectively and without serious adverse reactions by intravitreal MTX injections. They found bearable intraocular side-effects in their study.[16]

Although multiple and continuous injections of IVM, which were used to treat primary intraocular lymphoma in some studies,[17] can be associated with corneal epitheliopathy and maculopathy, three courses injections of intraocular MTX therapy, similar to the present study, have an excellent safety record both in animal models and in clinical practice.[18,19]

Similar to the present study, Khalil *et al*. found no significant changes in the mean IOP values with intravitreal MTX.[20] Some studies have recently demonstrated that using intravitreal anti-VEGF injection decreases NO and PGI2 production and induces vasoconstriction and a tendency for hypertension. Intravitreal anti-VEGF can also promote thrombosis formation and the tendency for cardiovascular and cerebral events.[4,5] Given these findings, MTX may be regarded as a new effective medication in treating DME.[21]

Although Falavarjani *et al*.[8] showed that the intravitreal injection of MTX results in improvements in a significant proportion of eyes with persistent DME, their study had some limitations, including the lack of a control group and evaluating only bevacizumab-unresponsive cases of DME. In contrast, the present study did not find significant differences in the clinical and paraclinical improvement of DME following the addition of IVM.

The present findings can be attributed to the small sample size of the study and the differences in the patients' characteristics in the two studies, as one study examined persistent DME cases and the other examined treatment-naive eyes.

The strengths of the present study include a controlled design for evaluating the efficacy and safety of a novel drug combination. The limitations of the study include the small sample size, the lack of a long-term follow-up, and the failure to evaluate pharmacological interactions between bevacizumab and MTX. Another limitation of our study was evaluation of patients with OCT monthly while intervals between injections in the combination group were 15 days. Unlike the results of previous studies, the present research found that IVM combined with IVB do not make an effective treatment for treatment-naive DME compared to IVB alone. Further controlled studies should be undertaken to confirm or refute the efficacy of this treatment for the management of DME in the long-term.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Schmidt-Erfurth U, Garcia-Arumi J, Bandello F, Berg K, Chakravarthy U, Gerendas BS, *et al*. Guidelines for the management of diabetic macular edema by the European society of retina specialists (EURETINA). Ophthalmol 2017;237:185-222.
2. Maroufizadeh S, Almasi-Hashiani A, Hosseini M, Sepidarkish M, Omani Samani R. Prevalence of diabetic retinopathy in Iran: A systematic review and meta-analysis. Int J ophthal 2017;10:782-9.
3. Ehrlich R, Harris A, Ciulla TA, Kheradiya N, Winston DM, Wirostko B. Diabetic macular oedema: Physical, physiological and molecular factors contribute to this pathological process. Acta ophthalmol 2010;88:279-1.
4. Falavarjani KG, Nguyen QD. Adverse events and complications associated with intravitreal injection of anti-VEGF agents: A review of literature. Eye (Lond) 2013;27:787-4.
5. Bandello F, Casalino G, Loewenstein A, Goldstein M, Pelayes D, Battaglia Parodi M. Pharmacological approach to diabetic macular edema. Ophthalmic Res 2014;51:88-95.
6. Kianersi F, Rezaeian-Ramsheh A, Pourazizi M, Kianersi H. Intravitreal diclofenac for treatment of refractory uveitis-associated cystoid macular oedema: A before and after clinical study. Acta ophthalmologica 2018;96:e355-e360.
7. Das A, McGuire PG, Rangasamy S. Diabetic macular edema: Pathophysiology and novel therapeutic targets. Ophthalmol 2015;122:1375-94.
8. Falavarjani KG, Golabi S, Modarres M. Intravitreal injection of methotrexate in persistent diabetic macular edema: A 6-month follow-up study. Graefes Arch Clin Exp Ophthalmol 2016;254:2159-64.
9. Photocoagulation for diabetic macular edema. Early treatment diabetic retinopathy study report number 1. Early treatment diabetic retinopathy study research group. Arch Ophthalmol 1985;103:1796-806.
10. Saghaei M. Random allocation software for parallel group randomized trials. BMC Med Res Med 2004;4:26.
11. Jampol LM, Bressler NM, Glassman AR. Revolution to a new standard treatment of diabetic macular edema. Jama 2014;311:2269-70.
12. Taylor SR, Habot-Wilner Z, Pacheco P, Lightman S. Intravitreal methotrexate in uveitis. Ophthalmol 2012;119:878-9.
13. Soheilian M, Movaseghi M, Ramezani A, Peyman GA. Pilot study of safety and effect of combined intravitreal bevacizumab and methotrexate for neovascular age-related macular degeneration. Eur J Ophthalmol 2011;21:77-82.
14. Smith JR, Rosenbaum JT, Wilson DJ, Doolittle ND, Siegal T, Neuwelt EA, *et al*. Role of intravitreal methotrexate in the management of primary central nervous system lymphoma with ocular involvement. Ophthalmol 2002;109:1709-16.
15. Samson CM, Waheed N, Baltatzis S, Foster CS. Methotrexate therapy for chronic noninfectious uveitis: Analysis of a case series of 160 patients. Ophthalmology 2001;108:1134-9.
16. Frenkel S, Hendler K, Siegal T, Shalom E, Pe'er J. Intravitreal methotrexate for treating vitreoretinal lymphoma: 10 years of experience. The Br J Ophthalmol 2008;92:383-8.
17. Reichstein D. Primary vitreoretinal lymphoma: An update on pathogenesis, diagnosis and treatment. Curr Opin Ophthalmol 2016;27:177-84.
18. Manna S, Banerjee RK, Augsburger JJ, Al-Rjoub MF, Donnell A, Correa ZM. Biodegradable chitosan and polylactic acid-based intraocular micro-implant for sustained release of methotrexate into vitreous: Analysis of pharmacokinetics and toxicity in rabbit eyes. Graefes Arch Clin Exp Ophthalmol 2015;253:1297-1305.
19. Taylor SR, Banker A, Schlaen A, Couto C, Matthe E, Joshi L, *et al*. Intraocular methotrexate can induce extended remission in some patients in noninfectious uveitis. Retina 2013;33:2149-54.
20. Khalil HE, El Gendy HA, Youssef HA, Haroun HE, Gheita TA, Bakir HM. The effectiveness of intraocular methotrexate in the treatment of posterior uveitis in behçet's disease patients compared to retrobulbar steroids injection. J Ophthalmol 2016;2016:5.
21. Chan ES, Cronstein BN. Molecular action of methotrexate in inflammatory diseases. Arthritis res 2002;4:266-73.