Original Article

Intravitreal injection of methotrexate in persistent diabetic macular edema: a 6-month study

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ABSTRACT

Background: Diabetic macular edema (DME) affects approximately 10% of patients with diabetes mellitus. This condition can cause blurred or distorted vision, which significantly affects the quality of life of these patients. We evaluated the therapeutic effects of intravitreal methotrexate (MTX) injections on persistent DME.

Methods: This prospective interventional case series included patients with confirmed persistent DME that was unresponsive to previous standard treatments. The patients underwent comprehensive eye examinations and macular imaging with optical coherence tomography (OCT). A single intravitreal MTX injection (400 µg MTX in 0.16 mL solution) was administered, followed by patient assessments at 1, 3, and 6 months after injection. Best-corrected distance visual acuity (BCDVA), intraocular pressure (IOP), macular thickness (MT), and central subfield thickness (CST) were measured at baseline and post-injection to evaluate treatment efficacy.

Results: We included 33 eyes of 30 patients with a mean (standard deviation [SD], range) age of 62.7 (8.3, 44 to 77) years, of whom 17 (56.7%) were men and 13 (43.3%) were women. All participants had type 2 diabetes mellitus, with a mean (SD, range) duration of 17.0 (6.8, 10 to 31) years. Most participants (n = 27 eyes, 81.8%) had non-proliferative diabetic retinopathy, and six eyes (18.2%) had regressed proliferative diabetic retinopathy. Four eyes (12.1%) had undergone prior macular laser photocoagulation. The mean (SD) number of prior intravitreal bevacizumab injections was 3.4 (0.8), and 29 eyes (87.8%) had received one intravitreal triamcinolone injection. During the study period, a statistically significant difference was observed in CST ($P < 0.05$); however, no statistically significant differences were observed in BCDVA, MT, or IOP ($P > 0.05$). Pairwise comparison revealed a significant decrease in CST at 6 months post-injection compared to the baseline value ($P < 0.05$). During the investigation period, no side effects of MTX, such as macular edema, retinal tears, vitreous hemorrhage, endophthalmitis, or vision loss, were observed.

Conclusions: A single intravitreal MTX injection significantly reduced CST in patients with persistent DME, without relevant safety concerns. However, no significant improvement in functional outcomes was observed. Therefore, there is no strong evidence to recommend its use as a treatment for pDME. Further studies, preferably randomized clinical trials with long-term follow-ups, are warranted to assess the long-term efficacy, safety, and potential benefits of intravitreal MTX for the treatment of persistent DME.

KEYWORDS

methotrexate sodium, type 2 diabetes mellitus, intravitreal injection, persistent diabetic macular edema, cystoid macular edema, persistant, diabetic retinopathies, visual acuities, intraocular pressures, optical coherence tomography, fovea centralis, macula luteas

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INTRODUCTION

Diabetic retinopathy is the leading cause of blindness among individuals aged < 60 years in the United States [1]. Diabetic macular edema (DME) affects approximately 10% of individuals with diabetes mellitus (DM) and occurs when retinal blood vessels become more permeable. This leads to the accumulation of fluid in the retina, thickening of the macula, and blurred or distorted vision that significantly affects the quality of life of these patients [2-4].

Current treatment options for macular edema include laser treatment along with improved management of the underlying disease and coexisting conditions; intravitreal triamcinolone and bevacizumab injections, which have short-term efficacy but notable risks; and surgical approaches that remain controversial and have not yielded long-term benefits [5-9]. However, some patients with DME do not respond to routine treatments, leading to a diagnosis of persistent DME (pDME) [10].

Methotrexate (MTX) is administered to treat various malignant tumors and autoimmune diseases. Intravitreal injection of 400 μg MTX is used for eye pathologies associated with inflammation-driven macular edema [11, 12]. It has both antineoplastic and anti-inflammatory properties and inhibits the enzyme dihydrofolate reductase, which is necessary for folate synthesis. This inhibition leads to a decrease in DNA synthesis and cell proliferation [11]. Additionally, MTX inhibits leukocyte chemotaxis and the secretion of tumor necrosis factor-alpha, interleukin-8, and interleukin-6 by monocytes. MTX also has anti-inflammatory effects through the release of extracellular adenosine [13, 14].

Promising results of MTX administration for the prevention and treatment of proliferative vitreoretinopathy have been reported [14]. Currently, there is interest in the intraocular injection of MTX as a potential treatment for DME; however, convincing supportive evidence is scarce [12, 13, 15]. A pilot study comparing monthly intravitreal injections of a combination of bevacizumab and MTX to intravitreal bevacizumab alone found no significant benefit with the addition of MTX in treating DME [15]. Another study showed that multiple intravitreal MTX injections reduced central macular thickness (MT) in some cases of pDME; however, only a small percentage of patients experienced significant visual improvement [13].

We investigated the effects of a single intravitreal injection of MTX in patients with pDME who failed to respond to common treatments. Because there is no established regimen for intravitreal MTX use in the treatment of pDME, this study focused on the efficacy of a single intravitreal dose.

METHODS

This prospective interventional case series recruited patients with type 2 DM and pDME who attended the ophthalmology clinic at Amir-al-Momenin Hospital, Rasht, Iran, between January 2020 and May 2023. The study adhered to the ethical principles outlined in the Helsinki Declaration and was approved by the Research Ethics Committee of Guilan University of Medical Sciences, Rasht, Iran. The study protocol was also approved by the Research Council of the Faculty of Medicine (Ethical Code: 928). All participants provided written informed consent. Patient confidentiality was strictly maintained throughout the study.

Because MTX injection is a non-approved treatment for DME, and to follow ethical guidelines, we used convenience sampling for case selection. We included patients aged ≥ 18 years with clinically significant DME and central subfield thickness (CST) (the circular area with 1-mm diameter centered on the fovea) [16] > 250 μm, best-corrected distance visual acuity (BCDVA) of 20/200 or better, and pDME (defined as a lack of improvement of at least one line in BCDVA or a minimum 10% reduction in CST after three consecutive intravitreal injections of bevacizumab and one combined injection of bevacizumab and triamcinolone at 4 – 6 week intervals). Alternatively, patients may have received three consecutive intravitreal injections of bevacizumab in addition to macular photocoagulation laser treatment.

We excluded individuals who had undergone eye surgery within the past year; received laser treatment within the last 4 months; required any intraocular surgery, injection, or laser photocoagulation during the follow-up period; exhibited traction on the macula during clinical and OCT examinations; had a significant media opacity of the eye making the acquisition of clear images difficult; were on systemic medications such as steroids, anti-tuberculosis treatment, or any medication that may affect retinal thickness; had a history of retinal or optic nerve diseases involving glaucomatous optic disc or ischemic optic neuropathy; had a history of eye trauma; and were lactating or pregnant women.

All recruited patients underwent a thorough ocular examination including BCDVA measurement (LED Vision Chart; LC-1300B, AnnoTek, Piramoon Raditech Co., Shiraz, Iran) with values converted to the logarithm of the minimum angle of resolution (logMAR), anterior segment examination using a slit lamp (SL-3C slit lamp,

Topcon, Tokyo, Japan), intraocular pressure (IOP) measurement using Goldmann applanation tonometry (Haag Streit AT900, Koeniz, Switzerland), and fundus examination under a slit lamp using a 90 D lens (Volk Optical Inc., Mentor, OH, USA) and a binocular indirect ophthalmoscope (Omega 100; Heine Optotechnik, Herrsching, Germany) using a 20 D lens (Volk Optical Inc.).

The MT and CST were measured using optical coherence tomography (OCT) (Cirrus HD-OCT, Model 4000; Carl Zeiss Meditec, Inc., Dublin, CA, USA) using the macular OCT program. A skilled technician performed OCT imaging after pupillary dilation with 1% tropicamide (Mydrax; Sina Daru, Tehran, Iran) to achieve a minimum diameter of 5 mm. The circular scan revolved around the macula while the studied eye was fixed with an internal fixation light. The macula was assessed three times by a single scan at 256 points encompassing a fixed diameter of 3.4 mm around the macular center. Scans with low image quality or noticeable misalignment of the measurement circle position were excluded. Ultimately, the MT and CST were obtained from the data reported in the OCT printouts. All patients were referred to the hospital's laboratory department to collect venous blood samples for analysis of total cholesterol, triglyceride [17], fasting blood glucose, and glycated hemoglobin (HbA1c) levels [18].

Intravitreal MTX injection was administered as previously described [13]. After sufficient application of 10% povidone–iodine solution in the operating room, a 30-gauge insulin syringe was used to tap the anterior chamber (0.1 mL). An intravitreal injection of 400 µg MTX in 0.16 mL of solution (Haupt Pharma Gmbh, Wolfratshausen, Germany) was administered at a point 4 mm posterior to the limbus. All injections were administered by an ophthalmologist (N.R.). The post-injection protocol included fundoscopic examination under a slit lamp using a 90 D lens and under a binocular indirect ophthalmoscope using a 20 D lens, IOP measurement using Goldmann applanation tonometry, and prescription of antibiotic eye drops (Chlobiotic 0.5%, Sina Darou, Tehran, Iran) 4 times daily for 7 days and corticosteroid eye drops (betamethasone 0.1%, Sina Darou) 4 times daily for 7 days. Follow-up visits were scheduled at 1 day, 1 week, 1 month, 3 months, and 6 months after injection to monitor for complications and drug efficacy. All examinations were performed by a single experienced ophthalmologist at baseline and follow-up. BCDVA and IOP were measured and OCT imaging was performed at each visit. Changes in BCDVA, MT, and CST were used as parameters to evaluate the efficacy of intravitreal MTX injections.

Data were analyzed using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY, USA). The normality of the sample distribution was tested using the Shapiro – Wilk test. Frequencies and percentages are used to describe qualitative variables. Descriptive statistics, including means and standard deviations (SDs), are used for quantitative variables. The Friedman test was used to compare the mean values of these variables at different time points. The Bonferroni – Dunn post-hoc test [19] was implemented for multivariable comparisons. Pearson's product-moment correlation was used to evaluate possible correlations between the mean age of the participants or the duration of DM and mean changes in BCDVA, MT, CST, and IOP. Statistical significance was set at a P-value < 0.05.

RESULTS

We included 33 eyes of 30 participants with a mean (SD, range) age of 62.7 (8.3, 44 to 77) years, of whom 17 (56.7%) were men and 13 (43.3%) were women. All had type 2 DM with a mean (SD, range) duration of 17.0 (6.8, 10 to 31) years.

Most participants (n = 27 eyes, 81.8%) had non-proliferative diabetic retinopathy, and six eyes (18.2%) had regressed proliferative diabetic retinopathy. Four eyes (12.1%) had prior macular laser photocoagulation. The mean (SD) number of intravitreal bevacizumab injections was 3.4 (0.8), and 29 eyes (87.8%) had received 1 intravitreal injection of triamcinolone.

The mean (SD) levels of fasting blood glucose, total cholesterol, triglycerides, and HbA1c were 141.3 (60.8) mg/dL, 155.7 (38.4) mg/dL, 144.9 (6.2) mg/dL, and 7.7% (1.4%), respectively, with total cholesterol and triglyceride levels within the normal ranges; the mean fasting blood glucose and HBA1c values were greater than the upper limits of normal ranges, as all participants had type 2 DM.

The data in Table 1 show that there were no statistically significant differences in BCDVA, MT, or IOP during the follow-up period of 6 months (all $P > 0.05$); however, the CST differed significantly ($P < 0.05$). Post-hoc analysis revealed a significantly thinner CST only at 6 months post-injection compared with the baseline measurement (pairwise comparison, $P < 0.05$) (Table 2).

The mean (SD) and median CST in the pre-treatment phase were 490.0 (101.1) µm and 479 µm, respectively, and at the sixth month they were 446.5 (166.4) µm and 432 µm, respectively.

At 6 months, BCDVA decreased in 10 eyes (30.3%), increased in 15 eyes (45.5%), and was unchanged in eight eyes (24.2%, $P = 0.424$) compared to the pre-injection measurements. At 6 months, MT was reduced in 20 eyes

Table 1. Comparison of BCDVA, MT, CST, and IOP between follow-up visits

Variable	Mean ± SD	*P*-value
BCDVA (logMAR)		
Before MTX injection	0.5 ± 0.3	0.314
At 1 month post- injection	0.5 ± 0.4	
At 3 months post- injection	0.6 ± 0.4	
At 6 months post- injection	0.7 ± 0.5	
MT (µm)		
Before MTX injection	369.8 ± 71.6	0.194
At 1 month post- injection	361.7 ± 67.3	
At 3 months post- injection	367.6 ± 68.1	
At 6 months post- injection	364.3 ± 77.3	
CST (µm)		
Before MTX injection	490 ± 101.1	**0.014**
At 1 month post- injection	470.4 ± 116.6	
At 3 months post- injection	481 ± 129.5	
At 6 months post- injection	446.5 ± 166.4	
IOP (mmHg)		
Before MTX injection	17.4 ± 2.4	0.075
At day 1 post- injection	18.2 ± 1.6	
At 1 month post- injection	17.9 ± 2.7	
At 3 months post- injection	18.0 ± 2.4	
At 6 months post- injection	16.7 ± 2.6	

Abbreviations: BCDVA, best-corrected visual acuity; MT, macular thickness; CFT, central subfield thickness; IOP, intraocular pressure; MTX, methotrexate; logMAR; logarithm of the minimum angle of resolution; µm, micrometers; mmHg, millimeter of mercury. Note: *P*-value < 0.05 is shown in bold.

Table 2. Pairwise comparison of CST between follow-up visits

Pair-wise comparison	Test statistics	*P*-value
CST at pre-injection versus 1 month post-injection	0.439	> 0.99
CST at pre-injection versus 3 months post-injection	0.369	> 0.99
CST at pre-injection versus 6 months post-injection	1.015	**0.008**
CST at 1 month versus 3 months post-injection	0.076-	> 0.99
CST at 1 month versus 6 months post-injection	0.576	0.420
CST at 3 months versus 6 months post-injection	0.652	0.242

Abbreviations: CST, central subfield thickness. Note: *P*-value < 0.05 is shown in bold.

(60.6%), increased in 12 eyes (36.4%), and unchanged in one eye (3%, $P = 0.216$). Furthermore, at 6 months, CST decreased in 24 eyes (72.7%) and increased in nine eyes (27.3%) ($P = 0.015$). We did not encounter any treatment-related side effects such as macular edema, retinal tears, vitreous hemorrhage, endophthalmitis, or vision loss during the 6-month follow-up.

The correlation between mean age of the participants and BCDVA changes after injection was statistically significant, whereby, individuals with ≥ 1 line increase in BCDVA tended to be younger than those with no change or a decrease in BCDVA ($r = +0.74$; $P = 0.044$). However, there was no significant correlation between the mean age of the participants and the mean changes in MT ($r = +0.11$; $P = 0.602$), CST ($r = +0.22$; $P = 0.125$), or IOP ($r = +0.25$; $P = 0.759$), or between the mean duration of DM and mean changes in MT ($r = +0.15$; $P = 0.330$), CST ($r = +0.17$; $P = 0.383$), or IOP ($r = +0.19$; $P = 0.287$).

DISCUSSION

We observed a significant decrease in CST after a single intravitreal MTX injection in eyes with pDME. However, no statistically significant changes were observed in BCDVA or MT. MTX injection was safe in our series, with no treatment-related side effects or increases in IOP during a follow-up period of 6 months.

Intraocular MTX has been used as a treatment for ophthalmic manifestations of various systemic diseases [12]. In addition to its anti-proliferative and anti-inflammatory effects [20], MTX may exert anti-fibrotic effects by inhibiting the production of type I collagen [21]. Many inflammatory mediators, such as transforming growth factor-beta, interleukin-6, interleukin-1-beta, and platelet-derived growth factor, are increased in the retinas of patients with DM [22-24]. These molecules phosphorylate junctional proteins, causing destruction of the blood-retinal barrier [12-14]. Intravitreal injection of vascular endothelial growth factor inhibitors influences vascular permeability and leakage in diabetic retinopathy. However, inflammation has been suggested as another main factor involved in the process of macular edema in patients with DM [15]. Systemically administered MTX has a half-life longer than that of corticosteroids, allowing for extended therapeutic effects [12, 25, 26]. MTX monotherapy in our series of patients with pDME resulted in a significant improvement in CST without a significant corresponding functional improvement.

Sabouri et al. reported a mean (SD) MT of 277.50 (11.96) μm and CST of 245.45 (20.39) μm for healthy eyes of similar racial backgrounds using Cirrus HD-OCT [27]. Considering these normal ranges, the mean MT and CST in our series were higher than normal at all post-treatment follow-up visits. Despite a significant decrease in CST at 6 months post-injection, the mean CST (446.5 [166.4]) was almost two times higher than its corresponding normal value in individuals with a similar racial background [27]. This may explain the lack of significant functional improvement in our series. However, further studies with randomized trial designs and longer follow-up periods or repeated intravitreal MTX injections may reveal a recovery in OCT parameters to within the normal ranges with resultant functional improvement.

Fazel et al. [15] compared the therapeutic effects of the intravitreal injection of combined bevacizumab and MTX with that of bevacizumab alone in patients with bilateral DME during a 3-month follow-up, and they observed no significant therapeutic benefit with the addition of MTX [15]. Likewise, Soheilian et al. [28] administered intravitreal MTX and bevacizumab combination therapy in seven eyes with choroidal neovascularization due to age-related macular degeneration. After the combined injection, eyes were examined every 1.5 months, and reinjections were performed using bevacizumab monotherapy. All treated eyes demonstrated improvement in BCDVA compared to baseline values. Central MT decreased in all but one patient, who had no reduction in BCDVA. However, there were no statistically significant differences between baseline and post-injection values of central MT. The authors concluded that combining MTX and bevacizumab is a safe treatment and may enhance the regression of neovascularization in age-related macular degeneration [28]. We injected a single dose of MTX in eyes with pDME and observed a significant anatomical improvement in treated eyes, with no corresponding functional improvement. Treatment was safe, with no side effects detected during the 6-month follow-up. Considering the contradictory treatment outcomes of MTX administered for different ocular entities [15, 28, 29] and the small sample sizes in the published research on this topic [15, 28], further studies are necessary to reach robust conclusions.

Taylor et al. [30] conducted a retrospective interventional study of 38 eyes of 30 patients with non-infectious uveitis to assess the long-term therapeutic effect of intravitreal MTX injections. They observed that intravitreal MTX effectively controlled intraocular inflammation and improved visual acuity in most eyes. Remission was prolonged in most cases, with a median time to relapse of 17 months. Additionally, intravitreal MTX reduced the requirement for immunosuppressive treatments [30]. In contrast, a similar MTX dose yielded no significant improvement in BCDVA in our series during the 6-month follow-up. This discrepancy in functional outcomes between our series and that of Taylor et al. [30] could be due to differences in basic ocular pathology, follow-up period, or number of injections.

Falavarjani et al. [13] studied 18 eyes of 16 patients with pDME unresponsive to bevacizumab injections. Intravitreal MTX resulted in a reduction in MT; however, the changes were not clinically significant during a 6-month follow-up. Some eyes demonstrated a significant reduction in CST during the follow-up; however, there was an overall increase in CST in a subset of eyes. Visual acuity did not significantly change [13]. Likewise, our study, having a larger sample size, a similar MTX treatment regimen, and the same follow-up duration, observed no significant improvement in BCDVA despite a significant change in CST. We observed no significant correlation of age or duration of DM with changes in MT and CST during the 6-month follow-up. However, we observed a significant correlation between mean age and changes in BCDVA, suggesting that age may influence treatment efficacy. Further studies are necessary to support this reasoning.

In a randomized clinical trial with 17 participants and at least 3 months of follow-up, Mackensen et al. [31] reported the superiority of interferon beta over MTX in terms of functional and anatomical outcomes in patients with intermediate uveitis with macular edema. Patients received either subcutaneous interferon beta (22 μg 3 times weekly for 2 weeks, then 44 μg 3 times weekly) or subcutaneous MTX (20 μg once weekly). Interferon beta yielded a mean decrease in MT of 206 μm versus a mean increase of 47 μm in eyes receiving MTX. At 3 months, eyes treated with interferon beta demonstrated a mean improvement in visual acuity of 0.31 logMAR (range: 0.02 to 0.96 logMAR) versus 0.09 logMAR (range: 0.12 to 0.38 logMAR) in eyes receiving MTX [31]. In our series, the mean (SD) BCDVA improved from 0.5 (0.3) logMAR at baseline to 0.7 (0.5) logMAR, although the difference was not statistically significant. Eyes demonstrated a statistically significant 43.5-μm reduction in CST at 6 months post-injection. However, the lack of an interferon treatment arm in our study, the dissimilar routes of administration, and differences in underlying ocular diseases preclude a direct comparison of these two studies.

The current study, having a similar MTX treatment regimen but a larger sample size, observed no short-term visual improvement despite a significant CST reduction in eyes with pDME. Although further supportive research is required, this may be related to chronic photoreceptor damage due to persistent chronic edema. One limitation of our study is that we injected only a single dose of MTX because its intraocular half-life is unknown. Establishing the intraocular half-life of MTX through further investigations would allow repeated injections at optimal intervals for greater efficacy. Other limitations include a short follow-up period and the lack of a control group for precise comparisons. Establishing the safety and efficacy of intravitreal MTX treatment for pDME will require multicenter studies with randomized trial designs, larger sample sizes, and longer follow-up periods.

CONCLUSIONS

Intravitreal MTX injection yielded a significant CST reduction in eyes with pDME. However, no statistically significant changes in visual acuity or MT were observed. Therefore, there is no strong evidence to recommend its use as a treatment for pDME. Further research, preferably randomized clinical trials with long-term follow-ups, is required to assess the long-term effects and potential benefits of intravitreal MTX in the treatment of pDME.

ETHICAL DECLARATIONS

Ethical approval: The study adhered to the ethical principles outlined in the Helsinki Declaration and was approved by the Research Ethics Committee of Guilan University of Medical Sciences, Rasht, Iran. The study protocol was also approved by the Research Council of the Faculty of Medicine (Ethical Code: 928). All participants provided written informed consent. Patient confidentiality was strictly maintained throughout the study.

Conflict of interest: None.

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