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Original Article

Using skew-symmetric mixed models for investigating the effect of different diabetic macular edema treatments by analyzing central macular thickness and visual acuity responses

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Abstract

BACKGROUND: Diabetic Macular Edema (DME) is one of the major causes of visual loss and increase in central macular thickness (CMT). The aim of this study was to determine the efficacy of a single intravitreal injection of bevacizumab (IVB) alone or in combination with intravitreal triamcinolone acetonide (IVB/IVT) versus macular laser photocoagulation (MPC) as primary treatment for DME when confounders were considered.

METHODS: Skew-symmetric bivariate mixed modeling according to best corrected visual acuity (BCVA) and CMT was done on the data of 103 diabetic patients from ophthalmic research center of Labbafinejad medical center (Tehran, Iran) to determine the best DME treatment by adjusting the effect of confounders.

RESULTS: Although there was no significant difference between IVB/IVT ($p > 0.05$), these two treatments increased BCVA and decreased CMT better than MPC ($p < 0.05$). The following three groups showed better treatment responses: 1) women, 2) patients with more diabetes duration, 3) patients whose CMT were higher and VA were lower at the beginning of the clinical trial.

CONCLUSIONS: Using skew-symmetric mixed effect model as updated statistical method in presence of asymmetric or outlier data, we received different results compared to the same investigation on this study by analyzing BCVA and CMT simultaneously. This research demonstrated the effect of IVB alone or in combination with intravitreal IVB/IVT on visual power and decreasing CMT during follow up.

KEYWORDS: Best-Corrected Visual Acuity, Central Macular Thickness, Diabetic Macular Edema, Skew-symmetric Mixed Models.

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Diabetic retinopathy is one of the most common side-effect of the diabetic patients and also one of the important reasons for visual impairment. Thus, the probability of visual acuity loss is higher in diabetic patients in comparison with normal population.[1,2] Nevertheless, 60-90 percent of those who have diabetes I and II show retinopathy after two decades and only half of them visit ophthalmologist because of the crucial symptoms.[3,4] Accordingly, identifying significant risk factors of retinopathy, its on-time diagnosis and control can hamper its progress.

It was shown that the following factors can accelerate the progress of retinopathy; the duration of diabetes, the patient's age, sex, body mass index, smoking and hyperlepidemia.[1,5,6] One of the most important factors is the duration of having diabetes, especially more than 10 years. In a recent research it was shown that

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80% of diabetic patients have retinopathy after 15 years.[7] Diagnosis and treatment of Diabetic Macular Edema (DME), that happens at the early stages of diabetes, can assist treatment of retinopathy.[8,9]

Among the medical studies, clinical trials are the most important and complete ones. In these studies, if it is possible to measure the parameters repeatedly during time, the results will be more reliable and the research can disclose facts that were not evident before.[10] Since the repeated measurements are mostly correlated, it is important to use the appropriate statistical analysis method. Longitudinal modeling is a recent technique enabling to analyze these types of clinical trials. In these studies, the patient features analyzed during time similar to different responses of treatment in different subjects creates a variable which is important to take into account in statistical analysis.[11] On the other hand, in some clinical trials there is more than one parameter to consider. Modeling each variable independently can potentially hide the inter-dependence of the analyzed parameters resulting in misleading hypothesis.[12] One of the mostly used methods to infer multi-variable repeated measurements is Multivariate Mixed Longitudinal Analysis that on the one hand, takes into account the correlation among measurands and the inter-subject variability and on the other hand, considers all the variable simultaneously.[13] Multivariate mixed models takes into account the randomness in the model, models the intra-subject variability and results in more accurate findings.[14] These models are called mixed models because there are mixtures of random and fixed effects in the model. One of the most important assumptions in the multivariate mixed models is the normality of random effects. Although satisfying this assumption facilitates the statistical inference, in many cases, the asymmetric data (due to the outliers and those that are far away from the recorded data) violates the normality assumption in the random effect. They introduce zero-deviated Kurtosis and Skewness in the distribution in comparison with the normal one and bias the variance to higher values.[15]

Normality assumption in traditional methods such as the correlation analysis paired sample t-test (to analyze the response before and after intervention) or its expanded version such as variance analysis in repeated (>2) measurements, has limited its usage in the analysis of the real recorded data.[14] Because in clinical trials on a specific disease, the existence of subject(s) with non-normal values is inevitable. The first and easiest solution in analyzing this kind of data is omitting asymmetric data that usually ends up with incorrect interpretation.[15] Thus, using a distribution that can be fitted to the recorded data because of its non-symmetrical and skewed nature is practically interested.[15] In the following we show the useful application of multivariate mixed models method to analysis ophthalmic randomized clinical trial to receive more precise results than the previous studies on this data set.

The data recorded from a randomized study on patients with DME were analyzed in this paper using a new statistical method. Accordingly, the best treatment among Intravitreal Bevacizumab (IVB), Intravitreal Bevacizumab/Intravitreal Triamcinolone (IVB/IVT) and finally, the versatile treatment of Macular Laser Photocoagulation (MPC) was identified after adjusting for confounders. Our study was motivated by the fact that many data sets considered in the literature seem to present non-normal behavior[11] which require data transformation or omitting these kinds of data in order to be better approximated by the normal distribution. Nowadays, using statistical models considering the real distribution of the data provides an appealing robust alternative to the usual symmetric process in repeated measurement models. Among the distributions that belong to the skewed class of distributions, we can receive more precise and efficient results by this method. In this article, we advocate the use of a subclass of skew-symmetric distributions which develops a more flexible modeling than the primary statistical models.

Methods

This randomized clinical trail study was performed on 150 patients in ophthalmic research center of Labbafinejad Medical Center (Tehran, Iran) during two years ending in 2007. Considering 90% power for detection of a 0.2-logMAR difference (equal to 2 Snellen lines) in visual acuity and being significant at the two-sided 5% level with an assumed standard deviation (SD) of 0.33, 50 eyes in each group were required.[15] We selected 103 patients' eye with complete information from these 150 eyes.

The informed written consent was obtained from all patients and the protocol was approved by the ethical committee of ophthalmic research center of Labbafinejad Medical Center (Tehran, Iran). In this study, patients were randomly allocated into three following groups: 1) The IVB group, patients who received 1.25 mg IVB (32 subjects); 2) the IVB/IVT group, patients who received 1.25 mg of IVB and 2 mg of IVT (32 subjects); and 3) the MPC group, patients who underwent focal or modified grid laser (33 subjects). To decrease the side effect of IVB, half doze was used instead of normal 4 mg injection. In the case of two eyes examination, each eye was studied separately and the best corrected visual acuity (BCVA) and central macular thickness (CMT) of each patient were measured during the survey.

BCVA was measured by the Snellen chart and was recorded in logarithm of the minimum angle of resolution (logMAR) scale. Retinal thickness was measured in a circle (3.5 mm in diameter) centered on the fixation point. Mean thickness on the 1 mm circle centered on the fovea (CMT) was considered for statistical evaluation.

Exclusion criteria were previous panretinal or focal laser photocoagulation, prior intraocular surgery or injection, history of glaucoma or ocular hypertension, VA of 20/40 or better or worse than 20/300, presence of iris neovascularization, high-risk proliferative diabetic retinopathy, and significant media opacity. Monocularity, pregnancy, serum creatinine ≥ 3 mg/dl, and uncontrolled diabetes mellitus were also among the exclusion criteria.[15]

The experiment was conducted in a triple-blind manner that patients, experimenters and data analyzers were not aware of the specific diagnostic methods. For this reason, a non-therapeutic light was shed on the patients' macula for 20 sec in IVB and IVB/IVT groups. The injection was also simulated for MPC group.[16]

Statistical modeling was based on the data recorded in weeks 6, 12, 24, 36, and 48 which the patient's data was complete at these times. BCVA as a continuous variable and CMT as an ordinal variable were the response variables and the duration of diabetes (> 10 years) (DD) as the number of years having diabetes, sex and the baseline values of BCVA and CMT were considered as confounder variables. It should be noted that the CMT variable was ordered in three following groups:[16] < 300 μm, 300-399 μm and > 400 μm. Since the bivariate distribution of the recorded data was shown to be asymmetric or heavy-tailed based on the bivariate Mardia's skewness and kurtosis coefficients[16] of 0.88 and 6.7, asymmetric modeling was crucial.

After fitting different symmetric and skew-symmetric distribution and running the model, DICs (goodness of fit criteria of the models) were compared in different models. The results of comparing these criteria showed that the skew-symmetric models fitted better than the symmetric models. In addition, among asymmetric models, the distribution with more kurtosis was the best one.

The bivariate mixed linear model is shown in Eq.1 and Eq.2. In this model, the constant random variable (CRV) was used to compensate intra-subject variability while the bivariate model was chosen because of the relative correlation between CMT and BCVA. Furthermore, f is the Log link function for the ordinal response.

$$CMT = f\left(CRV + \beta_{11}(sex) + \beta_{12}(DD) + \beta_{13}(IVB/IVT) + \beta_{14}(MPC)\right) + Error \quad \text{Eq.1}$$

$$VABC = CRV + \beta_{21}(sex) + \beta_{22}(DD) + \beta_{23}(IVB/IVT) + \beta_{24}(MPC) + Error \quad \text{Eq.2}$$

Skew-symmetric distributions used in this model were either those that had more kurtosis in comparison with the normal one or those that had right skewness[15,17] such as skew-normal[1] or skew-t.[2] Although the density functions of the skewed distributions are complicated, they are of interest for researchers because of the lower error variance and higher precision.[17] In these models, traditional methods are not used to compute the coefficients. Instead, Bayesian methods are used. Statistical modeling and inference was written in WinBugs ver. 14.[18] Selection of the best fitted asymmetric model among different distributions was based on Deviance Information Criteria (DIC) which is a goodness of fit measure in Bayesian methods. Data were reported as frequency (percent) for qualitative variables and mean ± Standard Deviation (SD) for quantitative variables. Odds Ratios (OR) and their 95% confidence intervals (CIs) were presented as the measure of association and p-values < 0.05 were considered as statistically significant.

Results

In this study, 47.4% of participants were men and 52.6% were women. Their age was in the 47-57 years range. Their average diabetic duration was 10.24 $\pm$ 1.2 years (Mean $\pm$ SD). Among the patients, 92.3% and 7.7% had non-proliferative and proliferative retinopathy, respectively. BCVA values are shown according to LogMAR scale. Twelve percent of the whole data were asymmetric or outlier based on some primary statistical method like Z-score (Table 1).

All confounders were significant in our model ($p < 0.05$) (Table 2). These results are presented based on the best fitted model using DIC. Using this criterion, it was identified that the mixed asymmetric model of skew-t was the best model among the fitted models. This distribution is one of the important asymmetric distributions that can cover more outliers than the normal distribution. There was no significant difference between IVB and IVB/IVT groups for the treatment of DME ($p > 0.05$) while these two groups showed significantly better therapeutic effect in comparison with the PMC group ($p < 0.05$) (Table 2). These effects were on increasing BCVA as well as decreasing CMT. Considering the regression coefficients and the p-values, patients with longer diabetic duration showed better response to the treatment. In other words, the rate of decreasing CMT and increasing BCVA was higher for patients whose diabetic duration was higher in comparison with those with lower diabetic duration. It was also shown women showed better response to the treatment. Taking into account the baseline values, patients with lower BCVA and higher CMT showed better response to the treatment. It should be noted that since the CMT variable is ordinal, the odds ratio and its confidence interval was reported (Table 2).

Table 1. The summary statistics of best correlated visual acuity and central macular thickness at the beginning and during the follow-ups (n=103)

	CMT			BCVA		
Treatment Groups	**IVB mean ± SD**	**IVB/IVT mean ± SD**	**MPC mean ± SD**	**IVB mean ± SD**	**IVB/IVT mean ± SD**	**MPC mean ± SD**
Baseline	320 ± 118	350 ± 116	341 ± 149	0.71 ± 0.26	0.73 ± 0.28	0.55 ± 0.18
Week 6	318 ± 111	348 ± 109	340 ± 124	0.72 ± 0.18	0.74 ± 0.25	0.60 ± 0.22
Week 12	295 ± 117	340 ± 115	338 ± 122	0.75 ± 0.16	0.76 ± 0.22	0.54 ± 0.32
Week 24	290 ± 110	338 ± 105	335 ± 132	0.79 ± 0.17	0.77 ± 0.19	0.57 ± 0.29
Week 36	289 ± 115	335 ± 118	333 ± 121	0.79 ± 0.19	0.77 ± 0.20	0.57 ± 0.17
Week 48	286 ± 113	324 ± 116	332 ± 114	0.79 ± 0.23	0.78 ± 0.28	0.58 ± 0.24

CMT: Central Macular Thickness, **BCVA**: Best-Corrected Visual Acuity, **IVB**: Intravitreal Bevacizumab injection alone group, **IVB/IVT:** Intravitreal Bevacizumab injection in combination with Intravitreal Triamcinolone acetonide group, **MPC:** Macular laser Photocoagulation group

Table 2. The regression results of the bivariate mixed linear asymmetric model for predictors of correlated visual acuity and central macular thickness in diabetic patients

Outcome	Variables	Group	n	Mean ± SD	Odds Ratio (95% CI)	P-value
CMT	Sex	Men	49	0.63 ± 1.25	1.9(0.5-6.5)	<0.001
		Women	54	Reference Group		
	Diabetic Duration			0.65 ± 0.24	1.9(1.5-2.4)	<0.001
	Baseline measurements			1.45 ± 0.52	4.2(2.5-7.1)	<0.001
	Treatment Groups	IVB/IVT	32	0.23 ± 0.57	1.2(0.7-2.2)	0.075
		MPC	33	1.02 ± 0.49	2.7(1.7-4.5)	<0.001
		IVB	32	Reference Group		
BCVA	Sex	Men	49	0.14 ± 0.73	-	<0.001
		Women	54	Reference Group		
	Diabetic Duration			-0.41 ± 0.76	-	<0.001
	Baseline measurements			-1.02 ± 0.43		<0.001
	Treatment Groups	IVB/IVT	32	-0.54 ± 0.55	-	0.067
		MPC	33	-0.73 ± 0.59	-	<0.001
		IVB	32	Reference Group		

CMT: Central Macular Thickness, BCVA: Best-Corrected Visual Acuity, IVB: Intravitreal Bevacizumab injection alone group, IVB/IVT: Intravitreal Bevacizumab injection in combination with Intravitreal Triamcinolone acetonide group, MPC: Macular laser Photocoagulation group
CI : Confidence Interval

Discussion

Using the specific design method in the analysis of clinical trials, it is possible not only to control the confounding variables but also to investigate the possible variation of the outcomes during time.[19] Non-normal data usually exists in medical records and it is rare to have normal data all the time. By removing asymmetric data that are non-normal data or outliers, it is possible to reduce the sample size so much that makes it difficult to analyze the data and correctly interpret the parameters.[20] Other proposed statistical methods used to analyze non-normal data, for example non-parametric analysis, have lower power in comparison with the parametric methods.[15] Therefore, in recent years, incorporating statistical methods that not only take into account the data correlation in repeated measurements but also tolerate the non-normality, has been of great interest among investigators.

Longitudinal data analysis is recently used to more accurately analyze the clinical trials. In these methods, since there is a correlation between repeated measures, it is important to use approaches that take into account the data correlation as well as time-dependent effects of the treatment methods.

According to the results of the current study, intravitreal bevacizumab injection alone or in combination with intravitreal triamcinolone acetonide improved the BCVA and significantly reduced the CMT that the association was not strictly proven to be significant in the previous researches using primary statistical methods.[16] These improvements are much clearer up to the week 24. Similar studies showed that the effect of bevacizumab injection is retained up to two weeks.[21] In the literature, other investigators showed that intravitreal bevacizumab injection improves the vision in diabetic patients but intravitreal bevacizumab injection in combination with intravitreal triamcinolone acetonide does not improve the patients' vision or reduce the CMT.[22,23] Statistical analysis using repeated measurement on the same data set also showed intravitreal bevacizumab injection

alone or in combination with intravitreal triamcinolone acetonide improved only the patients' vision.[15]

The significancy of the patient's sex and diabetic duration (> 10 years) on the patient's responses to the treatment is in line with the previous studies.[1,5,6] However, unlike the previous researches in which the effect of sex was not directly declared, we proved that women showed better responses to the treatments. We also showed that the patient's response to the treatment is better if the diabetic duration is higher which is in agreement with the previous researches.[5,24]

Therefore, according to our statistical analysis, intravitreal bevacizumab injection alone or in combination with intravitreal triamcinolone acetonide was a suitable substitute to macular laser photocoagulation as a primary treatment for diabetic macular edema. In addition, the aforementioned treatment methods had more positive effect on the patients that had lower BCVA and higher CMT as the baseline values.

In this paper, we not only modeled clinical outcomes in the presence of confounding parameters including disease duration and sex, but we also did not remove any asymmetric data or outliers that enabled to increase the power of tests in comparison with other similar studies. Considering the bivariate analysis, two outcomes of BCVA and CMT were analyzed simultaneously that indeed gave the opportunity to analyze their relationship that increased the accuracy of the inference in comparison with the previous investigations. In spite of all aforementioned advantages, this updated statistical analysis has a lot of computational difficulties and requires programming software which is hard to use.

Conclusions

No significant difference was observed in this research between intravitreal bevacizumab injections alone or in combination with intravitreal triamcinolone acetonide. However, both of these methods significantly showed better improvement in comparison with that of macular laser photocoagulation as a traditional treatment of diabetic macular edema. This improvement was shown in increasing the BCVA and decreasing the CMT during one year clinical follow-up. This research showed that when we have non-normal data in clinical trials, skew-symmetric mixed effect models may lead to different results compared to symmetric mixed models. With regard to the advantages of this updated statistical method, we can use all data set including asymmetric data.

Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

MM carried out the design and coordinated the study, and run all modelling studies and prepared the manuscript. AK and IK provided assistance in the design of the study, technical advice on modelling and revised the manuscript. FZ provided assistance for all statistical results. MS coordinated and carried out all the experiments and provided the technical advice on medical discussion. All authors have read and approved the content of the manuscript.

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