Original Article

First and Second Order Generalized Estimating Equations and Their Application in Analyzing Longitudinal Microleakage Data

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Abstract

Background and Aim: Longitudinal data are frequently obtained in medical studies. When the main aim of a study is marginal modeling of the mean and the correlation structure is considered as a nuisance parameter, the first- order generalized estimating equations (GEE1) is usually an appropriate option. However, when the modeling of correlation structure is considered the aim of a study, the second- order generalized estimating equations (GEE2) may be the first choice for analyzing the available data. The aim of the study was to evaluate application of first- and second-order generalized estimating equations to analyze longitudinal microleakage data.

Materials and Methods: In this study, the GEE1 and GEE2 methods were used to analyze the data from a study of microleakage in two root- end filling materials (CEM and MTA) in two different thicknesses and two diameters at three different times of measurement (one day, one week and one month after treatment). The obtained results from these statistical approaches were compared in continuous and binary (presence or absence) microleakage data.

Results: The results from the GEE1 and GEE2 methods showed that time of measurement, material type, diameter and thickness of filling material had significant effects on (continuous) microleakage rate. In addition, in binary microleakage data, these methods revealed that only time and material type were the significant factors. The correlations between measurements were not significant in continuous data, while they were significant in binary response microleakage data.

Conclusion: Since the correlations between pairs of measurements were not significant in continuous microleakage data and the obtained estimates were similar in both GEE1 and GEE2 methods, so the simpler GEE1 method seems to be adequate for these data. In contrast, in binary microleakage data, significant correlations were found between measurements. Therefore, in this case the GEE2 methodology may be used to estimate the correlation structure more efficiently.

Key Words: Microleakage, longitudinal study, GEE1, GEE2

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Introduction

Correlated medical responses are frequently seen in studies related to paired organs of the body, and in longitudinal studies. In these studies, the principle sampling unit is a group or a cluster of subjects (sampling unit), and one observation for each subject in a cluster is recorded. In longitudinal studies, the response variable and a collection of covariates for each subject (unit) are observed or measured at different times. Since these repeated measurements are obtained from each subject under study, thus these measurements should be considered as correlated observations [1,2]. Therefore, traditional methods should not be used in longitudinal data analysis [3], and the correlation between observations must also be considered to achieve correct results and valid conclusions [1, 2, and 4].

There are two objectives in longitudinal data analysis. First; modeling the marginal response probabilities as a function of covariates (marginal modeling), and second; modeling the correlation between the response pairs (modeling the correlation structure). In the first case, the correlation structure is a nuisance parameter, and the first-order Generalized Estimating Equations (GEE1) gives a suitable solution, but in the second, the second-order Generalized Estimating Equations (GEE2) produces an analytical solution, using correlation coefficient or odds ratio as a measure for the correlations [5, 6]. When data are quantitative, correlation coefficient is usually used, and when the data are binary, the odds ratio is used as a measure for correlations [7].

There is a long history of attempts to save and maintain teeth using various methods, and to date, a variety of treatments have been developed for preservation of teeth or remaining tissues. Endodontics is one of the most common treatment methods, aiming to maintain the treated tooth in a healthy and efficient state. This aim is achieved by early diagnosis, appropriate treatment strategy, cleaning and shaping the root canal cavity, and a full and dense 3-D filling with filling material of specific properties [8]. A major failure factor of endodontic treatment is penetration of bacteria or their toxins through the crown or root canal to the periodontal space around the root. Leakage of these pathogens can cause widespread contamination of the canal, both before root canal filling (in intervals between sessions), and after obturation and before permanent restoration of the crown. Therefore, root canal filling material should be chosen to have impermeable sealing properties to prevent leakage and subsequent contamination of the canal. This is even more important when tooth is still immature and the canal space is wider than normal. In such cases, a new technique called apical plug is performed for filling the root-end, using retro-filling material (root-end filling). A retro-filling material should possess properties like good compatibility with tooth sidewalls (creating a good seal), dimensional stability, ability to induce cement ogenesis, and bio-compatibility, without solubility, toxicity, or erosion. But, none of the materials available have all these properties, and none is able to create a 100% seal. Thus, by examining the properties of existing material, researchers are always trying to develop and introduce more suitable materials [9, 10].

Various materials have been used in periapical surgery. Mineral Trioxide Aggregate (MTA) is one such material, which was first introduced in 1993 as a root-end filling with good bio-compatibility. Due to satisfactory results achieved, this material is now considered one of the best endodontic biomaterials, and is used by dentists worldwide. However, it has its flaws as well including difficulty of use, prolonged setting time, weak anti-microbial properties, discoloration of teeth, and cost [11, 12]. Considering advantages and disadvantages of MTA, a material has recently been developed by one of the authors of this manuscript (Dr Asgari) called Calcium Enriched Mixture (CEM). An application of this new material is treatment of apical plug for open-apex teeth. Therefore, this study aims to use first and second order Generalized Estimating Equations for analysis of microleakage data, and introduction of a more suitable material for root-end filling of open-apex teeth with consideration for the thicknesses and diameter factors.

Materials and Methods

Microleakage data: In this manuscript, the Data from a joint study by Endodontic Research Center at Shahid Beheshti University of Medical Sciences and Dentistry School of Qazvin University of Medical Sciences was used. In this experimental study, two different filling materials with two different thicknesses (3 and 5 mm), and two different diameters (1.1 and 1.7 mm) were used for assessment of factors affecting the microleakage level. Each combination (2×2×2 modes) was investigated on 15 teeth (15 replicates), so 120 samples were studied in total. Then, microleakage level was measured on three occasions; the first day, the first week, and the first month (360 measurements in total), using a liquid filtration system with measuring unit in micro-liters [13]. In addition to microleakage numerical data, microleakage binary data were also used (by converting numerical data to two states of, with and without microleakage).

Data analysis: Since the repeated measurement of each tooth microleakage creates correlated responses, thus the analysis of such data requires methods that consider these correlations [14, 15]. To determine the simultaneous effects of 4 independent variables (microleakage measuring time, type of filling material, thicknesses, and diameter of material), the marginal modeling and the first [16, 17], and second [18, 20] order Generalized Estimating Equations approaches were used. The SPSS software was employed for marginal modeling using the GEE1 method, and MAREG software was used for the marginal modeling using the GEE2 approach. P-values les than0.05 were considered statistical significant.

Data structure: Assume there are t times of measurement for each subject (unit) in a longitudinal study. Hence, ith subject (i= 1 … N) is observed on occasions, t=1, 2 … t_i, where $t_i \leq t$. For simplicity, assume t_i=t. So, the response variable associated with ith subject at time t can be shown as y_{it} and each subject has t vector of covariates x_{it} with p×1 dimension. Therefore, the t×p matrix of X_i=[x_{i1}, …, x_{it}] is the covariates matrix for ith subject.

Marginal model and correlation structures: marginal modeling approach is one of the most common methods for analyzing longitudinal data sets. A marginal model for longitudinal data analysis can be written as:

$$g\left(\mu_{it}\right) = x'_{it}\beta$$

Where:

β = Regression coefficients vector,

x_{it} = Covariate vector

μ_{it} = mean response given x_{it}

The link function g(.) relates the expectation of responses to the covariate vector, and type of link function depends on the type of response variable. Correlation parameters for the vector of repeated measures (cluster) are assumed to be independent of the regression parameters. The important assumption in this model is assuming a correlation between repeated observations for each sampling unit. Different structures (shown below) of this correlation are included in the model:

A) Independent structure: It assumes no correlation between responses in a cluster.

B) Exchangeable structure: It assumes equal correlation for each pair of data in a cluster.

C) First order auto-regressive structure (AR1): It assumes that correlation between data diminishes with increasing the lag in measurement intervals.

D) Stationary K-Dependent structure: It is similar to AR1, the only difference is that after time interval K, zero correlations are considered.

E) Unstructured structure: A distinct correlation parameter for each pairs of responses or two observations in a cluster is considered.

Estimating the Regression parameters (β) in the marginal model can be performed by either of the GEE1 or GEE2 approaches.

First order Generalized Estimating Equations: In this method, regression parameters are estimated by solving the following equation:

$$U(\beta) = \sum_{i=1}^{N} \frac{\partial \mu'_i}{\partial \beta_i} V_i^{-1}\left(y_i - \mu_i\right) = 0$$

The ith subject covariance matrix is

$$V_i = A_i^{\frac{1}{2}} R_i A_i^{\frac{1}{2}}$$

Where A_i is a diagonal matrix, and the elements on the main diagonal is given by;

$Var(Y_{it}|X_i) = \phi v(\mu_{it})$

R_i is the working correlation matrix that determines data correlation structure.

Second order Generalized Estimating Equations: A set of estimating equations are now introduced that result in consistent estimates for both the regression and correlation parameters. These estimating equations are called second order generalized estimating equations. Generally, in this method there are $t(t$-1)/2 second order expressions in the form of $z_i' = (z_{i12}, z_{i13}, \ldots, z_{i(t-1)t})$ which can be modeled using $\ell_i(\alpha) = E(z_i)$ and an appropriate link function. Correlation coefficient or odds ratio can be used as the measure of correlation [20]. For instance, in continuous data analysis, estimates of the regression and correlation parameters can be found by solving the following equation system:

$$U\begin{pmatrix}\beta\\ \alpha\end{pmatrix} = \sum_{i=1}^{N}\begin{pmatrix}\frac{\partial\mu_i}{\partial\beta'} & 0\\ \frac{\partial\ell_i}{\partial\beta'} & \frac{\partial\ell_i}{\partial\alpha'}\end{pmatrix}'\begin{pmatrix}V(y_i) & Cov(y_i, z_i)\\ Cov(z_i, y_i) & V(z_i)\end{pmatrix}^{-1}\begin{pmatrix}y_i - \mu_i\\ z_i - \ell_i\end{pmatrix} = 0$$

Where $\ell_i(\alpha)$ is the correlation coefficient for the repeated observations.

Results

Analysis of microleakage quantitative data: To investigate simultaneous effects of type of filling material, thicknesses, diameter, and time of measurement on the level of microleakage, the marginal modeling using the GEE1 (in which, the correlation structure is considered as a parameter in the model) and GEE2 methods (in which, the correlation between observations is modeled separately) [20, 21] were utilized.

The following is the utilized marginal model:

$$E(Y_{it}) = \beta_0 + \beta_1 Material + \beta_2 Height + \beta_3 Diameter + \beta_4 Time$$
$$i = 1,2,\ldots,120 \qquad t = 1,7,30\,days$$

Where, y_{it} shows the microleakage level of the ith tooth at the tth time of measurement [22, 24]. The results of this model are shown in table 1. The obtained results from the marginal modeling using the GEE1 method for microleakage data indicate that the time trend has significantly influenced the microleakage level (p=0.001). Also, the time parameter estimate of -0.003 means an average daily reduction in microleakage level of 0.003 ml. In addition, type and diameter of the root-end filling material significantly influence microleakage level (p=0.001). According to these results, CEM filling material showed less microleakage level than MTA, and, level of microleakage was lower in 1.1 mm diameter than in 1.7 mm diameter of filling material. Also, filling material thicknesses made a significant difference in the level of microleakage (P=0.014), with this level being lower in 5 mm thicknesses than in 3 mm thicknesses .

Now, consider the marginal modeling using the GEE2 method for microleakage data. In this method, the correlation between pairs of microleakage measuring times (day one, week one, and month one) is estimated using the Pearson's correlation coefficient between repeated observations. Results of this model are shown in table 2.

The results of analysis of microleakage level data using GEE2 marginal modeling revealed that time trend has significantly reduced microleakage level (P=0.003). Also, the two filling materials were significantly different in terms of their effects on microleakage level (P<0.001), and according to the results obtained, the filling material CEM reduced microleakage level more than did MTA filling material. The thickness and diameter of filling material also had significant effects on microleakage level (P=0.013 and P<0.001, respectively), and microleakage was less at 5 mm thicknesses of filling material than at 3 mm thicknesses. Also, with 1.1 mm diameter of filling material microleakage level was less than what it was with 1.7 mm diameter. However, no significant correlations was found between pairs of microleakage measurements (between the first day and the first week, the first day and the first month, or the first week and the first month) (P>0.05).

Analysis of binary microleakage qualitative data: In this step of data analysis, the marginal modeling using the GEE1 and GEE2 methods were utilized To investigate the simultaneous effect of type of

Table 1. Results of the marginal modeling using the first and second order Generalized estimating equations

Variable	Category	Estimate	SE	P
Material type	CEM	-0/091*	0/023*	0<0/001
	MTA		Reference category	
Material thickness	3 mm	0/057	0/023	0/014
	5 mm		Reference category	
Material diameter	1/1 mm	-0/094	0/023	0<0/001
	1/7 mm		Reference category	
Time	--	-0/003	0/001	0/001

*** Values are in ml**

Table 2. Results of the marginal modeling using the first and second order Generalized Estimating Equations for the microleakage level data

Variable	Category	Estimate	SE	P
Width from origin	--	0/227	0/026	--
Material type	CEM	-0/091	0/023	0<0/001
	MTA		Reference category	
Material thicknesses	3 mm	0/057	0/023	0/013
	5 mm		Reference category	
Material diameter	1/1 mm	-0/094	0/023	0<0/001
	1/7 mm		Reference category	
Time	--	-0/003	0/001	0/003
α_1 **(first day and first week)**	--	-0/113*	0/185	0/221
α_2 **(first day and first month)**	--	0/063	0/183	0/488
α_3 **(first week and first month)**	--	-0/17	0/185	0/205

*** Correlation between microleakage levels in day 1 and week 1**

filling material, thicknesses, diameter, and time on microleakage, First, marginal modeling with GEE1 estimating method for binary microleakage data was considered. The following is the utilize marginal model:

$$\log\left[\frac{P(Y_{it}=0)}{P(Y_{it}=1)}\right] = \beta_0 + \beta_1 Material + \beta_2 Height + \beta_3 Diameter + \beta_4 Time$$

$$i = 1,2,\ldots,120; \qquad t = 1,7,30 days$$

In which, y_{it} indicates the presence (code 1) or absence (code 0) of microleakage in the ith tooth at the tth time of measurement [25]. The obtained results for this model are presented in table 3. Results of the analysis of microleakage data using the marginal modeling with GEE1 showed that the time trend has significantly influenced on the presence of the microleakage (p=0.001). This means that the odds of absence of microleakage increased by 0.03 daily (OR=1.03). Also, the two filling materials differed significantly in terms of microleakage ($p<0.001$), and the odds of absence of microleakage was over 10 times with CEM as that with MTA (OR=10.19). Therefore, compared with MTA, CEM is recommended. The thicknesses of filling material also influenced microleakage significantly ($p=0.042$), and odds of absence of microleakage at 3 mm thicknesses was 0.46 less than that at 5 mm thicknesses (OR=0.54). Therefore, it is recommended to use 5 mm thicknesses of filling material. However, the diameter of the filling material did not show a significant effect on the presence of microleakage ($p=0.149$).

Finally, we present the obtained results from the marginal modeling using the GEE2 for the binary microleakage data. In this method, the odds ratio (OR) is used as the measure of correlation between pairs of binary responses, and the correlation between microleakage at three times of measurement (the first day, the first week, and the first month) is estimated using paired odds ratio between repeated measures, assuming presence of dependence be tween these observations. The obtained results for Analysis of microleakage data using GEE2 method revealed that time trend significantly influenced the presence of microleakage ($p<0.001$). Also, there was a significant difference between two filling materials in terms of the presence of microleakage ($p<0.001$), and the odds of absence of microleakage with CEM was 10 times higher than with MTA (OR=9.8). Therefore, it is recommended to use CEM filling material instead of MTA. However, filling materials' thickness and diameter did not significantly influence the presence of microleakage ($p=0.064$ and $p=0.238$, respectively). The association between the first day and the first week observations was significant ($p=0.004$), with value of 3.49 for the odds ratio of microleakage between the first day and the first week. Also, association between the first day and the first month observations was significant ($p=0.001$), with an estimated odds of 11,36 for the presence of microleakage in the first day compared to first month.

Table 3. Results of the marginal modeling using the first order Generalized Estimating Equation for microleakage data

Variable	Category	Estimate	SE	Odds ratio	P
Width from origin	--	-1/730	0/311	--	--
Material type	CEM	2/231	0/300	10/19*	<0/001
	MTA		Reference category		
Material thicknesses	3 mm	-0/614	0/302	0/54	0/042
	5 mm		Reference category		
Material diameter	1/1 mm	0/431	0/299	1/54	0/149
	1/7 mm		Reference category		
Time	--	0/028	0/008	1/03	0/001

*** The presence of microleakage is considered as the reference category**

Discussion

To find consistent regression coefficients in GEE2 methodology, both the mean and correlation structures should be properly identified. However, the main advantage of GEE1 is that, only correct identification of mean structure is required, and eve

this model are shown in table 4.

with correlation structure not correctly identified, regression parameter estimates still remain consistent. Therefore, if in GEE2 method, significant efficiency is not obtained, GEE1 method can always provide acceptable regression parameter estimates [26].

Table 4. Results of the marginal modeling using the second order Generalized Estimating Equations for the presence of microleakage data

Variable	Category	Estimate	SE	Odds ratio	P
Width origin	--	-1.700	0.314	--	--
Material type	CEM	2.282	0.307	9.80	<0.001
	MTA		Reference category		
Material thicknesses	3 mm	-0.560	0.302	0.57	0.064
	5 mm		Reference category		
Material diameter	1.1 mm	0.353	0.300	1.42	0.238
	1.7 mm		Reference category		
Time	--	0.032	0.008	1.03	<0.001
α_1 **(first day and first week)**	--	1.25*	0.435	3.49**	0.004
α_2 **(first day and first month)**	--	2.043	0.534	11.36	0.001
α_3 **(first week and first month)**	--	0.478	0.489	1.62	0.328

Laing et al in a simulation study showed that if correlation parameters are regarded as nuisance parameters, or if the number of clusters is large in comparison with the cluster size, the GEE1 method will be more efficient than the GEE2 for estimating the regression coefficients. Conversely, in cases where correlation parameters are important and their estimates is the researcher's intention, or if the number of clusters is small, then GEE2 is the preferred option [27].

Analysis of microleakage data using both the first and second order Generalized Estimating Equations methods were performed, and obtained results indicate that type, thicknesses, and diameter of filling material significantly influence level of microleakage. In that, microleakage level is less with CEM filling material compared to MTA, and with 5 mm thicknesses, there is less microleakage compared with 3 mm thicknesses, also, 1.1 mm diameter produces less microleakage than 1.7 mm diameter. Results obtained with these methods indicate how time influences microleakage level, and that, this level decreases with passage of time. But, the results obtained using GEE2 method showed that correlation of microleakage levels was not significant between any of two measuring occasions (between first day and first week, first day and first month, or first week and first month).

Considering the obtained results from microleakage level data using GEE1 and GEE2 showed equal standard deviation for the estimates and regression parameters were significant similarly with both the methods. In addition, the correlation between observations was not significant in GEE2 method, therefore use of GEE1 method (that is theoretically and practically simpler) would be sufficient.

Analysis of binary microleakage data using both the first and second order Generalized Estimating Equations also was performed. Results obtained for both methods showed that time significantly influenced the presence of microleakage, which meant, the odds of absence of of microleakage (since tooth filling day) increased with passage of time. Also, there is a significant difference between the two filling materials in terms of presence of microleakage, and non-existence chance of microleakage is higher with CEM material than with MTA. Hence, CEM filling material is recommended. However, filling material diameter did not significantly affect presence of microleakage. With GEE1 method, thicknesses of the filling ma

-terial showed a significant effect on presence of microleakage, and the odds of absence of microleakage was less at 3 mm thicknesses than at 5 mm thicknesses. But, with GEE2 method, filling material thicknesses did not show any significant effect. Also, results of GEE2 showed significant association between first day and first week, and also between first day and first month.

Given the results from the GEE1 and GEE2 methods for presence of microleakage data, it is clear that standard deviations of estimates are the same and significance of regression parameters are also the similar with both methods. But, since correlation between repeated measures in GEE2 was significant, use of GEE2 method will produce more explicit estimates of these correlations.

Apical microleakage is an important factor in the etiology of root canal treatment failure. For successful treatment of roots of immature teeth, minimal canal preparation and then, placement of filling material with apical plug technique is suggested. A variety of materials are used for this purpose, which should be capable of creating a proper and compatible seal. MTA is an expensive material with difficult clinical application. Hence, it seems logical to produce an alternative material in this country that is inexpensive and widely available. So, it is necessary to conduct investigations and compare alternative materials with the original [28].

Many studies have been conducted in the field of microleakage, but comparative association of these studies and the present one is a difficult task. Since in these studies different time intervals, different root-end filling materials, and different microleakage measurement methods have been used. In a study by Razmi et al with the aim to compare microleakage levels using MTA and CEM materials, results revealed that CEM had lower leakage than MTA. These results are in agreement with the results obtained in this study that showed less microleakage with CEM compared with MTA [12]. Also, in an investigation by Asgari et al, sealing properties of CEM and MTA as root filling materials were the same, and better than that of IRM. In their study, investigation of microleakage level in CEM and its comparison with MTA and IRM has shown that the created seal in these three materials were IRM<MTA< CEM respectively. But there was no significant difference between CEM and MTA. So, it became clear that the seal property created by CEM and MTA was the same and both better than IRM [29]. Also, Zafar et al in their study evaluating sealing capability of three filling materials MTA, CEM, and AH26 concluded that, sealing capability of CEM was better than the rest [30].

A notable point in the above studies is that most of them used classic statistical tests (like, t-test and ANOVA test) for comparing different groups. But, the important point with this study was application of new and relatively complex statistical models for the analysis of data and subsequent presentation of more accurate results for comparison between groups. Accordingly, it is suggested that for the analysis of data that is normally collected over time in the form of repeated measurements, advanced statistical models should be used, in which simultaneous effects of several factors on the response variable, correlation between data, and influence of time are taken into account.

Conclusion

When modeling the mean is of utmost importance (correlation parameter is considered as nuisance), GEE1 could be the best choice for estimating regression parameters, without the need for modeling correlation parameter. But, if correlation modeling is the main goal, GEE2 is the best method for estimating regression and correlation parameters simultaneously. Our findings indicate that in treatment of apical plug, it is better to use CEM for filling root-end of teeth with open apex.

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