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ORIGINAL ARTICLE

Dealing with hierarchical data in periodontal research

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Abstract Site-specific clinical periodontal data are usually plentiful, typically hierarchical, and generally valuable information. Summarizing these data on a subject level for easy application of standard statistical tests leads to loss of most of the information. In addition, well-known fallacies may make interpretation difficult if not impossible. In this study, an attempt is made to apply, in a non-technical way and as a tutorial, a rather complex multilevel model of gingival thickness, which provides unbiased estimates of fixed effects and a variance/covariance matrix with considerable information as regards data structure. When applying multilevel modeling, random effects should generally be reported in a proper way, since they might reveal new insights into subject and tooth variation, correlations between covariates, and even problems with the chosen model.

Keywords Hierarchical data · Gingival dimensions · Periodontal phenotype · Random effects model · Tutorial

Introduction

How to statistically analyze collected data very much depends on its structure. Periodontal data are usually plentiful observations made in one oral cavity. In order to describe the periodontal situation, sites (gingival units) around teeth within patients or subjects are considered by using several variables. Observations may be even repeated in a longitudinal way. This is a typical hierarchical situation with lower and upper levels.

A suitable armamentarium for the study of fixed (estimates of covariates) and random parameters (variances and covariances) is provided by multilevel modeling [9]. The methods are well-known for decades and implemented in major statistical software packages such as SAS, STATA, and others; for a comprehensive review of software programs and packages that are designed or can be used for multilevel analyses, see de Leeuw and Kreft [3]. Easy-to-apply special software such as MLwiN has only recently been developed. The aim of this communication is to give a rather non-technical description of the basic principles of multilevel modeling and, as an educational example, the application of multilevel models to data on the thickness of facial gingiva.

The data

Large intra- and interindividual differences in width of gingiva have frequently been described, especially in the second half of the twentieth century; see, for an extensive review, Schroeder [19]. On the other hand, gingival thickness was systematically assessed only after the development of non-invasive, ultrasonic, measuring devices [5]. A first attempt of defining gingival phenotypes by clustering subjects with certain combinations of gingival thickness and width and crown shape [12] was later put into perspective [13]. This was possible by splitting the total variance of gingival thickness into two different components, one at the tooth and one at the subject level. It turned out that the variance at the subject level, which, by definition, defines part of a proclaimed, largely genetically determined ‘gingival phenotype’ [12], was very small,

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about 4% of the total variance. But should a small subject variance be ignored?

The data treated in the present example had been collected in a group of 40 periodontally healthy young adults (21 female) with good oral hygiene [15, 16]. A thorough mucogingival examination consisted of measurements of thickness and width of gingiva, periodontal probing depth, bleeding on probing, and ratios of crown width to its length of incisors, canines, and premolars. The latter were not further considered in this study. All calculations were done using a special software (MLwiN version 2.10 Beta, Centre of Multilevel Modeling, Bristol University, Bristol, UK).

Disregarding the subject level

The educational question in this study is: Is gingival thickness related to gingival width? A very simple expression of the relationship, disregarding sampling in different subjects, may be a linear regression model as described by the equation

$$y_i = \beta_0 + \beta_1 x_i + e_i; e_i \sim N\left(0, \sigma^2\right), \quad (1)$$

where teeth, indexed by i, were sampled. The response y is gingival thickness as measured with the ultrasonic device with a resolution of 0.1 mm, and x is the predictor, gingival width, as measured with a periodontal probe to the nearest millimeter. Both variables were centered on their respective means; see Kraemer and Blasey [10] for a comprehensive review of advantages of appropriate centering and a number of serious consequences of not centering. The residuals e_i are assumed to have normal distribution with variance σ^2, of course with the further assumption of being independently distributed. Neither β_0 (estimate, −0.002; standard error, 0.015) nor β_1 (−0.003; 0.011) are significantly different from 0 (which has of course to be expected for β_0 due to centering). The variance σ^2 is 0.273 (0.011). The relationship between gingival thickness and width (centered on their respective means) is graphically displayed in Fig. 1.

However, since several teeth were sampled in a given subject, the assumption of independence is not justified. Measurements of gingival thickness at two teeth sampled in a certain individual will be more similar than those at teeth sampled in different subjects, even if adjusted for gingival width (x_i).

A random intercept model

Thus, a more elaborate model may be a natural extension of Eq. 1:

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + u_j + e_{ij}; u_j \sim N\left(0, \sigma_u^2\right); e_{ij} \sim N\left(0, \sigma_e^2\right), \quad (2)$$

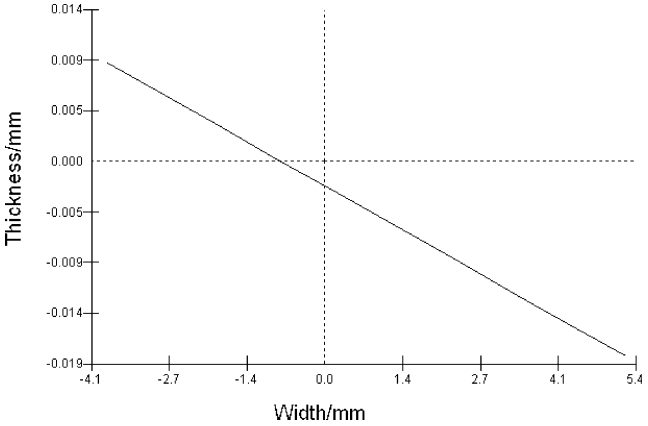

Fig. 1 Regression of gingival thickness (y-axis) on gingival width (x-axis, both in mm). Subject effects are disregarded (model 1). Neither the intercept nor the regression coefficient differs significantly from 0

with i indexing teeth and j subjects. Subject effects are described by the residuals u_j. This yields a group of parallel regression lines with different intercepts as is shown in Fig. 2.

The random intercept model in Eq. 2 is also known as 'variance components' model since, given subject and tooth residuals are independent, the total (unexplained) variance is the sum of the between-subject variance σ_u^2 and the between-tooth variance σ_e^2. In this random effects model, subject effects are modeled as a random variable depending on a single parameter, the variance σ_u^2. Subjects are randomly sampled from a larger population about we wish to make inferences.

When considering the present data, this model points to a significant influence of gingival width; the regression coefficient β_1 is −0.036 with a standard error of 0.011. While the mean intercept β_0 is again close to zero (estimate, −0.004; standard error, 0.028), subjects' intercepts vary with σ_u^2 of 0.024 (0.007). The tooth level variance σ_e^2 is 0.251 (0.011). The likelihood ratio test statistic (with one additional parameter, thus 1 degree of freedom) for this and the model described in Eq. 1 is 41.52 ($p<0.001$), pointing to a better fit of the more elaborate model to the data.

But does this model in fact describe the reality in such a way that one can, with justification and some confidence, predict the outcome, gingival thickness?

A random coefficient model

In the above random intercept model, the (negative) relationship between gingival thickness and width was assumed to be the same for all subjects. But should we expect this in a biological system, in particular when considering the by far non-significant relationship in the model described in Eq. 1? It is much more likely that, in reality, the relationship with gingival width varies from

subject to subject. A random coefficient model with different slope predictions for subjects' regression lines may be written as

$$y_{ij} = \beta_0 + \beta_{1j}x_{ij} + u_{0j} + e_{ij}; \beta_{1j} = \beta_1 + u_{1j}, \quad (3)$$

where $(u_{0j},\ u_{1j})$ is assumed bivariate normal with $var(u_{0j}) = \sigma^2_{u_0}$, $var(u_{1j}) = \sigma^2_{u_1}$, and $cov(u_{0j}, u_{1j}) = \sigma_{u_{01}}$.

Now, the coefficient of x (gingival width) is assumed to be random across subjects. Its mean is β_1, its variance $\sigma^2_{u_1}$, and covariance $\sigma_{u_{01}}$. In this model, the mean intercept β_0 is again very close to zero (−0.001; standard error, 0.026). Intercepts for different subjects vary significantly (p= 0.004) around the mean with a variance $\sigma^2_{u_0}$ of 0.017 (0.006). The mean coefficient for x (gingival width) is largely attenuated (−0.028) and, due to the larger standard error of 0.019, no longer significant. Coefficients vary around the mean with variance $\sigma^2_{u_1}$ of 0.009 (0.003). The covariance $\sigma_{u_{01}}$ is small (−0.002) and, due to its large standard error of 0.003, not significant. The tooth level variance is again further attenuated to 0.236 (0.010). The likelihood ratio test statistics for this and the random intercept model is 40.18 with 2 degrees of freedom. Thus, the random slope model describes the situation significantly (p<0.001) better than the previous model. A graphical representation of the data under consideration is shown in Fig. 3.

Model extension

So far, only gingival width has been considered as a possible predictor of gingival thickness. It turned out that its influence, if any, is rather low. We can assume, however, that thickness of gingiva may be related to periodontal probing depth at the tooth level and gender at the subject level as well. Tooth-level covariate probing depth may again differ from subject to subject. Therefore, it is assumed to be random across subjects. The final model of facial gingival thickness (y_{ij}), which is entirely based on the model given in Eq. 3, i.e., includes gingival width (x_{1ij}) but is also supplemented with covariates probing depth (x_{2ij}) and gender (x_{3j}), is shown below (all variables centered, estimates with standard errors in parentheses):

$$y_{ij} = \beta_{0j} + \beta_{1j}x_{1ij} + \beta_{2j}x_{2ij} + 0.102(0.041)x_{3j} + e_{ij}$$
$$\beta_{0j} = -0.102(0.045) + u_{0j}$$
$$\beta_{1j} = -0.037(0.018) + u_{1j}$$
$$\beta_{2j} = 0.302(0.036) + u_{2j}$$

$$\begin{pmatrix} u_{0j} \\ u_{1j} \\ u_{2j} \end{pmatrix} \sim N(0,\Omega_u) : \Omega_u = \begin{pmatrix} \sigma^2_{u_0} : 0.011(0.005) & & \\ \sigma_{u_{01}} : -0.001(0.002) & \sigma^2_{u_1} : 0.008(0.003) & \\ \sigma_{u_{02}} : 0.012(0.005) & \sigma_{u_{12}} : -0.009(0.004) & \sigma^2_{u_2} : 0.021(0.011) \end{pmatrix}$$

$$e_{ij} \sim N(0,\sigma^2_e)\ \sigma^2_e = 0.210(0.009)$$

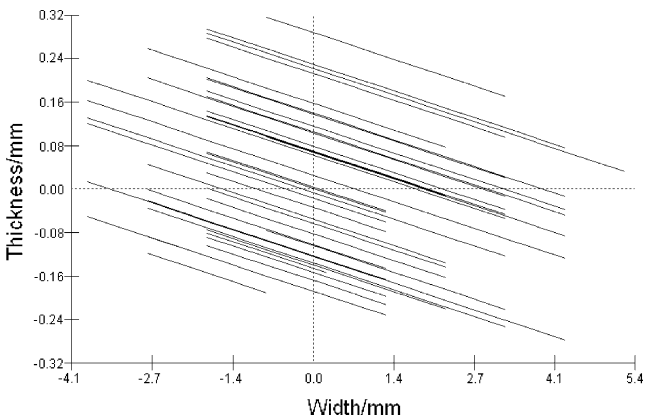

Fig. 2 Parallel regression lines for 40 subjects representing regressions of gingival thickness on gingival width according to a random intercept model (model 2)

Thus, the model reveals that both tooth-related covariates and subject-related gender have a more or less profound influence on gingival thickness. The random part of the model including the subject level covariance matrix Ω_u indicates that gingival width and periodontal probing depth significantly vary around their mean with variances 0.008 (standard error, 0.003) and 0.021 (0.011), respectively. The covariance matrix also reveals that probing depth and gingival width are negatively correlated with covariance $\sigma_{u_{12}}$ of −0.009 (0.004). A correlation coefficient of −0.678 (p=0.043) can be calculated according to the standard formula. Furthermore, probing depth is positively correlated with the intercept with a correlation coefficient of 0.743 (p=0.030). In a graph of the subject prediction lines, a fanning out pattern would be expected due to the positive intercept/slope covariance at the subject level (and positive correlation). Figure 4 indicates this phenomenon.

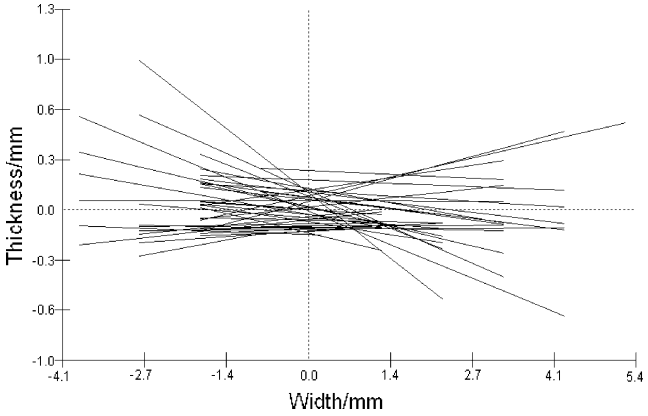

Fig. 3 Non-parallel regression lines representing regressions of gingival thickness on gingival width according to the random coefficient model. Each subject provides a regression line with a different slope (model 3)

The likelihood ratio statistics for this and the previous model with gingival width as the only covariate is 140.77 with 5 degrees of freedom ($p<0.001$). Thus, a highly significant improvement of the model in terms of fitting the observed data can be stated.

Discussion

The dataset that was modeled in this tutorial had originally been acquired in order to confirm previous observations of the author [12] with regard to a proposed gingival phenotype [15]. In that paper, cluster analysis was used in order to group subjects. Certain conditions at anterior teeth in the maxilla were taken into consideration: gingival width and thickness, as well as shape of teeth, say, slender or squared [15]. Cluster analysis seemingly revealed different combinations, such as thick and wide gingiva associated with squared tooth shape, in contrast to thin and narrow gingiva and slender teeth. However, cluster analysis is an entirely explorative measure for getting a quick impression of structure in the data. Notably, it has very low external validity, meaning that it does not apply for the whole population but rather for the data under consideration. In an independent dataset and based on a variance components model, we could later demonstrate that gingival thickness may in fact be influenced by subject-related factors but to a very low extent [13]. In the original paper, cluster characteristics were defined at upper anterior teeth. Thus, the question may arise to what extent gingival width is actually related to its thickness on individual teeth.

When modeling the original data [15] by considering their hierarchical structure in this study the (true) relationship between gingival thickness and width may be demonstrated only when assuming both width and thickness to be random, i.e., varying at the subject level. Under the assumption of having studied a random sample and assuming normal distribution of the data, graphical representation of this relationship is displayed in Fig. 3, yielding a rather complex situation in this population of young adults with healthy periodontal conditions.

The final model indicates that men have, on average, 0.1 mm thicker facial gingiva. There was a slight overall negative influence of gingival width on thickness, but the respective variance was significant and no correlation between gingival width and the subjects' intercepts was ascertained. Periodontal probing depth was the main factor associated with gingival thickness with an increase of the former by 0.3 mm for each millimeter increase of the latter. But variance at the subject level was significant, too. There was a significant positive covariance between probing depth and subjects' intercepts (see Fig. 4) and a negative covariance between probing depth and gingival width. It is certainly possible to further extend the model by adding more tooth- or subject-related covariates, but for the sake of comprehensiveness of this tutorial, that is not outlined in this paper.

While measurements of gingival dimensions, thickness and width, are assumed to follow a normal distribution, possible extensions of multilevel modeling include generalized linear models [9], such as the logistic regression model for categorical responses, Poisson regression for counts, or, for more complex data structures, cross-classified models. While these issues are beyond the scope of this tutorial (see Leyland and Goldstein [11] for comprehensive treatment), it is paramount to note that the choice of the link function requires correct assumptions about the distribution of the data. Moreover, while variance at level 1 is commonly assumed to be constant, there are situations where the level 1 variance depends on covariates, i.e., presence of heteroscedasticity. A respective example has recently been published [18].

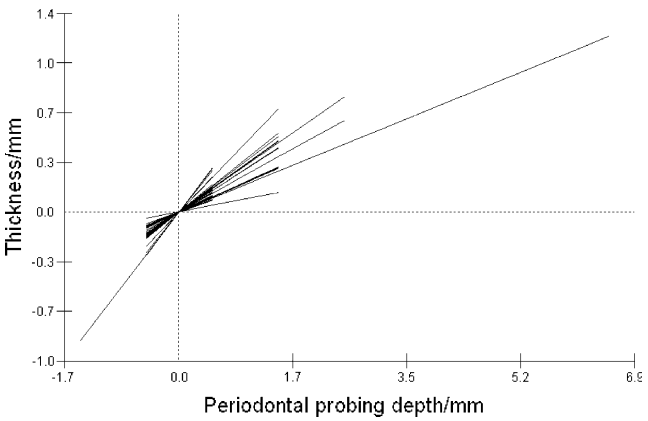

Fig. 4 Regression lines representing regressions of gingival thickness on probing depth (mm) according to the final random coefficient model. Note the fanning out of the subject prediction lines pointing to the positive intercept/slope covariance at the subject level

In Periodontology, a variance components model of probing parameters was first presented about 20 years ago [21]. Since then, multilevel modeling has been applied in a few early analytical [1, 2] as well as educational papers [6, 7] in Periodontology. Multilevel modeling has since then been applied in numerous periodontal studies mainly in order to attain correct estimates of site- and subject-related fixed effects in situations where more than one site was studied in a given subject. However, the type of model has rarely been specified or random effects reported. While multilevel modeling allows estimating fixed effects operating at different levels, in particular the study of random effects that have long been regarded as undesired nuisance ('error terms') may reveal new insights into the dynamics within the biological system of the periodontium under undisturbed or interventional conditions [8, 14, 17]. There might be problems with the determination of proper sample sizes, which have to be chosen at each level of the nesting hierarchy, as well as unbalanced samples where the numbers of lower level units vary among higher level units [20].

In the present tutorial, all calculations were done and graphs created using a special software program. In a recent extensive review [3], it was concluded that, without doubt, MLwiN may be the most comprehensive special program for conducting multilevel analyses. It cannot, however, be compared with the larger SAS package or STATA, which may allow the analysis of more complex models. In any way, the advent of rather easy-to-handle specialized software such as MLwiN has opened-up an entirely new field of questions regarding hierarchical dental data, which can be asked first, then maybe answered.

The present estimates were obtained using iterative generalized least squares (IGLS). In particular, in more complex models, these estimates may be used as starting values for Bayesian estimation via Markov chain Monte Carlo (MCMC) methods for further improving fixed and random parameters' estimates. In the example in this paper, estimates obtained by MCMC did not differ in any substantial way, thus strongly confirming the robustness of the IGLS estimation procedure.

As a first conclusion, the main advantage of multilevel modeling in periodontal research is the possibility of unbiased dealing with the true, hierarchical, structure of the data. While correct estimates of fixed effects are obtained, the random part of the model (variances and covariances) may provide new and deeper insights into phenomena and mechanisms at the level of interest, the periodontal site. New study designs should take into consideration the tremendous power of these techniques. Multilevel modeling should not only be applied in an attempt to simply obtain the correct (unbiased) estimates of fixed effects, for example, in a situation where in a few cases multiple observations in certain patients were recorded. In general, random effects should not be considered as nuisance as is the case in more commonly used marginal models [4]. They are not 'errors' but valuable information regarding data structure. Random effects should therefore properly be reported, since they may reveal new ideas about subject and tooth variation, correlations between covariates, and sometimes even problems with the chosen model. In particular, collaboration of professional statisticians and clinical researchers is urgently needed to significantly improve clinical research.

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Conflict of interest The author declares that he has no conflict of interest.

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