Multi-Level Statistical Models in Studies of Periodontal Diseases

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PERIODONTAL DATA TYPICALLY have a hierarchical structure, with sites grouped within individuals, and individuals grouped within communities. Also, the occasion may be regarded as another level since the acquired knowledge indicates that periodontal disease activity may vary over time. Conventional statistical tests are based on unilevel analysis of data. However, this approach to statistical analysis is often inconvenient in periodontal research because of the variation in the outcome variables between the various levels in the hierarchy. Lately there have been important developments in the statistical theory which have made available powerful statistical techniques for analyzing multilevel or hierarchical data. This report describes a new approach for analyzing periodontal data and uses an illustrative example to build a model which explains part of the variability in the response variable. The results from this analysis are then compared to results from an earlier report which uses unilevel methods and the findings discussed. The present multilevel approach has several advantages over unilevel methods, mainly due to its statistical validity and efficiency. Further, it permits the incorporation of explanatory variables measured at the site and the subject levels, and those which vary across the time points. Multilevel analyses have a promising potential and are expected to have a significant impact on periodontal research. *J Periodontol 1992; 63:690–695.*

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The main objectives of periodontal research have been the promotion of dental health through the prevention of periodontal destruction and the successful treatment of already established lesions. Investigations exploring the etiology and nature of periodontal diseases and clinical trials assessing the efficacy of different therapeutic modalities on periodontal health have been complicated by the distinctive characteristics of this disease. In this respect, epidemiological surveys show that chronic periodontitis is prevalent in industrialized as well as in developing societies, and among different age groups. However, results from these studies also suggest that only a minority of otherwise healthy individuals will ever experience rapidly progressing disease involving many teeth, whereas more often active disease process may affect few teeth at certain durations during the lifetime in other individuals.[1,2] Moreover, the rate and pattern of the disease process may differ from site to site within the same individual, and between different individuals.[3]

During the last few decades there have been important advances in our understanding of the pathogenesis of periodontal diseases.[4,5] However, we still lack key information about the principal triggering agent(s) and the factors which affect the development and progression of the disease. This lack of knowledge can simply be demonstrated by the difficulty of precisely predicting those individuals who are prone to acquire the disease, or its outcome in sites already afflicted. Hence, the bulk of results from research in this field indicates that periodontitis is a multifactorial disorder, and that the development of the disease may be attributed to an imbalance in the host-parasite interaction resulting in tissue destruction and loss of periodontal support.[6]

With the increase of knowledge there has also been an improvement in the design of studies of periodontal diseases. This together with the availability of computer hard- and software has resulted in a notable increase in the amount of collected data. Statistical analyses have gradually become important integral tools for interpreting the data in periodontal research. These, however, have evolved over the last decades from methods comprising simple contingency tables used initially to study frequency distributions to more sophisticated multivariate techniques. Concomitant with the refinement of these methods and their application in the field of periodontology we have become increasingly attentive to some fundamental statistical issues which have often been overlooked.[7,8]

Periodontitis may be regarded as a heterogeneous disease process.[9] This implies that the disease activity and the outcome from the disease may vary significantly among sites,

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subjects, and over time. Therefore, unilevel analyses which use either the site or the subject as their computational unit are often inappropriate.[7,8] Non-independence of different sites within the same subject cannot be ignored. Evidence endorsing this view involves the existence of systemic factors which may influence all sites of the subject. Such factors include, for instance, age, gender, diet, host resistance factors, oral hygiene habits, and so forth. On the other hand, there are other factors specific to the site, such as the diverse oral and periodontal flora, presence of local factors pertaining to plaque retention, the anatomical features of the site, etc.

In periodontal research the measurement of disease outcome is usually made at one level, namely the site. Nevertheless, we would often like to assess the effect of explanatory variables, some of which are measured at the subject level, while others are measured at the site level, and maybe some others that are measured longitudinally at different time points.

Furthermore, in periodontal research the analyzed data are frequently unbalanced. Thus, the number of examined sites may not always be equal in all patients. This may be due to missing teeth, unreadable sites for radiographic measurements, or lost samples during laboratory procedures. Also, some teeth may be lost during the course of study, and some subjects may not be available at all examinations. Similarly, the units might not have been distributed randomly among different treatment groups.

RECENT ADVANCES IN MULTILEVEL MODELLING

Lately there have been some important developments in the statistical theory concerning the analysis of hierarchical data. These developments have made available powerful statistical techniques for analyzing such data which we expect to have a significant impact on periodontal research. Periodontal data have a hierarchical structure in that the response variables are usually assessed on sites belonging to groups of teeth from patients (individuals) or, for example, repeated measures where measurement occasions are grouped or "clustered" within mouths. In this communication we intend to describe some of these developments and give an example of how these may be used in studies of periodontal diseases. These techniques, known as multilevel modelling, can be regarded as an extension of ordinary least-squares regression. We shall illustrate a basic multilevel model by introducing a 2-level analysis first then proceed with a 3-level model.

Illustrative Example

In a survey of 142 subjects comprising the employees of an industrial plant in Oslo, Norway, Albandar[10] studied predictors of periodontal disease progression as assessed on serial periapical radiographs taken at baseline, and at 2 and 6 years. Within these subjects, repeated measurements from 5,579 sites were made. The alveolar bone height at the interproximal areas of teeth was assessed to the nearest 0.1 mm by one examiner on the radiographs which were magnified 10 fold.

A number of potential site- and subject-level explanatory variables were then assessed. In the following text explanatory variables which are measured at the site-level are designated as x_m, and those measured at the subject-level are designated as z_l.

The site-level variables included the initial bone level at the site (x_1), presence of local factors pertaining to plaque retention which included calculus, overhanging amalgam and poor crown restorations (x_2), and presence of proximal fillings without overhanging margins (x_3). The subject-level explanatory variables were age in years (z_1), gender (z_2), the mean bone level at baseline (z_3), number of missing teeth (z_4), proportion of proximal sites with restorations (z_5) and those with plaque retention factors (z_6), and whether the subject had rheumatoid arthritis (z_7).

MULTILEVEL MODELS

A Simple 2-Level Model

To start with we consider one year's data and use the bone level at 6 years as the response variable. It can be seen that the clusters in the present material are the subjects. Suppose we want first to explore the relationship between the proximal bone height after 6 years (y_i for site i) and the initial values of bone height at the site (x_{1i}). Ordinary regression would estimate a single equation by pooling all 5,579 sites and expressing the response variable — the alveolar bone height after 6 years — as a linear function of the explanatory variable, initial bone height

$$y_i = \beta_0 + \beta_1 x_i + e_i \qquad (1)$$

where β_0 and β_1 are parameters to be estimated, and e_i is a random variable usually assumed to be normally distributed with mean 0 and constant variance. A multilevel model provides a generalization of equation (1). Changing the suffixes so that y_{ij} is the alveolar bone height at 6 years of the i - th site in the j - th subject, we explicitly incorporate subject membership into the model by writing

$$y_{ij} = \beta_{0j} + \beta_{1j} x_{ij} + e_{ij} \qquad (2)$$

where the suffixes j on the parameters now indicate that a different relationship applies to each subject. Now, it would, in principle, be possible to estimate 142 pairs of values of β_{0j} and β_{1j} using the standard extension of equation (1) known as analysis of covariance; perhaps assuming the β_{1j} to be equal. In practice, however, this would be cumbersome, and not feasible at all if the number of subjects was very large. The key technical advance in multilevel modelling is to assume that these parameters vary randomly across subjects (that is, at the subject level). Thus

$$\beta_{kj} = \beta_k + u_{kj} \qquad (k = 0,1) \qquad (3)$$

where β_k is the grand mean of the subject regression coef-

ficients β_{kj}, and the terms u_{kj} are random variables usually assumed to be normally distributed with mean 0 and variance that is independent of j. Generally, also, there will be a correlation between the random terms u_{0j} and u_{1j}, but random terms at different levels (e_{ij}, u_{kj}) are assumed to be independent of each other.

Extension of equations (2) and (3) to incorporate multiple -predictor variables, indexed by l, is straightforward

$$y_{ij} = \alpha + \beta_1 x_{1ij} + \ldots + \gamma_1 z_{1j} + \ldots + (u_j + e_{ij})$$

or

$$y_{ij} = \alpha + \Sigma_m \beta_m x_{mij} + \sum_{l=1}^{l=2} \gamma_l z_{lj} + u_j + e_{ij} \quad (4)$$

We also note that in this model the sites are assumed to be sampled at random within subjects, independently. That is the identification, i, of a site simply indexes a sample position. In reality, of course, we can identify the same sites for subjects, and this information can be used to improve the analysis. The appropriate multilevel model in this case, however, is complicated and we do not consider it further. An alternative formulation is to fit a separate "dummy" variable for each site. This would then yield a standard unilevel analysis of covariance model. But since the number of sites is usually large this alternative approach will be cumbersome.

A Basic 2-Level Model for Repeated Measures Longitudinal Data

If we take equation (2) and define x_{ij} as the age at the i - th measurement occasion on the j - th subject, and y_{ij} as above, the model now postulates a linear relationship between alveolar bone level and age, with the intercept and slope coefficients varying from subject to subject. Where a linear relationship is insufficient we can then add a quadratic, cubic, or higher order terms. In addition, we can incorporate further explanatory variables, such as the ones listed above, in order to study how the relationship varies across such groups. Consider the following model

$$y_{ij} = \beta_{0j}^{(1)} + \beta_{1j}^{(1)} x_{ij} + \beta_{2j} x_{ij}^2 + e_{ij} \quad \text{for males}$$

$$y_{ij} = \beta_{0j}^{(2)} + \beta_{1j}^{(2)} x_{ij} + \beta_{2j} x_{ij}^2 + e_{ij} \quad \text{for females}$$

$$\beta_{0j}^{(k)} = \beta_0^{(k)} + u_{0j}^{(k)}$$

$$\beta_{1j}^{(k)} = \beta_1^{(k)} + u_{1j}^{(k)}$$

$$\beta_{2j} = \beta_2 + u_{2j}$$

$$\text{var}\,(u_{0j}^{(k)}) = \sigma_0^{(k)^2}, \ \text{var}\,(u_{1j}^{(k)}) = \sigma_1^{(k)^2}, \ \text{var}\,(u_{2j}) = \sigma_2^2$$

$$k = 1,2$$

Note also that the e_{ij} with variance σ_e^2 represent the within-subject variation about each subject's own growth curve.

This model states that the intercept and slope coefficients for males and females differ on average and also have different variances (and covariances) across subjects. The level 1 variance, the quadratic coefficient and its variance are assumed to be the same for both sexes. If we fit such model we can go on to test whether the variances are different.

There are numerous elaborations possible for this basic model. We can consider nonlinear growth models,[11] and models where σ_e^2 is allowed to vary across time and groups.[12] We can also generalize to the multivariate case, for example where we wish to analyze several measurements simultaneously, perhaps at different sites. An example is given by Goldstein.[13]

A 3-Level Model

In the original data set of Albandar[10] the same sites were examined longitudinally at 3 successive occasions at 0, 2, and 6 years. In order to exploit the data, the present analysis makes it possible to define a 3-level model. The lowest level is the examination year (occasion), the next level is the site, and the third level is the subject. Thus, the total variation in the alveolar bone level during the study period may be decomposed into 3 components, the variance between examinations, that between sites within each subject, and that between different subjects. We can write such a model as

$$y_{kij} = \alpha + \Sigma_m \beta_m x_{mkij} + \sum_{l=1}^{l=3} \gamma_l z_{lkj} + v_j + u_{ij} + e_{kij} \quad (5)$$

where x and z are site-level and subject-level variables, and the subscripts k, i, and j refer to year, site, and subject, respectively. And the random variables distributions are specified as follows

$$v_j \sim N(0,\sigma_v^2),\ u_{ij} \sim N(0,\sigma_u^2),\ e_{kij} \sim N(0,\sigma_e^2)$$

RESULTS

The results from a 2-level variance analysis using the data on periodontal disease progression over 6 years are listed in Table 1. In this model level 1 is the site and level 2 is the subject and the response variable is the radiographic bone height at the end of the observation period. The initial bone height and the presence of local plaque retention factors showed significant effect ($P < 0.0001$) on the bone height at the site at 6 years, whereas the mean initial bone height for the subject showed only a moderate effect ($P < 0.07$). The rest of the explanatory variables had insignificant effects.

There was a significant between- as well as within-subject variability in the bone height. Furthermore, the effect of initial bone height on subsequent bone height seemed to vary significantly among sites. There was also a significant negative correlation between the within-subject variability of subsequent bone height and the variability caused by the initial bone height.

Table 2 shows the results when the same data were analyzed by means of a 3-level model. Here, level 1 is the occasion, level 2 is the site, and level 3 is the subject. The response variable is the radiographic bone height at the

Table 1. The Alveolar Bone Height at the Interproximal Areas of Teeth at the Last Examination (6 years) Related to Potential Explanatory Variables (Parameter estimates from a 2-level variance analysis are shown)

Explanatory Variables	Estimate	S.E.	Z Statistic
Fixed effects			
Constant (α)	0.675	0.155	4.4*
Initial bone height (β_1)	0.983	0.040	24.9*
Plaque retention factors (β_2)	0.324	0.070	4.6*
Proximal fillings (β_3)	−0.026	0.070	0.4
Age (γ_1)	−0.010	0.011	0.9
Gender (γ_2)	0.069	0.274	0.2
Mean bone height at baseline (γ_3)	0.229	0.153	1.5†
Number of missing teeth (γ_4)	0.011	0.043	0.2
% Restorations (γ_5)	0.005	0.008	0.6
% Plaque retention factors (γ_6)	0.004	0.008	0.5
Rheumatoid arthritis (γ_7)	0.100	0.532	0.2
Random effects			
Subject level:			
Constant (σ_u^2)	0.219	0.084	
Site level:			
Constant (σ_{e0}^2)	0.596	0.131	
Initial bone height (σ_{e1}^2)	0.109	0.032	
Constant/initial bone height (σ_{e10})	−0.176	0.066	

*$P<0.0001$.
†$P<0.07$.

Table 2. The Alveolar Bone Height at 2 and 6 Years Related to Potential Explanatory Variables. (Parameter estimates from a 3-level variance analysis are shown)

Explanatory Variables	Estimate	S.E.	Z Statistic
Fixed effects			
Constant (α)	−0.684	0.257	2.7*
Initial bone height (β_1)	0.927	0.065	14.4†
Plaque retention factors (β_2)	0.193	0.042	4.7†
Proximal fillings (β_3)	0.008	0.039	0.2
Age (γ_1)	0.026	0.002	11.0†
Gender (γ_2)	−0.043	0.258	0.2
Mean bone height at baseline (γ_3)	−0.037	0.154	0.2
Number of missing teeth (γ_4)	−0.008	0.038	0.2
% Restorations (γ_5)	−0.006	0.009	0.7
% Plaque retention factors (γ_6)	−0.0001	0.008	0.01
Rheumatoid arthritis (γ_7)	−0.062	0.510	0.1
Random effects			
Subject level:			
Constant (σ_u^2)	0.39	0.165	
Initial bone height (σ_{e1}^2)	0.028	0.017	
Constant/initial bone height (σ_{e10})	−0.084	0.043	
Site level:			
Constant (σ_{e0}^2)	0.405	0.069	
Initial bone height (σ_{e1}^2)	0.042	0.011	
Constant/initial bone height (σ_{e10})	−0.115	0.033	
Occasion level:			
Constant (σ_{e0}^2)	0.063	0.019	
Initial bone height (σ_{e1}^2)	0.043	0.008	
Constant/initial bone height (σ_{e10})	−0.037	0.016	

*$P<0.01$.
†$P<0.0001$.

serial examinations. The initial bone height, presence of local factors pertaining to plaque retention, and age had significant effects ($P < 0.0001$) on the subsequent bone height during the study period, whereas the rest of the explanatory variables had insignificant effects.

Analysis of the random effects revealed significant variability in the bone height and a correlation between initial bone height and subsequent bone height at each of levels 1, 2, and 3.

DISCUSSION

In the present communication we specified 2 basic multilevel models which we believe will be suited for analyzing repeated measures longitudinal studies of periodontal data. The first model is a 2-level type which decomposes the variance into site-specific and subject-specific components; whereas the second is a 3-level model and partitions the variance into occasion-specific, site-specific, and subject-specific components.

Analyzing the data set of the present study by the 2-level model showed that initial bone height at a site and the presence of local plaque retaining factors are significant factors for predicting the future height of alveolar bone at that site. Hence, when the same data were analyzed by the 3-level model it showed that these two factors and age are significant predictors of the subsequent bone height over 6 years.

Both models indicated that a significant component of the variance was still unexplained after all explanatory variables were entered in the models. The 2-level model suggested that the within-subject proportion of the unexplained variance was much larger than the between-subject variance. However, the 3-level model was more informative since the residual variance is modelled separately on the 3 levels. This model showed that the within-subject residual variance was almost as large as the between-subject variance, followed by the inter-examination variance. This implies that there are other important explanatory variables specific to all 3 levels which need to be identified and entered in the present models in order to construct a model with a better fit.

Inspecting the random part of the 3-level model suggested that approximately 42% of the total variance is a between-subject component, while 46% and 12% are within-subject and between-occasion components, respectively. In addition, the variance components at all 3 levels were significantly larger than zero. This indicates that a multilevel model for analyzing the present data is recommended. The negative covariances between initial bone height and the intercept at levels 1 and 2 imply that the within- and the between-subject variability in the subsequent alveolar bone height are smaller the higher the bone height at baseline. At level 1 the observed correlation means that the between-occasion variance is a complex (quadratic) function of initial bone height.

The present material has earlier been analyzed by unilevel statistical methods.[10,14] In accord with the present results, Albandar et al.[10,14] reported that initial periodontal status and presence of plaque retaining factors at a site, such as calculus and faulty dental restorations, together with the age of individual are significant predictors of the future

periodontal state at that site. However, in contrast to the present findings, the above cited reports concluded that also gender and rheumatoid arthritis are significant predictors of the response variable. Furthermore, the unilevel analyses suggested that if bone loss or plaque retaining factors were present at other sites within the same individual they may predict the response variable.[10] Hence, these 2 variables were insignificant when multilevel analyses were used (Tables 1 and 2).

These differences in the results stress the importance of using methods of analysis designed for hierarchical data. It is known that if a single level analysis is carried out when a multilevel structure is really present, significance tests are artificially inflated (that is, results seem more significant than they really are) and confidence intervals are too small. In addition, of course, it is impossible in a single level analysis to explore the hierarchical structure of the data. Yet, single level analysis may be used if exploring the data fails to reveal such hierarchical pattern.

It has been shown that periodontitis is a heterogeneous phenomenon.[9] The outcome from an active process may vary between sites of the same individual, between different individuals, and also longitudinally over time. The multilevel models account for the heterogeneity of periodontal response. Study populations need not be treated as homogeneous, and indeed the methods yield statistical tests of whether the groups that have been represented in the model are different from each other. In this way it is feasible to study the effect of, for instance, a novel therapeutic method by testing whether there really was variation among sites in the regression parameters β_{0j} and β_{1j}. Another example is studying the effect of several surgical techniques on periodontal healing. Here we can use the previous methods, or we may define the treatment groups as a new level, and then study the variation between the groups.

Multilevel analyses have other advantages over conventional unilevel methods of analysis. The study material may well be unbalanced. Thus, the number of observations need not be equal in all subjects. Moreover, the observations may be measured at different time points. So different sites may have different numbers of observations during the course of the study. Therefore, the method exploits the study data efficiently and the problem of missing observations is minimized. Further, multilevel analyses are highly flexible, and it is indeed possible to define complex models for random effects on all levels and model a broad array of covariance structures.

It may be noted here that for analyzing the present material the 3-level model was more useful than the 2-level model. First, it exploited the data in a better way than did the 2-level model. Owing to model specifications only data from first and last examinations (baseline and 6 years) were used in the first model, whereas data from all 3 examinations (baseline, 2, and 6 years) were used in the second model. Second, the 3-level model is more efficient since it enables the investigator to study the within-subject and the between-subject variability in the response variable and the variability between different examination episodes. In this way the variability over time can be studied by the introduction of explanatory variables that change longitudinally and which may be measured at the successive occasions. In the present example, age was the only explanatory variable whose value was assessed with the outcome variable at the serial examinations. Similarly, it is easy to assess other changeable predictors at various occasions concurrently with the outcome variable and enter them in the model. Such variables can, for instance, be the number of subgingival microorganisms, local mediators of inflammation, presence of bleeding, plaque control measures, etc.

Another approach to modelling repeated measurements is to model the response at all the occasions, not just the final two as here, and using the first occasion as a covariate. We would then wish to allow the age coefficient to vary across subjects (level 3) and possibly at level 2 also. This leads to the usual "growth curve" type analysis, described, for example, by Goldstein.[12] In the present case a more useful interpretation of the data is obtained by "conditioning" on the initial measurement.

When several measurement occasions are available a mixed model, with initial measurement as a covariate and subsequent measurements following a growth curve model is possible. When this is fitted to the present data, however, it becomes impossible to obtain estimates of the relevant parameters, since there are only 3 measurements.

As with all statistical models, it is important to verify that the assumptions are satisfied, at least approximately. In the present case the normality assumption was looked at by studying the distributions of residuals and looking at different models. Further illustrations of such procedures are given in Goldstein.[12]

There have been some recent reports of using statistical methods for dependent data in periodontal trials.[15-17] However, to our knowledge statistical methods which analyze the effect on periodontal disease progression of explanatory variables which may change longitudinally have to date not been reported in the dental literature. In the present report we described a statistical approach which utilizes recent developments in the statistical theory to analyze hierarchical data. The method is efficient and highly flexible and permits incorporating explanatory variables measured at the site and the subject levels and at different time points. These methods seem to possess a promising potential in studies of periodontal diseases.

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