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DIFFERENT METHODS OF ANALYZING MULTIPLE SAMPLES REPEATED MEASURES DATA

Garima Shukla[1] and Vinod Kumar[2]
Department of Mathematics, Statistics and Computer Science
G. B. Pant University of Agriculture and Technology, Pantnagar, India
E mail: [1]aim_garimashukla@yahoo.com ; [2]vinod_kumarbcb@yahoo.com
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Abstract

Three methods of analysis viz. Standard ANOVA, Repeated Measures ANOVA and Linear Mixed Model were used to analyze two sets of data due to Cole and Grizzle (1966) and Crowder and Hand (1990) by using SAS 9.2. Four groups of dogs (dataset 1) were found to differ significantly with respect to blood histamine levels under all the three methods of analysis except linear mixed approach under H-F covariance structure, whereas different groups of pigs (dataset 2) were found to differ insignificantly with respect to body weights under all the abovesaid methods. On the basis of the values of AIC, AICC and BIC unstructured covariance structure was found best.

Key Words: Repeated Measures, Sphericity, Mixed Model, Covariance Structure, Fit Statistics.

1. Introduction

Repeated measurements are observations of the same characteristics, which are made in several periods on the same experimental units. The typical repeated measures experiment in animal research consists of animals randomly assigned to treatments, groups and with responses measured on each animal over a sequence of time points. The objectives of repeated measures data analysis are to examine and compare response trends over time. This can involve comparisons of treatments at specific times, or averaged over time. It can also involve comparison of time within a treatment. The important feature of such experiments that requires special attention in data analysis is the correlation pattern among the responses on the same individual (animal) over time. Repeated measures designs have commonly used in animal science (Littell et al., 1996; Akbas et al., 2001). Some special methods of statistical analysis are needed for repeated measures data because of the covariance structure. Present study consists of different methods for the analysis of repeated measures which are standard ANOVA, repeated measures ANOVA and mixed model methodology. The repeated measures ANOVA approach is a useful alternative to the unstructured multivariate approach. A traditional approach to the analysis of repeated measurements is to perform a standard ANOVA, as if the observations are independent and to determine whether additional assumptions or modifications are required to make the analysis valid. This method is commonly called “Repeated Measures ANOVA”. The variance of the difference between the estimated means for any two different factor levels will be the same. This property is called sphericity. Repeated ANOVA is used safely when sphericity assumption (an assumption about the structure of the covariance matrix in a repeated measures design) is provided. If sphericity is violated, the *p* values need to be adjusted

upwards. The first step in each test is to estimate something called epsilon. The value of epsilon depends on the number of levels (k) on the repeated measure factor.

$$\text{Lower bound of epsilon} = 1/(k\text{-}1)$$

So, more are the levels on the repeated measures factor, the worse is the potential for violations of sphericity. In general MANOVA is less powerful than repeated measures ANOVA and therefore should probably be avoided. However, when sample sizes are reasonably large ($n > 10+k$) and epsilon is low (< 0.7), MANOVA may be more powerful and should probably be preferred. Mixed model methodology for repeated measures design is used straightforwardly for data with or without missing observations (Eyduran and Akbas, 2010). Mixed model methodology enables statisticians to specify different covariance structures in repeated measures designs where both random and fixed effects are included in the model. Therefore, mixed model methodology is potentially the most powerful tool. In mixed models we can use all of the data we have. If a score is missing, it is just missing and we don't have to be consistent about time. In these models, we do not have to assume sphericity or compound symmetry in the model. The aim of this study was to compare three different methods viz, Standard ANOVA, Repeated Measures ANOVA and Linear Mixed Model Approach with respect to the analysis of two sets of data.

2. Material and Methods

In the present study, two sets of data due to Cole and Grizzle (1966) and Crowder and Hand (1990) respectively published in Davis (2002) were used for the analysis and comparison. The dataset 1 consisted of information about 16 mongrel dogs which were treated with drug morphine and trimethaphan with histamine supply intact and depleted for each. So, there were four groups of dogs viz. morphine intact, morphine depleted, trimethaphan intact and trimethaphan depleted. Blood histamine levels were measuresd at three different time periods (min1, min3 and min5). The dataset 2 consisted of information about 15 guinea pigs which were treated with three treatments control, low dose and high dose. Body weights in grams of 15 guinea pigs were measured at six different time periods (week1, week3, week4, week5, week6 and week7). For analyzing both the datasets, we have used statistical software SAS using PROC GLM with REPEATED statement and PROC MIXED with REPEATED and RANDOM statements.

2.1 Standard ANOVA

The analysis of variance is a powerful statistical tool for tests of significance. Standard analysis of variance (ANOVA) i.e. One way ANOVA is used to test the homogeneity among groups. In this case linear mathematical model will be:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \qquad (2.1)$$

where y_{ij} are the independent observations.

μ is general mean effect given by :

$$\mu = \frac{\sum_{i=1}^{k} n_i \mu_i}{N}$$

α_i is the effect of the i[th] treatment.

ε_{ij} are i.i.d. N(0, σ^2),i.e., E(ε_{ij})=0 and V(ε_{ij})=0 $\forall$ I and j.

Hypotheses to be tested are

$H_0 = \alpha_i = 0$

H_1=At least two of the effects of treatment are different.

Finally the ANOVA is prepared and value of F is calculated under H_0.

Source of Variation	d.f.	S.S.	M.S.	F_{cal}
Treatment	k-1	SST	MS_T	$\frac{MS_T}{MS_E}$
Error	n-k	SSE	MS_E	
Total	n-1	TSS		

Table 1 : ANOVA Table for one-way classified data

We reject H_0 at α % level of significance if $F_{cal} > F_{(k-1,\ n-k,\ \alpha)}$, otherwise accept H_0.

2.2 Repeated Measures ANOVA model for Multiple Samples

Let us assume that repeated measurements at t time points are obtained from s groups of subjects. Let n_h denote the number of subjects in group h, and let $n = \sum_{h=1}^{s} n_h$.

Let y_{hij} denote the response at time j from the i[th] subject in group h for h=1,2,……..,s, i=1,2,……..n_h and j=1,2,……t. The simplest model for this case is

$$y_{hij} = \mu + \gamma_h + \tau + (\gamma\tau)_{hj} + \pi_{i(h)} + e_{hij} \qquad (2.2)$$

where, μ is the overall mean and γ_h is fixed effect of group h, with $\sum_{h=1}^{s} \gamma_h = 0$. In addition, τ_j is the fixed effect of time j, with $\sum_{j=1}^{t} \tau_j$ =0, and $(\gamma\tau)_{hj}$ is the fixed effect for the interaction of the h[th] group with j[th] time. The constraints on the interaction parameters are

$$\sum_{h=1}^{s}(\gamma\tau)_{hj} = \sum_{j=1}^{t}(\gamma\tau)_{hj} \quad = 0 \tag{2.3}$$

The parameters $\pi_{i(h)}$ are random effects for the i[th] subject in the h[th] group. The $\pi_{i(h)}$ are assumed to be independently normally distributed with mean zero and variance σ_{π}^{2}. Finally the e_{hij} parameters are independent random error terms, with $e_{hij} \sim N(0, \sigma_e^2)$.

In terms of the parameters of the general model given by (2.2), we have

- $\mu_{ij} = \mu + \gamma_h + \tau_j + (\gamma\tau)_{hj}$;
- $\pi_{ij} = \pi_{i(h)}$;

(1.3)

- $e_{ij} = e_{hij}$.

The sums of squares may be obtained on the basis of following decomposition of the deviations $y_{hij} - \bar{y}_{...}$ of each observation about the overall mean:

$$y_{hij} - \bar{y}_{...} = (\bar{y}_{h..} - \bar{y}_{...}) + (\bar{y}_{hi.} - \bar{y}_{h..}) + (\bar{y}_{..j} - \bar{y}_{...}) + (\bar{y}_{h.j} - \bar{y}_{h..} - \bar{y}_{..j} + \bar{y}_{...}) + (y_{hij} - \bar{y}_{h.j} - \bar{y}_{hi.} + \bar{y}_{h..}) \tag{2.4}$$

Where $\bar{y}_{...} = \dfrac{\sum_{h=1}^{s}\sum_{i=1}^{n_h}\sum_{j=1}^{t} y_{hij}}{nt}$ is the overall mean.

$\bar{y}_{h..} = \dfrac{\sum_{i=1}^{n_h}\sum_{j=1}^{t} y_{hij}}{n_h t}$ is the mean for group h.

$\bar{y}_{h.j} = \dfrac{\sum_{i=1}^{n_h} y_{hij}}{n_h}$ is the mean for group h at time j, and

$\bar{y}_{hi.} = \dfrac{\sum_{j=1}^{t} y_{hij}}{t}$ is the mean for the i[th] subject in group h.

The various sums of squares viz. sum of squares due to groups, subjects(group), time, group*time and residuals are then defined respectively as follows:

$$SS_G = t\sum_{h=1}^{s} n_h(\bar{y}_{h..} - \bar{y}_{...})^2$$

$$SS_{S(G)} = t\sum_{h=1}^{s}\sum_{i=1}^{n_h}(\bar{y}_{hi.} - \bar{y}_{h..})^2$$

$$SS_T = n\sum_{j=1}^{t}(\bar{y}_{..j} - \bar{y}_{...})^2$$

$$SS_{GT} = \sum_{h=1}^{s}\sum_{i=1}^{n_h}\sum_{j=1}^{t}(\bar{y}_{h.j} - \bar{y}_{h..} - \bar{y}_{..j} + \bar{y}_{...})^2$$

$$SS_R = \sum_{h=1}^{s}\sum_{i=1}^{n_h}\sum_{j=1}^{t}(y_{hij} - \bar{y}_{h.j} - \bar{y}_{hi.} + \bar{y}_{h..})^2 \qquad (2.5)$$

Note that SS_G, SS_T and SS_{GT} are equal to the sum of squares from a two factor ANOVA model (assuming that all n.t observations are independent) with effects for group, time and the group*time interaction. The residual sum of squares SS_R is due to the subject effect nested within the cross classification of group*time.

The F statistic for testing for differences among groups is given by

$$F = \frac{MS_G}{MS_{S(G)}} = \frac{SS_G/(s-1)}{SS_{s(G)}/(n-s)} \qquad (2.6)$$

with (s-1) and (n-s) d.f. This test requires the assumption that the within group covariance matrices are equal. In general, this assumption is required for all tests of between-subjects effects.

The F statistic for testing differences among time points is given by

$$F = \frac{MS_T}{MS_R} = \frac{SS_T/(t-1)}{SS_R/(n-s)(t-1)} \qquad (2.7)$$

with (t-1) and (n-s)(t-1) d.f. Similarily, the F statistic for testing significance of group*time interaction is given by

$$F = \frac{MS_{GT}}{MS_R} = \frac{SS_{GT}/(s-1)(t-1)}{SS_R/(n-s)(t-1)} \qquad (2.8)$$

with (s-1) (t-1) and (n-s)(t-1) d.f. Both of these tests require the assumption that the within-group covariance matrices are equal and that the sphericity condition is satisfied. In general, these assumptions are required for all tests of within-subjects effect. The ANOVA table is given Table 2.

Source of Variation	d.f.	S.S.	M.S.	F_{cal}	E(MS)
Group	(s-1)	SS_G	SS_G/(s-1)	F= $\frac{MS_G}{MS_{S(G)}}$	$\sigma_e^2 + t\sigma_\pi^2 + D_G$
Subjects (Group)	(n-s)	$SS_{S(G)}$	$SS_{S(G)}$/ (n-s)		$\sigma_e^2 + t\sigma_\pi^2$
Time	(t-1)	SS_T	SS_T/(t-1)	F= $\frac{MS_T}{MS_R}$	$\sigma_e^2 + D_T$
Group*Time	(s-1)(t-1)	SS_{GT}	SS_{GT}/(s-1)(t-1)	F= $\frac{MS_{GT}}{MS_R}$	$\sigma_e^2 + D_{GT}$
Residual	(n-s)(t-1)	SS_R	SS_R/(n-s)(t-1)		σ_e^2

Table 2 : Repeated measures ANOVA table for multiple samples

SAS Statements for standard ANOVA and repeated Measures ANOVA using PROC GLM and REPEATED

```
PROC GLM options;
CLASS variable;
MODEL options;
REPEATED independent variable/printe nom;
RUN;
```

2.3 The Mixed Model

Let $\mathbf{y}= (y_{i1.....}y_{it1})'$ be the t_1*1 vector of responses from subject i for i=(1……n). The general linear mixed model for longitudinal data is

$$\mathbf{y_i}=X_i\boldsymbol{\beta}+Z_i\boldsymbol{\gamma_i}+\boldsymbol{\varepsilon_i} \quad (2.9)$$

where X_i is a t_i*b model (design) matrix for subject i.

$\boldsymbol{\beta}$ is a b*1 vector of regression coefficients.

$\boldsymbol{\gamma_i}$ is a g*1 vector of random effects for subject i,

Z_i is a t_i*g design matrix for random effects and $\boldsymbol{\varepsilon_i}$ is a t_i*1 vector of within-subject errors.

$\boldsymbol{\gamma_i}$'s are assumed to be independent $N_g(\mathbf{0_g},B)$ and $\boldsymbol{\varepsilon_i}$'s are assumed to be independent $N_t(\mathbf{0_t},W_i)$. In addition the $\boldsymbol{\gamma_i}$ and $\boldsymbol{\varepsilon_i}$'s are assumed to be independent.

Thus the vectors $y_1,y_2,\ldots,y_n$ are independent $N_{ti}(X_i\boldsymbol{\beta},V_i)$ where

$$V_i=\mathbf{Z_i}B\mathbf{Z_i}'+W_i \quad (2.10)$$

The matrices X_i, Z_i and W_i are subject specific.

The model is very general because subjects can have varying number of observations and because the observation times can differ among subjects. The within subject covariance matrix W_i is assumed to depend on i only through its dimension t_i i.e. any unknown parameters in W_i do not depend on i. A wide variety of covariance structures for $\boldsymbol{\gamma_i}$ and $\boldsymbol{\varepsilon_i}$ can be considered. In particular, the MIXED procedure of SAS implements more than 20 distinct covariance structures.

For statistical analysis of abovesaid dataset 1 and dataset 2, we have used SAS software. PROC MIXED was used for the linear mixed model with repeated measurements and different covariance structures were fitted using RANDOM and REPEATED statements in PROC MIXED.

SAS Statements in the MIXED procedure

$$\mathbf{y}=X\boldsymbol{\beta}+Z\boldsymbol{\gamma}+\boldsymbol{\varepsilon}$$

where X specified in the MODEL statement for fixed effects.
Z specified in the RANDOM statement for random effects.
$\boldsymbol{\varepsilon}$ specified in the REPEATED statement for non-default structure.

For compound symmetry (by RANDOM statement)

```
PROC MIXED options;
CLASS variables;
MODEL dependent=fixed-effects/options;
RANDOM random effects/options;
RUN;
```

For compound symmetric structure with the REPEATED statement

```
PROC MIXED option;
CLASS variables;
MODEL dependent=fixed effects/options;
REPEATED fixed effects/option=random effects TYPE=CS R CORR;
RUN;
```

For unstructured covariance structure with REPEATED statement

```
PROC MIXED option;
CLASS variables;
MODEL dependent=fixed effects/options;
REPEATED fixed effects/option=random effects TYPE=UN R CORR;
RUN;
```

For heterogeneous compound symmetric covariance structure with REPEATED statement

```
PROC MIXED option;
CLASS variables;
MODEL dependent=fixed effects/options;
REPEATED fixed effects/option=random effects TYPE=CSH;
RUN;
```

For unstructured covariance structure with REPEATED statement

```
PROC MIXED option;
CLASS variables;
MODEL dependent=fixed effects/options;
REPEATED fixed effects/option=random effects TYPE=UN R CORR;
RUN;
```

For Huynh-Feldt structure with REPEATED statement

```
PROC MIXED option;
CLASS variables;
MODEL dependent=fixed effects/options;
REPEATED fixed effects/option=random effects TYPE=HF;
RUN;
```

For Toeplitz covariance structure with REPEATED statement

```
PROC MIXED option;
CLASS variables;
MODEL dependent=fixed effects/options;
REPEATED fixed effects/option=random effects TYPE=TOEP;
RUN;
```

3. Results

In datasets 1 and 2, we have carried out analysis of the data due to Cole and Grizzle (1966) and Crowder and Hand (1990). We have tested homogeneity among four groups of dogs with respect to blood histamine levels in dataset1 and homogeneity among three groups of 15 guinea pigs divided into groups on the basis of three treatments (Control, Low dose and High dose) with respect to their body weights in dataset 2 by using standard ANOVA techniques. We conclude for dataset 1 Pr>F corresponding to different time points are less than 0.05 (level of significance), hence different groups of dogs differ significantly with respect to blood histamine levels at all the time points and for dataset 2 Pr>F corresponding to different time points are greater than 0.05 (level of significance), hence it is concluded that different groups of pigs do not differ significantly with respect to body weights at all the time points.

Repeated measures ANOVA of dataset 1 as well as dataset 2 reveals that the sphericity condition is not satisfied in both the cases, which is a pre-requisite for applicability of F-test, although Mauchly's test has low power for small sample sizes. Therefore, it may be reasonable to use unstructured multivariate approach in both the cases.

Linear mixed model approach was applied under five different covariance structures viz. CS, Unstructured, CSH, H-F and Toeplitz. Four fit statistics -2 res log likelihood, AIC, AICC and BIC were calculated under every covariance structure using SAS. Tables 3 and 4 summarize the values of F statistic with the corresponding p-

values for dataset 1 and 2 respectively against group, time and group*time effects under different covariance structures.

Effect	CS		Unstructured		CSH		H-F		Toeplitz	
	F	p	F	p	F	P	F	P	F	P
Group	5.28	0.0149	5.28	0.0149	5.13	0.0164	2.63	0.0980	4.40	0.0262
Time	4.59	0.0206	2.79	0.1015	2.56	0.0980	4.59	0.0206	3.66	0.0410
Group *time	1.86	0.1295	2.19	0.1169	1.72	0.1604	1.86	0.1295	1.64	0.1804

Table 3: F-values along with the corresponding p-values under different covariance structures for dataset 1

Effect	CS		Unstructured		CSH		H-F		Toeplitz	
	F	p	F	p	F	P	F	P	F	P
Group	1.06	0.3782	1.06	0.3782	1.09	0.3672	0.39	0.6858	1.18	0.3409
Time	52.6	< 0.0001	59.4	< 0.0001	66.7	< 0.0001	52.6	< 0.0001	29.6	< 0.0001
Group *time	1.80	0.0801	3.83	0.0157	1.67	0.1079	1.80	0.0801	2.21	0.0294

Table 4: F-values along with the corresponding p-values under different covariance structures for dataset 2

The Tables 5 and 6 show the values of fit statistics (-2 log likelihood, AIC, AICC & BIC) for dataset 1 under different covariance structures.

Cov. Structure	-2 Res log likelihood	AIC	AICC	BIC	No. of cov. Parameters
Compound symmetry	46.0	50.0	50.3	51.5	2
Unstructured	-1.6	10.4	13.3	15.0	6
Heterogeneous Compound symmetry	8.4	16.4	17.6	19.4	4
Huynh-Feldt	14.1	22.1	23.4	25.2	4
Toeplitz	40.3	46.3	47.1	48.6	3

Table 5: The values of fit statistics for dataset 1

Cov. Structure	-2 Res log likelihood	AIC	AICC	BIC	No. of cov. parameters
Compound symmetry	720.0	724.0	724.5	725.5	2
Unstructured	661.4	703.4	721.8	718.2	21
Heterogeneous compound symmetry	704.4	718.3	720.1	723.3	7
Huynh-Feldt	696.3	710.3	712.0	715.2	7
Toeplitz	689.4	710.4	711.7	714.6	6

Table 6 : The values of fit statistics for dataset 2

Akaiki's Information Criterion (AIC), Burnham-Handerson Criterion (AICC) and Shwartz's Bayesian Information Criterion (BIC) were used to determine the most suitable covariance structure for mixed model approach in repeated measures design. The covariance structure for which the AIC, AICC and BIC values are minimum, is accepted as the best covariance structure. Hence, from this point of view, unstructured covariance structure may be considered best among these five structures for both the datasets.

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References

1. Akbas Y., Firat, M. Z. and Yakupoglu, C. (2001). Comparison of different models used in the analysis of repeated measurements in animal science and their SAS applications, Agricultural Information Technology Symposium, Sutcu Imam University, Agricultural Faculty, Kahramanmaras, p. 20-22, September 2001.
2. Cole, J. W. L. and Grizzle, J. E. (1966). Applications of multivariate analysis of variance to repeated measurements experiments, Biometrics, 22: p. 810-828.
3. Crowder, M.J. and Hand, D.J. (1990). Analysis of Repeated Measures, New York: Chapman and Hall.
4. Davis, C. S. (2002). Statistical Methods for the Analysis of Repeated Measurements, Springer-Verlag New York, Inc.
5. Eyduran, E. and Akbas, Y. (2010). Comparison of different covariance structures used for experimental design with repeated measurement, The Journal of Animal and Plant Sciences, 20(1), p. 44-51.
6. Keselman, H.J., Algina, J. and Kowalchuk, R. A. (2001). The analysis of repeated measures designs: A review, British Journal of Mathematical and Statistical Psychology, 54, p. 1-20.
7. Kieffer, Kevin M. (2002). On analyzing repeated measures designs with both univariate and multivariate methods: a primer with examples, Multiple Linear Regression Viewpoints, 28(1), p. 1-17.

8. Kowalchuk, R. K., Keselman, H. J., Algina, J. and Wolfinger, R. D. (2004). Measurements with mixed model adjusted F-tests, Educational and Psychological Measurement, 64 (2), p. 224-242.
9. Little, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., and Schabenberger, O. (2006). SAS for mixed models, Cary, NC SAS Institute Inc.
10. Tewari, P. and Shukla, G. (2011). Approach of linear mixed model in longitudinal data analysis using SAS, Journal of Reliability and Statistical Studies, 4 (1), p. 73-84.