Journal of Statistical and Econometric Methods, vol.5, no.3, 2016, 1-10
ISSN: 1792-6602 (print), 1792-6939 (online)
Scienpress Ltd, 2016

Multiple Comparison Testing for Experimental Chemotherapy Based on Multivariate Covariance Analysis

Adelodun, Olusegun Ayodele[1]

Abstract

This paper considers the practical problem in experimental chemotherapy where several treatment groups are compared with a control group in a multivariate covariance analysis and the observed data of mice are subjected to multiple comparison test. Mice infected with 50 cercariae of *Schistosoma mansoni* in unequal group sizes are challenged with some doses of *Zingiber officinale* extracts, *Cremophore* and *Praziquantel*. Data are available for the number of male and female parasites, weights of liver and intestine, and egg per gramme liver and intestine tissues. Proposed herein are multiple testing procedures such as, Tukey, Scheffe and Bonferroni based on two-sample statistics, each comparing an individual treatment with the control, for determining which treatments are more effective than the control. Newman-Keul's test corrobarates exactly with Bonferroni test's result, in the further analysis.

[1] Institute of Education, Obafemi Awolowo University, Ile-Ife. Nigeria.
E-mails: segunadelodun@yahoo.com; adelodun@oauife.edu.ng

Article Info: *Received* : June 27, 2016. *Revised* : July 23, 2016.
Published online : September 1, 2016.

Mathematics Subject Classification: 62H99

Keywords: Schistosoma mansoni; Multivariate Covariance Analysis; Experimental Chemotherapy

1 Introduction

Drug research often involves repeated multivariate outcomes on a small number of subjects for which there is interest in identifying outcomes that exhibit change in their levels over time as well as to characterize the nature of that change. Multiplicity is a challenging statistical issue in drug discovery, and a particular example is multivariate covariance study. Biomedical and drug research often involves the analysis of multiple outcomes recorded on repeated occasions [1].

2.1 Post-hoc Testing of ANOVAs

Multiple comparison procedures are commonly used in an analysis of variance after obtaining a significant omnibus test result, like the ANOVA F-test. The significant ANOVA result suggests rejecting the global null hypothesis, H_0 that the means are the same across the groups being compared. Multiple comparison procedures are then used to determine which means differ. In a one-way ANOVA involving K group means, there are $k(k - 1)/2$ pairwise comparisons.

A number of methods have been proposed for this problem, some of which are: Single-step procedures such as Tukey–Kramer method (Tukey's HSD) (1951) and Scheffe method (1953); Multi-step procedures based on Studentized range statistic such as Duncan's new multiple range test (1955), Nemenyi test, Bonferroni–Dunn test, Student Newman-Keuls, Dunnett's test (1955).

Choosing the most appropriate multiple-comparison procedure for your specific situation is not easy. Many tests are available, and they differ in a number of ways [6].

2.2 Multiple Comparison Procedures

We consider the three procedures for analysis models which permit the family confidence coefficient to be controlled. The three methods are Tukey, Scheffe and Bonferroni.

The Tukey method of multiple comparisons applies when:

i) All factor level sample sizes are equal, i.e. $n_j = n$

ii) The family of interest is the set of all pairwise comparisons of factor level mean, i.e. the family consists of estimates of all pairs $\mu_j - \mu_j'$

This method is inappropriate for the data because of unequal sample sizes.

The Scheffe method of multiple comparisons applies when:

i) Regardless whether or not the factor level sample sizes are equal

ii) When the family of statements is the set of estimates of all possible contrasts.

The Bonferroni method of multiple comparisons applies when:

i) The factor level sample sizes are equal or unequal, and for pairwise comparisons as well as for general contrasts

ii) The family of interest is the particular set of estimated contrasts specified by the researcher.

2.3 Difference Between Scheffe and Bonferroni Methods

Both Scheffe and Bonferroni methods of multiple comparisons are appropriate for the considered data. However, the latter is preferred because it gives narrower confidence limits [7].

If the family consists of *s* statements, the Bonferroni inequality implies that the confidence is at least $1-\alpha$ that all of the following confidence intervals are correct:

$$\hat{L}_i - Q\,s\left(\hat{L}_i\right) \le L_i \le \hat{L}_i + Q\,s\left(\hat{L}_i\right), \quad i = 1, 2, ..., s$$

where $Q = t\left(1-\alpha/2s; \mathrm{n_T} - r\right)$.

3 Results and Discussion

These will be shown clearly under the following:

3.1 Estimation of the Difference Between Two Factor-Level Means

The means of the factor level are given as

$$\mu_1 = 5.056,\ \mu_2 = 5.746,\ \mu_3 = 5.852,\ \mu_4 = 5.685,\ \mu_5 = 2.924,\ \mu_6 = 6.115,\ \mu_7 = 5.839$$

Table 1 reveals the complete paired difference table $\hat{\mu}_1 - \hat{\mu}_j$ for the point estimates.

Table 1: The point estimates of the paired differences for e1 data

$\hat{\mu}_1 - \hat{\mu}_j$	μ_1	μ_2	μ_3	μ_4	μ_5	μ_6	μ_7
μ_1	0	-.691	-.796	-.629	2.132	-1.055	-.783
μ_2		0	-.105	.062	2.822	-.369	-.093
μ_3			0	.167	2.927	-.264	.012
μ_4				0	2.760	-.431	-.155
μ_5					0	-3.191	-2.915
μ_6						0	.276
μ_7							0

The point estimates of the treatment effects vary from 2.924 for *E15* (*Praziquantel)* to 6.116 for *E16 (Cremophore).* This leads to paired differences varying from 2.132 *(*for *E11* and *E15)* to 3.191 *(*for *E16* and *E15)* for comparisons involving *praziquantel*.

3.2 Bonferroni Method of Multiple Comparisons Between Two Factor-Level Means

Using the procedure of estimation of contrast on the Bonferroni inequality, we consider

The contrast between group *E11* and *E12*.

Put $L_1 = \mu_1 - \mu_2,$ then $\hat{L}_1 = \frac{101.117}{20} - \frac{114.929}{20} = -.691$ where

$C_1 = 1;\ C_2 = -1$

Estimated variance, $s^2\left(\hat{L}_1\right) = .607\left(\frac{1}{20} + \frac{1}{20}\right) = .061;$ $\therefore\ s\left(\hat{L}_1\right) = .246$

By linear interpolation, $Q = t\left(.999, 106\right) = 3.289$

The confidence intervals are $-.691 - .810 \le L_1 \le -.691 + .810$

which gives $-1.501 \le L_1 \le .120$

Table 2 shows the upper diagonal matrix of the paired difference in the interval estimates.

We conclude that for those intervals that include the point 0 on Table 2, the estimated paired differences are not statistically significant. The significant cases are prominent for column 5 or row 5 involving *praziquantel.* The only other significant one is the comparison of 6 *(cremophore)* with 1 *(ginger chloroform).* Thus, the multiple comparison procedure permits us to infer, with a *95*% family confidence for the chain of conclusions, that *praziquantel* (5) leads to highest parasite mortality while *ginger chloroform* (1), *isoquinoline* (2), *chalcone 3* (3), *chalcone 4* (4), *cremophore* (6) and control (7) are substantially less effective and do not differ much among themselves. However, *ginger chloroform* (1) is the distant second best, *chalcone 4* (4) is the third best, *isoquinoline* (2) is the fourth best, followed by *chalcone 3* (3), then *cremophore* (6) and control group (7).

Table 2: Matrix for the interval estimates of the paired difference for e1 data

$\hat{\mu}_1 - \hat{\mu}_j$	μ_1	μ_2	μ_3	μ_4	μ_5	μ_6	μ_7
μ_1	$\begin{pmatrix}0\\0\end{pmatrix}$	$\begin{pmatrix}-1.501\\.120\end{pmatrix}$	$\begin{pmatrix}-1.868\\.276\end{pmatrix}$	$\begin{pmatrix}-1.565\\.307\end{pmatrix}$	$\begin{pmatrix}1.256\\3.007\end{pmatrix}$	$\begin{pmatrix}-1.892\\-.227\end{pmatrix}$	$\begin{pmatrix}-1.594\\.029\end{pmatrix}$
μ_2		$\begin{pmatrix}0\\0\end{pmatrix}$	$\begin{pmatrix}-1.177\\.967\end{pmatrix}$	$\begin{pmatrix}-.874\\.998\end{pmatrix}$	$\begin{pmatrix}1.947\\3.698\end{pmatrix}$	$\begin{pmatrix}-1.202\\.464\end{pmatrix}$	$\begin{pmatrix}-.903\\.718\end{pmatrix}$
μ_3			$\begin{pmatrix}0\\0\end{pmatrix}$	$\begin{pmatrix}-1.003\\1.337\end{pmatrix}$	$\begin{pmatrix}1.805\\4.049\end{pmatrix}$	$\begin{pmatrix}-1.353\\.825\end{pmatrix}$	$\begin{pmatrix}-1.060\\1.084\end{pmatrix}$
μ_4				$\begin{pmatrix}0\\0\end{pmatrix}$	$\begin{pmatrix}1.768\\3.753\end{pmatrix}$	$\begin{pmatrix}-1.386\\.524\end{pmatrix}$	$\begin{pmatrix}-1.091\\.781\end{pmatrix}$
μ_5					$\begin{pmatrix}0\\0\end{pmatrix}$	$\begin{pmatrix}-4.087\\-2.295\end{pmatrix}$	$\begin{pmatrix}-3.791\\-2.040\end{pmatrix}$
μ_6						$\begin{pmatrix}0\\0\end{pmatrix}$	$\begin{pmatrix}-.557\\1.109\end{pmatrix}$
μ_7							$\begin{pmatrix}0\\0\end{pmatrix}$

3.3 Further Investigation of Treatment Effects

To collaborate the results of Bonferroni method of multiple comparison, we adopt the balanced Newman-Keul's test to compare the means of the plant extracts in a pairwise manner. This essentially provides a logical procedure for ordering the plant extracts by their effect on the organs.

Since the mean treatments of the seven plant extracts differ, we compute the mean corresponding to each plant extracts and arrange in ascending order.

Put

$$A = \mu_5 = 2.924,\ \text{B} = \mu_1 = 5.056, \text{C} = \mu_4 = 5.685, \text{D} = \mu_2 = 5.746, \text{E} = \mu_7 = 5.839,$$

$$F = \mu_3 = 5.852, \text{G} = \mu_6 = 6.115.$$

We now prepare the table of difference thus:

Table 3: Newman-Keul's test table

k	*k*-1	*k*-2	*k*-3	*k*-4	*k*-5
7	6	5	4	3	2
3.191	1.059	.431	.369	.276	.264
	2.927	.796	.167	.105	.012
		2.915	.783	.155	.093
			2.822	.691	.062
				2.760	.629
					2.132

where *k* is the number of means in the experiment.

To prepare the list of the least significant ranges, we calculate

$$R_7 = q_{95}(7,105)\, s_{\bar{y}} = 4.24(.195) = .826$$

$$R_6 = q_{95}(6,105)\, s_{\bar{y}} = 4.10(.195) = .799$$

$$R_5 = q_{95}(5,105)\, s_{\bar{y}} = 3.92(.195) = .764$$

$$R_4 = q_{95}(4,105)\, s_{\bar{y}} = 3.68(.195) = .717$$

$$R_3 = q_{95}(3,105)\, s_{\bar{y}} = 3.36(.195) = .655$$

$$R_2 = q_{95}(2,105)\, s_{\bar{y}} = 2.80(.195) = .545$$

Referring back to Table III, we consider each diagonal element and compare with $R_{k's}$ in order from 7 to 2.

$G - A = 3.191 > .826 = R_7$ Significant

$F - A = 2.927 > .799 = R_6$ Significant

$E - A = 2.915 > .764 = R_5$ Significant

$D - A = 2.822 > .717 = R_4$ Significant

$C - A = 2.760 > .655 = R_3$ Significant

$B - A = 2.132 > .545 = R_2$ Significant

$G - B = 1.059 > .826 = R_7$ Significant

$F - B = .796 > .799 = R_6$ Not Significant

$E - B = .783 > .764 = R_5$ Not Significant

$D - B = .691 > .717 = R_4$ Not Significant

$C - B = .629 > .655 = R_3$ Not Significant

$G - C = .431 > .826 = R_7$ Not Significant

$F - C = .167 > .799 = R_6$ Not Significant

$E - C = .155 > .764 = R_5$ Not Significant

$D - C = .062 > .717 = R_4$ Not Significant

$G - D = .369 > .826 = R_7$ Not Significant

$F - D = .105 > .799 = R_6$ Not Significant

$E - D = .093 > .764 = R_5$ Not Significant

$G - E = .276 > .826 = R_7$ Not Significant

$F - E = .012 > .799 = R_6$ Not Significant

$G - F = .264 > .826 = R_7$ Not Significant

4 Conclusion

We conclude that on one hand *praziquantel* $(A = \mu_5)$ differs significantly from others *(ginger chloroform, isoquinoline, chalcone 3, chalcone 4, cremophore* and the control group*).* On the other hand, *ginger chloroform* is significantly different in effect from *cremophore.* This corrobarates exactly with our result from the Bonferroni test.

References

[1] T.A. DeRouen, P.P. Hujoel, and L.A. Mancl, Statistical Issues in Periodontal Research, *Journal of Dental Research,* **74**, (1995), 1731–1737.

[2] Y. Hochberg and A.C. Tamhane, Multiple Comparison Procedures, New York: Wiley, 1987.

[3] Z.A. Andrade, and P. Azevedo De Brito, Curative chemotherapy and resistance to reinfection in murine schistosomiasis, *The American Journal of Tropical Medicine and Hygiene*, **31**(1), (1982), 116-121.

[4] A.W. Cheever, The intensity of experimental schistosome infections modulates hepatic pathology, *The American Journal of Tropical Medicine and Hygiene,* **35**(1), (1986), 124-133.

[5] F.G. Austin and P.J. Frappaolo, Schistosoma mansoni: Chemoprophylaxis studies with antipenetration compounds, The American Journal of Tropical Medicine and Hygiene, **22**(6), (1973), 743-747.

[6] Y. Hochberg, A Sharper Bonferroni Procedure for Multiple Tests of Significance, *Biometrika,* **75**, (1988), 800–802.

[7] J. Neter and W. Wasserman, *Applied linear statistical models: regression, analysis of variance, and experimental designs*, R.D. Irwin, 1974.