Journal of Biometrics & Biostatistics
ISSN: 2155-6180
Naqvi and Bring, J Biom Biostat 2019, 10:3
Research Article Open Access

Dose Response Relationship from Four-Way Cross Over Trials

Itrat Batool Naqvi[1]* and Johan Bring[2]

[1]Department of Statistics, FC College, Lahore, Pakistan
[2]Department of Statistics, Dalarna University, Högskolegatan 2, 791 31 Falun, Sweden

Abstract

This paper is an application of randomization test for clinical, four ways cross over, trials. The response variable was the proportion of nights with hypoglycaemic episode i.e., lowering the concentration of sugar in the blood. The hypothetical data has been used to examine how persuasively the probability of an episode depends on doses. We also observed how the power, of nonparametric randomization test, was affected when data possessed missing observations with varying sample sizes at 5% level of significance. One consequence in case of missing observations was the reduction of the power of the test, due to the reduction in the actual sample size. As a remedial mean imputation approach used, dose wise and found better power results.

Keywords: Hypoglycaemia; Clinical trial; Statistics

Introduction

In the clinical trial GLU-004 each subject took four different doses. One was placebo, not an active substance of medication, and three different doses of active treatment. In the experiment every subject was observed for three consecutive nights on each of four doses. Here, four doses means four-way and repetition of each dose for three consecutive nights on same subject represents cross over. Generally we can say it is a four-way cross over clinical trial. The definition of clinical trial is given by Meinert in Encyclopaedia of Biostatistics [1].

"*A clinical trial is the action or process of putting something to a test or proof at the bedside of the sick. However, broadly it refers to any testing done on human beings for the sake of determining the value of a treatment for the sick or for preventing disease or sickness.*"

Hypoglycemia

"Insulin is normally produced in the pancreas and helps the body's cells absorb glucose from the blood. Hypoglycemia is a condition when the level of glucose (sugar) in the blood drops below a certain point, about 3 mmol/L. There are number of symptoms of hypoglycaemia, like, shaking, perspiration, a feeling of weakness, rapid heartbeat, hunger, agitation but the worst symptoms are temporary loss of consciousness and coma" [2].

"One of the side effects of the insulin/diabetes treatment is known as hypoglycaemia. In the daytime the subject himself or the people around him can recognize early warning symptoms and treat the subject to assure that the blood glucose level rise to an acceptable level. But it can be more dangerous at night times because it can occur without even the subject is wakening up. The definition of an episode is hypoglycaemia event" [3].

Hence, hypoglycemic episode means that the sugar level goes down below 3 mmol/L. In our study "0" means no episode. Showing that glucose level does not go below 3 mmol/L during the whole night and "1" means glucose level goes below 3 mmol/L, causing hypoglycaemia. The response variable dichotomous (binary) is the proportion of nights with hypoglycaemic episode. The researcher interested to examine how the proportion of episode is affected by the dose (Table 1).

For one-night episode can take 0, 1, value i.e., Bernoulli trial. While, for three consecutive nights, episode can take 0, 1, 2 or 3 value for dose (i) where, i=1, 2, 3, 4 i.e., outcome data is binomially distributed. For the randomization test used a non-parametric test statistic, Spearman rank correction used, in order to examine the association between dose and response. In clinical trials it is common to have missing data. A remedial for missing data is imputation in this paper missing observations imputed through mean imputation approach.

Subject	Day	Dose	Episode
1	1	1	1
1	2	1	1
1	3	1	1
1	1	2	1
1	2	2	1
1	3	2	0
1	1	3	0
1	2	3	1
1	3	3	1
1	1	4	0
1	2	4	0
1	3	4	1
…	…	…	…

Table 1: Hypothetical data for one subject.

Design of the Paper/Methodology

In clinical trials, the interest sometime lies in the effectiveness of a new drug as compared to placebo. In this study one dose is placebo and three different doses of active treatments. Our interest is if the probability of getting an episode=1 is less given the active doses as compared to placebo? The following situation, about dose response relationship, has been used in this paper.

Doses are effective in higher order of magnitude i.e., dose 4 is the most effective dose as compare to rest of three doses. Dose 3 is better than dose 2 and placebo while, dose 2 is better than placebo. So, appropriate alternative hypothesis can be:

***Corresponding author:** Itrat Batool Naqvi, Department of Statistics, FC college University, Lahore, Pakistan, Tel: 9242992315818; E-mail: itratnaqvi@fccollege.edu.pk

Received February 04, 2019; **Accepted** March 30, 2019; **Published** April 06, 2019

Citation: Naqvi IB, Bring J (2019) Dose Response Relationship from Four-Way Cross Over Trials. J Biom Biostat 10: 428.

H_1: P (E=1/di)<P (E=1/dj); where i>j{for all i,j=1,2,3,4}

The null hypothesis states that all doses are equal so no matter which dose is using the probability of episode=1 given dose (i) is equal to the probability of episode=1 given dose (j); where i ≠ j {for all i,j=1,2,3,4}

H_0: P (E=1/di)=P (E=1/dj); i ≠ j{for all i,j=1,2,3,4}

Hence, for testing the hypothesis of a dose response relationship this research used one sided left tailed test which is appropriate since the alternative hypothesis implies a negative correlation.

Episode can take 0, 1, 2, 3 value for dose (i) where i=1, 2, 3, 4 (placebo and three active doses). Let x be the number of observations for each dose (i). Then X~bin (3, pi); where pi is the probability of an episode which is determined by alternative hypothesis.

P (E=1|dose 1)=p_1.

P (E=1|dose 2)=p_2.

P (E=1|dose 3)=p_3.

P (E=1|dose4)=p_4.

Estimate p_i.

$$\widehat{p_i} = \frac{x_i}{m_i}$$

x_i=number of episode observed,

m_i=total number of nights observed.

However, many possibilities can also be assumed but the analysis in this paper will be based on above assumption. The hypothetical data has been generated according to the specific dose response relationships mentioned above. According to purpose strategy the purpose of this paper we do not want to estimate the parameter(s) rather to access dose response relationship, through the non-parametric randomization test statistic Spearman's rank correlation. The power of the test with varying sample sizes, along with fix alpha at 5% level of significance has been examine for full, missing and imputed data sets.

Computational strategy

For computational purpose the R Statistical Software package was used [4]. The computational strategy is described as follows:

Step 1: Generate hypothetical data (in term of "0" and "1") binomially distributed data.

Where,

- n=No. of observations for each of four doses,
- x=No. of episode observed,
- *p*=probability of an episode given by alternative hypothesis.

Step 2: Calculate test statistics, Spearman's rank correlation (say τ) for each subject between dose and response. If we have "N" subjects, then step 2 is giving us N "τ values".

Step 3: Take the average of N "τ values" and store this average. This is observe value for test statistic which is named as *obsertabar* ($\bar{\tau}_{obs}$) in this research.

Now drive the distribution of τ under the null hypothesis, H_0.

H_0: *P (E=1|di)=P (E=1|dj); i ¹ j, "i,j=1, 2, 3, 4.*

Step 4: Randomly rearrange the doses for each subject while keeping constant the response values.

Step 5: Repeat step 2 and step 3 and store the average correlation coefficient, which is named as *tabarrandom* ($\bar{\tau}_{random}$) in this research.

Step 6: In order to get the distribution of *tabarrandom* repeat step 4 and step 5 at least 1,000 times.

Step 7: Take 5th percentile in the distribution of *tabarrandom* not 95th because our alternative hypothesis implies a negative correction. So we take the 5th percentile in distribution of *tabarrandom* which is named as *pest*; this *pest* gave critical value and by comparing it with *obsertabar* decision has been taken either to accept or reject the null hypothesis.

Decision criteria

If the value of *obsertabar* is less than the value of *pest*, reject null hypothesis otherwise accept it. If result is significant then assign decision to "1" otherwise "0".

IF (*obsertabar<pest*) then decision=1 otherwise decision=0.

Step 8: By repeating above 7 steps 500 times we can get 500 decisions. The proportion of "1's" from these 500 decisions represents *power of the test.*

The above computational steps also clarify the application of randomization test for four way crossover clinical trials. It is important to mention here that what we can expect about *obs*.

Two possibilities can be discussed in this regard.

a. If H_0 is true

If null hypothesis is true then it can be expect $\bar{\tau}_{obs} = 0$ i.e., doesn't matter which treatment has been used the probability of an episode remained unchanged.

b. If H_1 is true

If alternative hypothesis is true then we can expect $\bar{\tau}_{obs} < 0$. The doses are effective in higher order of magnitude. From simulated data we observed the distribution of $\bar{\tau}_{obs}$ is slight skewed below "0," which comply with our expectation (Figure 1).

In clinical trial it is common to have missing data, usually due to subject dropouts. For missing observation, we supposed that some subjects feel uncomfortable or habitually move a lot during the night. Resultantly the reading needle for hypoglycaemic episode dislocated from its position and we did not get the episode for that particular night.

With missing data one consequence was observed; the diminished of the power of the test. The power of the test was diminished due to the reduction in the actual sample size. One possible remedial for missing data is imputation.

There are several imputation approaches e.g., mean imputation, probabilistic imputation, multiple imputations, etc. For analysis purpose mean imputation approach has been used.

One of simple approach is the mean imputation approach, which is easy to understand as well. Suppose we have missing data on dose 1 then take the sum of all episodes observed on dose 1 and divide it by the total number of nights observed on dose 1 for all "N" subjects. Define this as *A1*. Impute *A1* for each missing episode for dose 1. Similarly impute *A2, A3*, and *A4* if observations are missing for dose 2, dose 3, and dose 4, respectively. This is called a mean imputation approach.

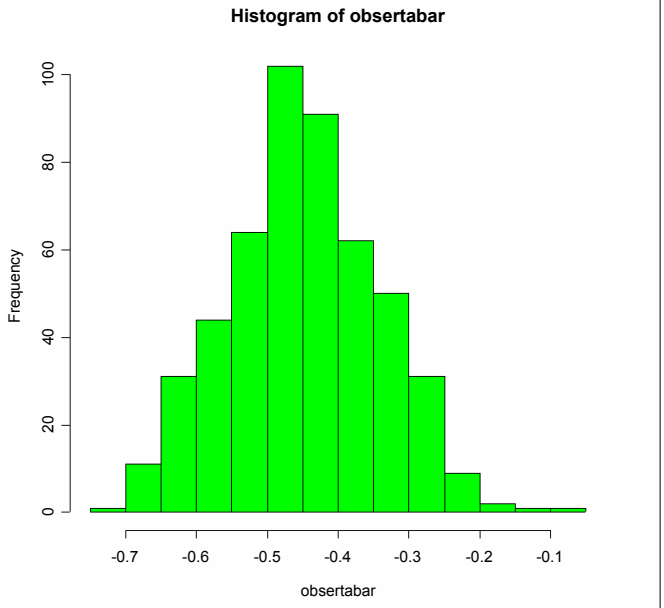

Figure 1: Histogram of obsertabar when H_1 is true.

Dose response relationship	Power of the test			Nature of data
	n=40	n=60	n=80	
Doses are effective in higher order of magnitude	86%	95%	99%	Full data
	75%	88%	95%	Missing (20%)
	82%	94%	97%	Imputed data

Table 2: Power analyses.

Power analysis

In the following table of this section power of the test discussed, when the dose response relationship determined by the alternative hypothesis under the proposed assumption (Table 2).

The power is increasing with increasing sample sizes in all three cases, but it diminished in missing data case as compare to full data. While imputed data showing improved power results as compare to missing data. Mean imputation is a reasonable approach for handling missing data problem. However, there is one key drawback of mean imputation approach i.e., too low variance of the statistic. In this connection we would like to quote the words of Dempster and Rubin [5]. "*The idea of imputation is both seductive and dangerous. It is seductive because it can lull the user into the pleasurable state of believing that the data are complete after all, and it is dangerous because it lumps together situations where the problem is sufficiently minor that it can be legitimately handled in this way and situations where standard estimators applied to the real data have substantial biases*" [6-9].

Results and Discussion

In this paper power of a four ways crossover trial based on hypothetical data (full, missing and imputed) with nonparametric randomization test, Spearman's rank correlation has been examined. Spearman's rank correlation measures the strength of the relationship between the dose (*di*) and proportion of nights with an episode (*pi*). It was obvious to observe with full observations data that, when the sample size increased power of the test improved. Because with increasing sample size, the true values get closer to their point estimate. The same phenomena of increasing power with increasing sample sizes were observed with missing and impute predicted cases. It was easily perceived that, why the power of the test was decreased in missing data case as compare to full data set with same sample size and same dose response relationship?

Because missing data reduced the actual sample size due to reduction in the original sample size the power was diminished as compare to full data. As a remedial of power loss mean imputation approach used and got improved power results [10-12].

Conclusion

However, to get precise estimates of dose-response relationship the data can be analysis with nonlinear mixed effect model. Then maybe it will more confidently report that doses are effective in higher order of magnitude in order to prevent hypoglycaemic episode.

References

1. Meinert CL (1998) Clinical trials, Overview. In: Encyclopedia of Biostatistics, p: 698.
2. Olsson U (2002) Generalized Linear Models. An applied approach, Studentlitteratur, Lund, Sweden, pp: 1-232.
3. Lan CW, Soon (2005) Hypoglycaemia (low blood sugar) in diabetes.
4. Good P (1994) Permutation Tests: A Practical Guide to Resampling Mehods for Testing Hypothesis, Springer Series in Statistics, Springer Nature, Switzerland.
5. Little RJA, Rubin DB (1987) Statistical Analysis with Missing Data. Journal of Educational Statistics 16: 150-155.
6. Edgingtion ES (1987) Randomization Tests. Statistics, textbooks and monographs (2ndedn), New York: Dekker, USA.
7. Armitage P, Colton T (2005) Encyclopedia of biostatistics (2ndedn), John Wiley & Sons, Chichester, England.
8. Daldaard P (2008) Statistics and Computing: Introductory statistics with R, Statistics and Computing, Springer Verlag, New York, USA, pp: 1-364.
9. Bland JM, Bland DG (1994) General practice: Statistical Notes one sided and two sided test of significance. British Medical Journal 309: 873-874.
10. Chaudhary SM, Kamal S (1996) Solved problems: Introduction to Statistical Theory Part 2, Ilmi Kitab Khana, Urdu Bazar.
11. Malin S (2004) A method of detecting episodes of hypo glycaemia when missing data occur and A simulation study for the comparison of the power of three multivariate procedures, Department of Mathematics, Uppsala University.
12. Grégoire G, Hamrouni Z (2002) Two Non-Parametric Tests For Change-Point Problems. IDOPT Project: It is a joint project of CNRS, INRIA, UJF and INPG. Journal of Nonparametric Statistics 14: 87-112.