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Test of dose-response relationship From a 4-way cross-over trial

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Abstract

This paper is an application of randomization test for clinical, four way cross over, trials. The response variable was the proportion of nights with hypoglycaemic episode i.e. lowering the concentration of sugar in the blood. I examined with hypothetical data how persuasively the probability of an episode depends on doses. I also observed how the power, of nonparametric randomization test, was affected by different dose-response relationship and varying sample sizes especially when data possessed missing observations. 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 I imputed missing observations, through mean imputation approach, dose wise and found better power results.

1. 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* (page 698) [3].

"Trial is from the Anglo-French trier, meaning to try. Broadly, it refers to the action or process of putting something to a test or proof. Clinical is from clinic, from the French clinique´and from the Greek klinike and refer to the practice of caring for the sick at the bedside. Hence, narrowly, 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."

The purpose of this paper is to check the power of a randomization test for repeated measures and also evaluate the effect of missing data. The analysis in this paper is based on hypothetical data. The response variable is the proportion of nights with hypoglycaemic episode, i.e. lowering the concentration of sugar in the blood, which will be clearer from following discussion.

- **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 3mmol/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." [9]

"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 nighttimes because it can occur without even the subject is wakening up. The definition of an episode is hypoglycaemia event." [10] Hence, hypoglycemic episode means that the sugar level goes down below 3mmol/L.

In our study "0" means no episode. Showing that glucose level does not go below 3mmol/L during the whole night and "1" means glucose level goes below 3mmol/L, causing hypoglycaemia. The response variable is the proportion of nights with hypoglycaemic

episode. Our interest is to examine how the proportion of episodes (response variable) is affected by the dose. We assumed independence between the nights.

Table1: Hypothetical data for one subject

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
…	…	…	…

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 I used a nonparametric test statistics, Spearman rank correction, 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 I have imputed missing observations through mean imputation approach (see section 5 and 6). Section 2 of this paper explains the aim of the paper, section 3 gives basic definitions, section 4 describes the statistical methods; while section 7 explores the size of the test, section 8 deals with power analysis and finally section 9 is a discussion.

2. Aim of the Paper

This paper aims to analyse dose response relationship through a randomization test, using a non-parametric test statistics. An appropriate measure of association can be Spearman's rank correlation, the non-parametric test statistic, which has been used in this paper.

In clinical trials, the interest sometime lies in the effectiveness of new drug as compare to placebo. In our study one dose is placebo and three different doses of active treatments. The following three situations, about dose response relationship, have 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.

- All of the active doses, 2, 3, 4, having same effect of preventing hypoglycaemic episode as compare to placebo.
- First effective dose has same effect as placebo, while dose 3 and dose 4 are more effective in higher order of magnitude.

However, more possibilities can also be assumed but the analysis in this paper will be based on above three assumptions. It should be mentioned here that I am not using real data. The hypothetical data has been generated according to the specific dose response relationships mentioned above. Our objective is not to estimate the parameter of the dose response relationship. I will analyse power of the test with varying sample sizes and different dose response relationships, with fix alpha at 5% level of significance. I also want to examine the size of the test. This paper also aims to study the power of the test when we have missing observations and how power of the test is affected by imputing new data.

In the light of above discussion the aim of this paper can be summarised as follow:

- Examine size of the test.
- Examine power of the test with different dose response relationship and varying sample sizes and observe which dose response relation give us better power results.
- What happens to the power of the test when data possess missing observations?
- Imputation for missing observations and finally analyse power of the test for full, missing, and imputed data sets.

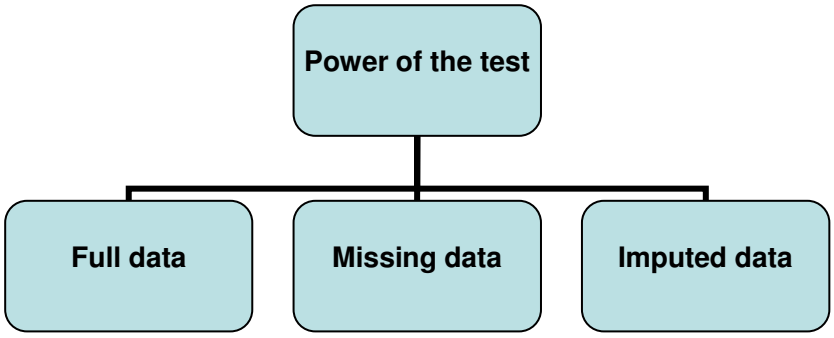


Figure1: Analysis of the power of the test for three data set

3. Basic Definitions

In the following sections some basic definitions will be explained.

3.1. Type I error

In hypothesis testing there is a risk of two kinds of error, which are known as type I and type II errors, denoted by α, β respectively. The probability of rejecting the null hypothesis when actually it is true, known as type I error also called alpha = α

$\alpha = P$ (reject $H_0 \mid H_0$ is true).

Alpha is also known as the "level of significance" for a hypothesis test. For analyses purpose I, used 5% level of significance in a one sided test. The choice of one-sided test instead of a two-sided test is motivated by the alternative hypothesis.

3.2. Type II error

The probability of accepting a false null hypothesis is known as beta type II error also called beta = β.
β = P (accept H_0| H_1 is true).

For example "In a court trial, the supposition of law is that the accused is innocent. This supposition of innocence may be regarded as a kind of null hypothesis H_0. Assume that accused is, in fact, guilty (i-e H_0 is false) but the finding of the judge says that accused is innocent; i-e the judge has accepted a false null hypothesis. By accepting a false hypothesis judge has committed a type II error." [7]

3.3. Power of the test

By definition power of the test is the probability of rejecting a false null hypothesis.
P (reject H_0 | H_1 is true) =1- P (accept H_0 | H_1 is true).

Power of the test = 1- β.

We can describe power phenomena with the help of simple illustration as follow, see figure 2.

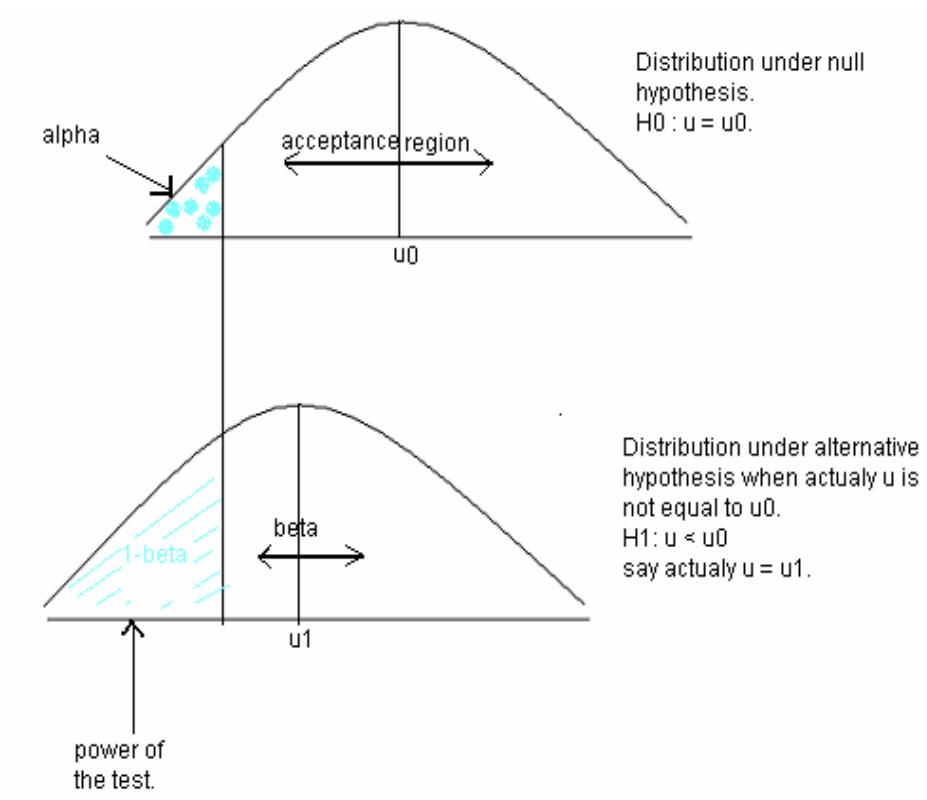


Figure2: Describing power phenomenon [7]

The above simple illustration describes that power of the test is inversely related with β. When probability of type II error decreases power of the test increases and vice versa. For analysis purpose I kept alpha constant at the 5% level and examined what happened to the power of the test with varying sample sizes and different dose response relationships.

3.4. Nonparametric test statistics

Many statistical methods are based on the assumption of normally distributed data. But in practice we have different type of response variables, for example response as a proportion, response as a rate, ordinal response etc. When the assumption of normality does not hold then one can think to model the data by GLIM. "Generalized linear models (GLIM) allow us to model the data using other distributions than normal." [8] But I didn't use this approach. However, I used a nonparametric test statistics, Spearman's rank correlation. Spearman's rank correlation is just sensitive by ordinal data. As I could rank my data so, I were able to use Spearman's rank correlation in order to investigate the association between dose and response.

"For large samples many nonparametric techniques can be viewed as the usual normal-theory-based procedures applied to ranks. The following table contains the names of some normal-theory-based procedures and their nonparametric counterparts." [11]

Table2: Normal theory based test with their nonparametric counterparts

Some Commonly Used Statistical Tests		
Normal theory based test.	**Corresponding nonparametric test.**	**Purpose of test.**
t test for independent samples.	Mann-Whitney U test; Wilcoxon rank-sum test.	Compares two independent samples.
Paired t test.	Wilcoxon matched pair's signed-rank test.	Examines a set of differences.
Pearson correlation coefficient.	Spearman rank correlation coefficient.	Assesses the linear association between two variables.
One way analysis of variance. (F test).	Kruskal-Wallis analysis of variance by ranks.	Compares three or more groups.
Two way analysis of variance.	Friedman Two way analysis of variance	Compares groups classified by two different factors.

3.5. Randomization test

In the words of Edgington [1] a randomization test is:

"A statistical test for which the significance of the experimental results is determined by permuting the data repeatedly to compute test statistics is called randomization test."

The randomization test can be described with the help of following steps.

- Step1.

  Define the hypotheses.

  H_0: $\mu = \mu_0$, $\mu \leq \mu_0$, $\mu \geq \mu_0$.

  H_1: $\mu \neq \mu_0$, $\mu > \mu_0$, $\mu < \mu_0$.

- Step2.

  Devise a test statistics.

- Step3.

  Calculate the test statistics, say $\tau_{observed}$, for given data set.

- Step4.

  Randomly rearranged the data (according to the null hypothesis)

- Step5.

  Calculate the test statistics for randomly rearranged data, say τ_{random}.

- Step6.

  Repeat step 4 and 5 e.g 1,000 times in order to get the distribution of $\tau_{random.}$

- Step7.

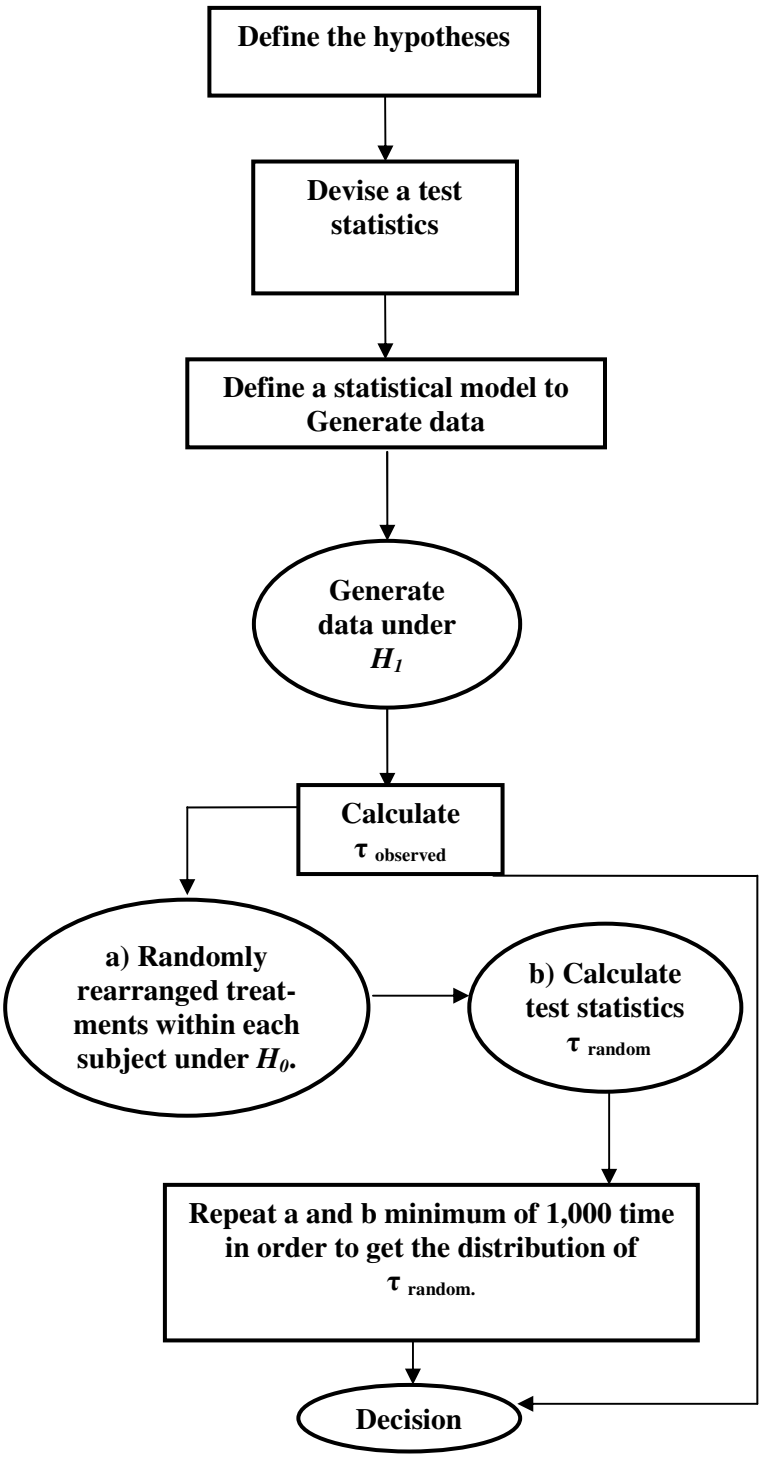
  Decision criteria

  For a two sided test if the value of $\tau_{observed}$ is smaller than the 2.5^{th} percentile or larger than the 97.5^{th} percentiles in the distribution of τ_{random} then one can reject the H_0 otherwise accept it.

  For one sided, upper tail, test if the value of $\tau_{observed}$ is greater than the 95th percentile of the distribution of τ_{random} then one can reject H_0 otherwise accept it.

  For one sided, lower tail, test if the value of $\tau_{observed}$ is less than the 5th percentile of the distribution of τ_{random} then one can reject H_0 otherwise accept it.

  The above steps can also be described with the help of following illustration (see figure3). It should be mentioned here that figure3 is describing the randomization test procedure specifically for this paper.



If the 5th percentile of the randomized τ is greater than observe τ (average of the "N" spearman rank correlations) then I can reject the null hypothesis otherwise accept it (see section 4.3 for detail discussion).

Figure3: Randomization test procedure specifically for this paper

4. Statistical Method

This section deals with statistical tools, hypotheses testing, statistical model, and computational strategy.

4.1. Hypotheses

Clinical trials often investigate the effectiveness of the treatments under study. Our interest is if the probability of getting an episode = 1 is less given the active doses? More than 90% clinical trials are analysed with two sided test, which dose not specify any direction, because we can't say anything about the new drug. While in a one side test the alternative hypothesis specifies a direction[1]. Bland J.M and Bland D.G. [6] said "this is sometime justified by saying that we are not interested in the possibility that the active dose is worse than no treatment."

However, we have four doses to be tested one is placebo and three active doses. The fourth dose is the highest active dose as compare to the rest of three but there can be the possibility of at least two active doses having the same effect. These different possibilities have already been discussed in aim of the paper. So, appropriate alternative hypothesis can be of less than equal to nature.

H_1: $P(E=1|d_i) \leq P(E=1|d_j)$; *where* $i>j$, *{and* $<$ *for some* $i \neq j$ *}* $\forall\, i,j = 1, 2, 3, 4$.

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 $i \neq j, \forall\, i,j = 1, 2, 3, 4$.

H_0: $P(E=1|di) = P(E=1|dj); i \neq j, \ \forall\, i,j = 1, 2, 3, 4$

H_1: $P(E=1|d_i) \leq P(E=1|d_j)$; *where* $i>j$, *{and* $<$ *for some* $i \neq j$ *}* $\forall\, i,j = 1, 2, 3, 4$.

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

4.2. Statistical model to be used

It has been discussed in section 1 that the outcome variable is dichotomous (binary) in nature, 0, 1. Where "0" represents glucose level does not go below 3mmol/L during the night and "1" represent glucose level goes below 3mmol/L and cause hypoglycaemia. At first glance it seems Bernoulli outcome data but we have repeated measures on same individual. Each subject takes four different doses and each dose has been observed for three consecutive nights, i.e. "several observations for each x-value." Hence, outcome data is binomially distributed.

[1] J.M Bland and D.G.Bland [6]. In a one sided test the alternative hypothesis does specify a direction; this is sometime justified by saying that we are not interested in the probability that the active dose is worse than no treatment. This possibility is still part of the test; it is part of the null hypothesis.

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.

$\hat{p}_i = x_i / m_i;\ (0 \leq \hat{p}_i \leq 1)$

x_i = the number of episode observed

m_i = total number of nights observed.

For every treatment group t; where t = 1, 2, 3, 4 the mean proportion of nights with hypoglycaemia episode can be estimated as:

$$\hat{\bar{p}}_t = \frac{\sum_{1}^{n_t} p_i}{n_t};$$

Here n_t is the total number of subjects in group t [10].

4.3. Computational strategy

For computational purpose the R statistical software package, [5] has been used. Readers are also encourage to read Thompson Laura A, (2005), S-plus (and R) Manual to Accompany Agresti's Categorical Data Analysis (2002) 2nd edition. The computational strategy is described as follow:

- Step1.

Generate hypothetical data (in term of "0" and "1") with rbinom (n, x, *p*) i.e. random 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.

(see appendix A)

- Step2.

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".

- Step3.

Take the average of N "τ values" and store this average. This is our observe value for test

statistic which is named as *observtabar* ($\bar{\tau}_{obs}$) in the program. Now we need to drive what we can expect if H_0 is true, i.e. the distribution of τ under the null hypothesis, H_0.

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

- Step4.

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

- Step5.

Repeat step2 and step3 and store the average correlation coefficient, which is named as *tabarrandom* ($\bar{\tau}_{random}$) in the program.

- Step6.

In order to get the distribution of *tabarrandom* repeat step4 and step5 atleast 1,000 times.

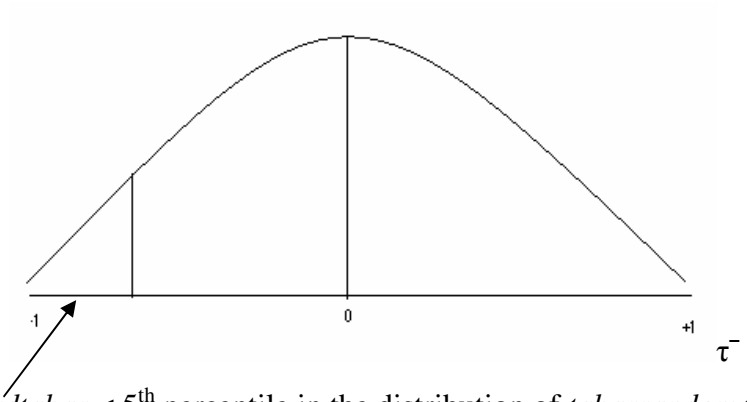
- Step7.

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* in the program. This *pest* is our critical value and by comparing it with *observtabar* we can get the decision either to accept or reject the null hypothesis.

<u>*Decision criteria*</u>

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.



(If *obserdtabar* < 5th percentile in the distribution of *tabarrandom* then we could reject H_0 otherwise accept it).

Figure4: Decision criteria for the randomization test

- 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 $\bar{\tau}_{obs}$. Two possibilities can be discussed in this regard.

a. If H_0 is true

If null hypothesis is true then we can 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.

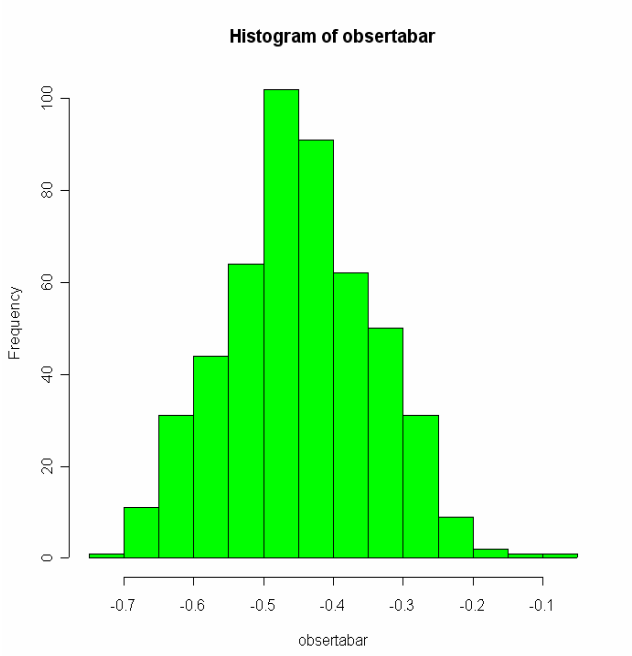


Figure5: Histogram of *observetabar* when H_1 is true

5. Missing Data

There are many types of missing data in practices e.g. missing at random, missing completely at random, etc. Little Roderick J.A &Rubin.B Donald [2] have classified the missing data mechanism as follow. [2].

"Let X and Y are two variables under study. Let X = age and Y = income. If the probability that the income is recorded varies according to the age of the respondent but dose not vary according to the income of respondent within an age group, then the data is MAR (missing at random). On the other hand if the probability that income is recorded is the same for all individuals, regardless of their age or income, then the data is MCAR (missing

completely at random)". However, for more discussion about missing data readers are encouraged to read Statistical Analysis with Missing Data. [2]

In clinical trial it is common to have missing data, usually due to subject dropouts. For missing observations I, also supposed that some subjects feel uncomfortable or habitually move alot 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. So, I can suppose it as MAR, missing at random, data because the missingness is related to subject. With missing data it was still possible to conduct the randomization test[2]. The simple way to tackle the missing data problem is to ignore the missing observations and work with full/complete data like listwise and pairwise deletion. It should be mentioned here that I did not delete the complete subject which possess some missing values while I, ignored the missing observations and calculate the correlation based on fewer observations.

There are some statistical software packages which by default posses the listwise deletion like SAS and SPSS and workout with complete cases, while some other packages like R and S-Plus can also tackle the missing data problems. When missing data was ignored 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. I took the remedial for missing data through impute prediction.

6. Imputation

There is several imputation approaches e.g. mean imputation, probabilistic imputation, multiple imputations, etc. For analysis purpose mean imputation approach has been used. One arbitrary approach dichotomy (binary) imputation has also discussed, but due to some drawbacks it did not seem an optimal imputation approach, which will be clearer from the coming discussion.

6.1. Dichotomy approach (an arbitrary approach)

This approach imputes either "1" or "0" for the missing observations in the data. This approach can be explained with the help of following table and forth coming paragraph.

Table3: Hypothetical missing data for three subjects

Subject	Day	Episode For dose1	Episode For dose 2	Episode For dose 3	Episode For dose 4
1	1	1	1	1	0
	2	NA	1	0	0

[2] Good Phillip, page 61, [4]. An unresolved problem in the analysis of clinical trials is the dropping out of subjects during the course of investigation. When such dropouts occur at random, we still may apply any of the standard permutation method.

	3	**0**	**NA**	**0**	**NA**
2	**1**	**0**	**0**	**1**	**1**
	2	**0**	**0**	**0**	**1**
	3	**1**	**1**	**NA**	**0**
3	**1**	**0**	**0**	**0**	**0**
	2	**1**	**1**	**NA**	**NA**
	3	**1**	**NA**	**1**	**0**
…	**…**	**…**	**…**	**…**	**…**

Here "NA" represents the missing observations in the data, "0" means no episode, and "1" means glucose level goes below 3mmol/L, causing hypoglycaemia. In column 5 and 6 the numbers of "0" are greater than the number of "1." So, I can impute "0" for the missing episodes for dose 3 and dose 4; while for dose 2, I can impute "1." But for dose 1 the numbers of "0" are equal to the number of "1." So, I can not impute "0" or "1" for missing episode for dose 1 i.e. the data still contain NA's. Hence, dichotomy approach is not an optimal imputation approach.

6.2. Mean approach

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 A_1. Impute A_1 for each missing episode for dose 1. Similarly impute A_2, A_3, and A_4 if observations are missing for dose 2, dose 3, and dose 4 respectively. This is called a mean imputation which has been used for analysis purpose in this paper.

7. Size of the Test

Before analysing the power of the test we examine the size of the test. If we suppose that the null hypothesis is true then the probability of mistakenly rejecting the null hypothesis is known as size of the test. This probability should be equal to α.

Table4: Size of the test.

Dose response relationship.	**Size of the Test.**		
	Total observations for four doses		
	n=80	**n=160**	**n=240**
All doses are equal.	**3%**	**4%**	**4%**

Where;

n = total number of observations for four doses.

For n=80 means total number of subjects, say N, equals to 20.

As each subject took four doses i.e. N=> 80/4=20. Same is true for the rest.

For n=160; N=40 and for n=240, N=60.

Dose response relation represents;

All doses are equal and it doesn't matter which dose has been used. The probability of an episode = 1 given $dose_i$ is equal to the probability of an episode = 1 given $dose_j$.

$P(E=1|dose_i) = P(E=1|dose_j)$; *where* $i \neq j$ *for all* $i,j = 1, 2, 3, 4$.

The results in table4 suggests that for n=80, size of the test = 0.03 i.e there is 3% chance of rejecting the null hypothesis when actually it is true. While for 60 subjects when the total numbers of observations for four doses are 240, a large sample size, the size of the test is 0.04. Here, I examined the large sample behaviour of the test statistics under the assumption that H_0 is true. I found as I were expecting that size of the test is close to or equal to the level of significance.

8. Power Analysis

In the following tables I discuss the power of the test under different assumptions. In the power analyse the dose response relationship were determined by the alternative hypothesis.

8.1. Full data power analyses

Power has been tested under different dose response relationship with varying sample sizes, while kept constant α at 5% level of significance.

Table5: Full data power analysis

Dose response relationship	Power of the test		
	Total no: of observations for four doses		
	n=40	n=60	n=80
Doses are effective in higher order of magnitude.	**86 %**	**95 %**	**99 %**
All active doses having same effect as compare to placebo.	**82 %**	**93 %**	**97 %**
Placebo and 1st active dose have same effect. While rest are more effective in higher order.	**81 %**	**92 %**	**98 %**

The power is increasing with increasing sample sizes in all three cases. But the first row of table5 is showing higher power results as compare to the rest of two cases. In first case I supposed that, doses are effective in higher order of magnitude i.e. dose four 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.Hence, from full data power results I can say that:

$P(E=1|dose_i) < P(E=1|dose_j) \ \forall \ i,j = 1, 2, 3, 4$ *and* $i>j$.

This dose response relationship gives better power results as compare to the others when I supposed that data generated under following relationship.

$P(E=1|dose_i) \leq P(E=1|dose_j)$; i>j{*and* < *for some* $i \neq j$ *}.*

8.2. Missing data power analyses

I assumed and test three different conditions about missing observations which will be clearer from following discussion.

Let for each subject "k" where k= 1,…, N

y = number of missing observations,

y ~ bin (n, π) where n=12 and

π = probability of missing observations.

- Let π = 0.1.

Then

E (y_k) =12*0.1=1.2; on the average one subject will give us one missing observation.

- Suppose π = 0.2.

Then

E (y_k) =12*0.2=2.4; on the average one subject will give us two missing observations.

- 10% and 20% missing

Another assumption about missing data is, let 15% of the subject, say group1, moves alot during the night and having higher missing probability as compare to rest of 85% of subjects, say group2.

Suppose π_1 = is the missing observations for group1 = 0.2

π_2 = is the missing observations for group2 = 0.1

On the average one subject of first group give 2.4 ≈ 2 missing observations while other group 1.2 ≈ 1 missing observation.

With these three assumptions I observed power of the test for missing data.

Table6: Missing data power analyses

Dose response relationship	**Power of the test**			**Probability of missing**
	Total no: of observations for four doses			
	n=40	**n=60**	**n=80**	
Doses are effective in higher order of magnitude	**80 %**	**92 %**	**97 %**	**10 %**
	75 %	**88 %**	**95 %**	**20 %**
	80 %	**92 %**	**97 %**	**10 % and 20 %**
All active doses having same effect as compare to placebo	**80 %**	**90 %**	**96 %**	**10 %**

I found reduced power as compare to full data because of wrong decisions, due to the reduced sample size. If we observe, in table6, the first and third rows are showing same results while the suppositions about missing data are different. Hence, for our analysis the result suggests, it dose not matter if I suppose that each subject have 10% probability of

missing observations or divide the subjects into two groups. However, if the probability of missing observations increased from 10% to 20%, examine first and second row of table6, then there is more reduction in the power of the test as compare to full data.

8.3. Comparison among full, missing and imputed data

As a remedial of power loss I imputed the missing observations through mean imputation approach and compare the three power results.

Table7: power analysis for three data sets.

Dose response relationship	Power of the Test			Nature of data
	Total no: of observations for four doses n=40	n=60	n=80	
Doses are effective in higher order of magnitude	86 %	95 %	99 %	Full data
	80 %	92 %	97 %	Missing 10 %
	75 %	88 %	95 %	Missing 20 %
	82 %	94 %	97 %	Imputed data

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 (especially in 20% missing). 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 I would like to quote the words of Dempster and Rubin (1983) from [2].

"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."

9. Discussion

I have examined power of a four ways crossover trial based on hypothetical data with nonparametric randomization test, Spearman's rank correlation. Spearman's rank correlation measures the strength of the relationship between the dose (d_i) and proportion of nights with an episode (p_i). It was obvious to observe with full observations data that when I increased the sample size, the power of the test improved. Because with increasing sample size the true values gets 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 I imputed the missing values by mean imputation approach and got improved power results. However, mean imputation has one key drawback of low variance (see section 8.3). From the power analysis we can say that doses are effective in higher order of magnitude. The probability of an episode observed "1" given dose (i) was less than the probability of an episode observed "1" given dose (j), where i>j for all of four doses.

But I did not observe any substantial reduction in the power results, which is clear from table5 results, when I tested that doses are not effective in higher order of magnitude. Hence, I can not deny that there is a possibility that atleast two doses having same intended status in order to prevent hypoglycaemic episode (see table5).

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

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Appendix A

Program Codes: for full observations

```
rm(list=ls())
f<-function(n="number of observations for each dose",x="no. of episode observed i.e 1",
p0="probability of placebo",p1="probability of first active dose",p2="probability of second
active dose",p3="probability of third active dose i.e dose 4")
{
decision<-numeric(500)
obsertabar<-numeric(500)
pest<-numeric(500)
for(M in 1:500)
 {
d0<-rbinom(n,x,p0)
d1<-rbinom(n,x,p1)
d2<-rbinom(n,x,p2)
d3<-rbinom(n,x,p3)
N<-n/3
 r1<-4*N
 response<-numeric(r1)
 j<-1
 k<-1
 for(i in 1:N)
 {
 response[seq(j,j+3,1)]<-c(mean(d0[seq(k,k+2,1)]),mean(d1[seq(k,k+2,1)]),
                           mean(d2[seq(k,k+2,1)]), mean(d3[seq(k,k+2,1)]))

 j<-j+4
 k<-k+3
 }
#Step2.
#Spearman rank correlation(τi) between dose and proportion of episode.
l<-1
 dose<-c(1, 2, 3, 4)
 response1<-numeric(4)
 estimate<-numeric(N)
 for(i in 1:N)
 {
 response1[1:4]<-response[seq(l,l+3,1)]
 r<-cor.test(dose,response1,method="spearman")
 names(r$estimate)<-NULL
 estimate[i]<-r$estimate
 l<-l+4
 }
#Step3.
#Average of  (τi) i-e obsertabar = τ-
obsertabar[M]<-mean(na.omit(estimate))
tabarrandom<-numeric(1000)
 for(L in 1:1000) # *step 5 in order to get distribution of randomized τ bar
{
 o<-1
 doserandom<-numeric(4)
 estimate1<-numeric(N)
 response2<-numeric(4)
 for(i in 1:N){
 doserandom<-sample(1:4,4) #Step 4.#random rearrangement of the treatment allocations for each
#subject in the favour of null hypothesis
 response2[1:4]<-response[seq(o,o+3,1)]
 r<-cor.test(doserandom,response2,method="spearman")
 names(r$estimate)<-NULL
 estimate1[i]<-r$estimate
 o<-o+4
 }
tabarrandom[L]<-mean(na.omit(estimate1))
 }
#hist(tabarrandom,br = 14, col = "green", border = "black") #distribution of randomized τ bar
pest[M]<-quantile(tabarrandom,0.05)#due to one sided alternative hypothesis ,5%
hist(obsertabar,br = 14, col = "green", border = "black")
 if(obsertabar[M]<pest[M]){decision[M]<-1}
 }
return(decision)
}
```

Description

- Step1.

First generating the random binomially distributed data (in terms of 0 and 1),

combing 12 values for each subject then taking the proportion of each 3 consecutive values (within 12 values) for each subject in this way we got 4 "response" values for each subject.

- Step2.

Calculating the test statistics, spearman rank correlation for each subject between dose and response (proportion of episode).If we suppose N=the no of subjects, then from step 2 we are getting N estimated (τ_i) values. Take the average for these N, (τ_i) values and store it. This is our observe/true value for the test statistics, called as obsertabar in the program.

- Step3.

In the favors of null hypothesis (i-e doses are independent).Randomly assigns the doses for each subject while keeping constant the response values.

- Step4.

Again calculating the spearman rank correlation for these randomly arranged doses with responses for each subject and taking the average of them, which is called "tabarrandom" in the program, and save it.

- Step5.

In order to get the distribution of tabarrandom repeating step 3 and 4, 1000 times.

- Step6.

As we are dealing with one sided test so take 5^{th} percentile of this distribution (tabarrandom) which is our critical value and by comparing it with "obsertabar" we will get one decision either to accept or reject our hypothesis.

if(obsertabar<pest) {decision<-1}

(5^{th} percentile) and not the 95th since our alternative hypothesis implies a negative correlation.

- Step7.

By repeating the whole above program 500 times we will get 500 decisions and the proportion of 1's from these 500 represent the power of the test.

Note.

Function command is generalizing this program i.e. user of it can give the arguments of his / her own choice e.g. we can give the function

#f (30, 1, 0.4, 0.3, 0.2, 0.1) and etc.

Program 2

Missing data program

In the clinical trial unfortunately it is usual to deal with the missing data due to subjects' dropout, one paradox assumption in our situation is the dislocation of the reading needle due to subject movement during the night and resultantly we didn't get the episode for that particular night.

Let suppose

y=the number of missing observations for each subject

N=total number of subjects then

y~b(n, pi) where pi is the probability of missing observation if we suppose

p = 0.1 then for each subject y~b(12,0.1)on the average each subject give us 1.2 missing observations and N subjects give us 1.2*N missing observations for one data set.

The rest of the procedure for this program is same as explained for full observations in first program.

Program 3

Impute prediction between subjects mean approach.

Observing the overall response of subjects for each of four doses and impute averages say A1, A2, A3, A4 of complete cases, ignoring NA, dose wise and impute missing observation with that averages if we got missing on any of four doses placebo, dose 2, dose 3, dose 4 respectively.

Note.

Rest of the procedure same like full and missing for coming programmes

Appendix B

B.1 Program Codes: for missing observations

```
rm(list=ls())
```

```
f<-function(n="number of observations for each dose",x="no. of episode observed i.e 1",
p0="probability of placebo",p1="probability of first active dose",p2="probability of second
active dose",p3="probability of dose 4"){
decision<-numeric(500)
obsertabar<-numeric(500)
pest<-numeric(500)
for(M in 1:500)
 {
d0<-rbinom(n,x,p0)
d1<-rbinom(n,x,p1)
d2<-rbinom(n,x,p2)
d3<-rbinom(n,x,p3)
N<-n/3
n1<-4*n #here 4*N is not valid because we are not taking proportion yet so,4*n=480#
response<-numeric(n1)
j<-1
k<-1
for(i in 1:N){
response[seq(j,j+11,1)]<-c(d0[seq(k,k+2,1)],
d1[seq(k,k+2,1)],d2[seq(k,k+2,1)],d3[seq(k,k+2,1)])
j<-j+12
k<-k+3
}
#missing observations#
#if y is probability of missing observation for each subject =0.1
#then average(y)=12*0.1=1.2 for one subject then for N subjects,
#1.2*N#
n2<-round(1.2*N)
vel<-c(1:n1)#vector of equal length of response#
rmo<-sample(vel,n2)#random missing draws from vel#
response[rmo]<-NA #assignment of missing to response#
l<-1
r1<-4*N #because now we are taking proportion of response so 4*N#
response1<-numeric(r1)
for(i in 1:r1)
{
response1[i]<-mean(response[seq(l,l+2,1)],na.rm=T)
l<-l+3
}
m<-1
response2<-numeric(4)
dose<-c(1, 2, 3, 4)
estimate<-numeric(N)
for(i in 1:N)
{
response2[1:4]<-response1[seq(m,m+3,1)]
r<-cor.test(dose,response2,method="spearman")
names(r$estimate)<-NULL
estimate[i]<-r$estimate
m<-m+4
}
obsertabar[M]<-mean(na.omit(estimate))
 tabarrandom<-numeric(1000)
 for(L in 1:1000) # *step 5 in order to get distribution of randomized τ bar
 {
 o<-1
 doserandom<-numeric(4)
 estimate1<-numeric(N)
 response3<-numeric(4)
 for(i in 1:N){
 doserandom<-sample(1:4,4)
 response3[1:4]<-response1[seq(o,o+3,1)]
 r<-cor.test(doserandom,response3,method="spearman")
 names(r$estimate)<-NULL
 estimate1[i]<-r$estimate
 o<-o+4
 }
 tabarrandom[L]<-mean(na.omit(estimate1))
 }
 #hist(tabarrandom,br = 14, col = "green", border = "black")
 pest[M]<-quantile(tabarrandom,0.05)#due to one sided alternative hypothesis ,5%
 hist(obsertabar,br = 14, col = "green", border = "black")
 if(obsertabar[M]<pest[M]){decision[M]<-1}
 }
return(decision)
}
```

B.2 Program Codes: for missing 10% and 20%

```
rm(list=ls())
```

```
f<-function(n="number of observations for each dose",x="no. of episode observed i.e 1",
p0="probability of placebo",p1="probability of first active dose",p2="probability of second
active dose",p3="probability of dose 4"){
decision<-numeric(500)
obsertabar<-numeric(500)
pest<-numeric(500)
for(M in 1:500)
 {
d0<-rbinom(n,x,p0)
d1<-rbinom(n,x,p1)
d2<-rbinom(n,x,p2)
d3<-rbinom(n,x,p3)
N<-n/3
n1<-4*n
response<-numeric(n1)
j<-1
k<-1
for(i in 1:N){
response[seq(j,j+11,1)]<-c(d0[seq(k,k+2,1)],d1[seq(k,k+2,1)],d2[seq(k,k+2,1)],
d3[seq(k,k+2,1)])
j<-j+12
k<-k+3
}
s1<-round(0.15*n1)#15% of total observations#
s3<-round(0.85*n1) #85% 0f the total observations#
s2<-s1+1
vel1<-c(1:s1)
vel2<-c(s2:n1)
pma<-round(0.15*N*2.4)#y(12,.20)=2.4,pma<- round(0.15*n1*0.20),20% missing
pmr<-round(0.85*N*1.2)#y(12,0.1)=1.2,pmr<- round(0.85*n1*0.1), 10% missing
rmo1<-sample(vel1,pma)
rmo2<-sample(vel2,pmr)
response[rmo1]<-NA
response[rmo2]<-NA
l<-1
r1<-4*N #because now we are taking proportion of response so 4*N#
response1<-numeric(r1)
for(i in 1:r1)
{
response1[i]<-mean(response[seq(l,l+2,1)],na.rm=T)
l<-l+3
}
m<-1
response2<-numeric(4)
dose<-c(1, 2, 3, 4)
estimate<-numeric(N)
for(i in 1:N)
{
response2[1:4]<-response1[seq(m,m+3,1)]
r<-cor.test(dose,response2,method="spearman")
names(r$estimate)<-NULL
estimate[i]<-r$estimate
m<-m+4
}
obsertabar[M]<-mean(na.omit(estimate))
 tabarrandom<-numeric(1000)
 for(L in 1:1000) # *step 5 in order to get the distribution of randomized τ bar
 {
 o<-1
 doserandom<-numeric(4)
 estimate1<-numeric(N)
 response3<-numeric(4)
 for(i in 1:N){
 doserandom<-sample(1:4,4)
 response3[1:4]<-response1[seq(o,o+3,1)]
 r<-cor.test(doserandom,response3,method="spearman")
 names(r$estimate)<-NULL
 estimate1[i]<-r$estimate
 o<-o+4
 }
 tabarrandom[L]<-mean(na.omit(estimate1))
 }
 #hist(tabarrandom,br = 14, col = "green", border = "black") #distribution of randomized τ bar
 pest[M]<-quantile(tabarrandom,0.05)#due to one sided alternative hypothesis ,5%
 hist(obsertabar,br = 14, col = "green", border = "black")
 if(obsertabar[M]<pest[M]){decision[M]<-1}
 }
return(decision)
}
```

Appendix C

Program Codes: for mean imputation (dose wise)

```
rm(list=ls())
f<-function(n="number of observations for each dose",x="no. of episode observed i.e 1",
p0="probability of placebo",p1="probability of first active dose",p2="probability of second
active dose",p3="probability of dose 4"){
decision<-numeric(500)
obsertabar<-numeric(500)
pest<-numeric(500)
for(M in 1:500)
 {
d0<-rbinom(n,x,p0)
d1<-rbinom(n,x,p1)
d2<-rbinom(n,x,p2)
d3<-rbinom(n,x,p3)
N<-n/3
n1<-4*n
response<-c(d0,d1,d2,d3)
n2<-round(1.2*N)
vel<-c(1:n1)
rmo<-sample(vel,n2)
response[rmo]<-NA
m<-function(i)
{
d<-trunc((i-1)/n)*n+1
d5<-(n-1)
s<-mean(response[seq(d,d+d5,1)],na.rm=T)
}
for(i in rmo){
con<-m(i)
response[i]<-con
}
j<-1
k<-1
l<-(n+1)
o<-(2*n)+1
p<-(3*n)+1
r1<-4*N
response1<-numeric(r1)
for(i in 1:N)
{
response1[seq(j,j+3,1)]<-c(mean(response[seq(k,k+2,1)]),
             mean(response[seq(l,l+2,1)]),mean(response[seq(o,o+2,1)]),
                          mean(response[seq(p,p+2,1)]))
j<-j+4
k<-k+3
l<-l+3
o<-o+3
p<-p+3
}
q<-1
 dose<-c(1, 2, 3, 4)
response2<-numeric(4)
estimate<-numeric(N)
for(i in 1:N)
{
             response2[1:4]<-response1[seq(q,q+3,1)]
r<-cor.test(dose,response2,method="spearman")
names(r$estimate)<-NULL
estimate[i]<-r$estimate
q<-q+4
}
obsertabar[M]<-mean(na.omit(estimate))
tabarrandom<-numeric(1000)
for(L in 1:1000) # *step 5 in order to get distribution of randomized τ bar
{
t<-1
doserandom<-numeric(4)
estimate1<-numeric(N)
response3<-numeric(4)
for(i in 1:N)
{
doserandom<-sample(1:4,4)
response3[1:4]<-response1[seq(t,t+3,1)]
r<-cor.test(doserandom,response3,method="spearman")
names(r$estimate)<-NULL
estimate1[i]<-r$estimate
t<-t+4
}
```

```
tabarrandom[L]<-mean(na.omit(estimate1))
}
#hist(tabarrandom,br = 14, col = "green", border = "black")
 pest[M]<-quantile(tabarrandom,0.05)#due to one sided alternative hypothesis ,5%
 hist(obsertabar,br = 14, col = "green", border = "black")
 if(obsertabar[M]<pest[M]){decision[M]<-1}
 }
return(decision)
}
```

Appendix D

Program Codes: for dichotomy approach

Codes for dichotomy approach within and between subjects.

Dichotomy approach (within subjects)

```
rm(list=ls())
f<-function(n="number of observations for each dose",x="no. of episode observed i.e 1",
p0="probability of placebo",p1="probability of first active dose",p2="probability of second
active dose",p3="probability of dose 4"){
decision<-numeric(500)
obsertabar<-numeric(500)
pest<-numeric(500)
for(M in 1:500)
 {
d0<-rbinom(n,x,p0)
d1<-rbinom(n,x,p1)
d2<-rbinom(n,x,p2)
d3<-rbinom(n,x,p3)
 N<-n/3
#N=total number of subjects
#n=no of response for each dose
n1<-4*n #here 4*N is not valid because we are not taking proportion yet #
response<-numeric(n1)
j<-1
k<-1
for(i in 1:N){
response[seq(j,j+11,1)]<-
c(d0[seq(k,k+2,1)],d1[seq(k,k+2,1)],d2[seq(k,k+2,1)],d3[seq(k,k+2,1)])
j<-j+12
k<-k+3
}
                            #missing observation#
#if y is probability of missing observation for each subject =0.1
#then average(y)=12*0.1=1.2 for one subject then for N subjects,
 #1.2*N#
n2<-round(1.2*N)
vel<-c(1:n1)#vector of equal length of response#
rmo<-sample(vel,n2)#random missing draws from vel#
response[rmo]<-NA
#in the following function we are imputing the missing observations after #considering the no:
#of 0's and 1's within subjects for each of four #doses#
cond<-function(i){
d<-trunc((i-1)/12)*12+1
s<-sum (response [seq(d,d+11,1)],na.rm=T)
b<-table (response [seq(d,d+11,1)])
names (b)<-NULL
if(s>sum(b)-s){return(1)}else
if (s<sum(b)-s){return(0)}else
{(return (NA))}}
for(i in rmo){
con<-cond(i)
response [i]<-con
  }
l<-1
r1<-4*N #because now we are taking proportion of response so 4*N#
response1<-numeric(r1)
for(i in 1:r1)
{
response1[i]<-mean(response[seq(l,l+2,1)],na.rm=T)
l<-l+3
}
m<-1
response2<-numeric(4)
dose<-c(1,2,3,4)
estimate<-numeric(N)
for(i in 1:N)
{
```

```
response2[1:4]<-response1[seq(m,m+3,1)]
r<-cor.test(dose,response2,method="spearman")
names(r$estimate)<-NULL
estimate[i]<-r$estimate
m<-m+4
}
obsertabar[M]<-mean(na.omit(estimate))
 tabarrandom<-numeric(1000)
 for(L in 1:1000) # *step 5 in order to get the distribution of randomized τ bar
 {
 o<-1
 doserandom<-numeric(4)
 estimate1<-numeric(N)
 response3<-numeric(4)
 for(i in 1:N){
 doserandom<-sample(1:4,4)
 response3[1:4]<-response1[seq(o,o+3,1)]
 r<-cor.test(doserandom,response3,method="spearman")
 names(r$estimate)<-NULL
 estimate1[i]<-r$estimate
 o<-o+4
 }
 tabarrandom[L]<-mean(na.omit(estimate1))
 }
#hist(tabarrandom,br = 14, col = "green", border = "black")
 pest[M]<-quantile(tabarrandom,0.05)#due to one sided alternative hypothesis ,5%
 hist(obsertabar,br = 14, col = "green", border = "black")
 if(obsertabar[M]<pest[M]){decision[M]<-1}
 }
return(decision)
}
```

.

Dichotomy approach (between subjects)

```
rm(list=ls())
f<-function(n="number of observations for each dose",x="no. of episode observed i.e 1",
p0="probability of placebo",p1="probability of first active dose",p2="probability of second
active dose",p3="probability of dose 4"){
decision<-numeric(500)
obsertabar<-numeric(500)
pest<-numeric(500)
for(M in 1:500)
 {
d0<-rbinom(n,x,p0)
d1<-rbinom(n,x,p1)
d2<-rbinom(n,x,p2)
d3<-rbinom(n,x,p3)
N<-n/3
n1<-4*n
response<-c(d0,d1,d2,d3)
n2<-round(1.2*N)
vel<-c(1:n1)
rmo<-sample(vel,n2)
response[rmo]<-NA
#in the following function we are imputing the missing observations after #considering the no:
#of 0's and 1's between subjects
cond<-function(i){
d<-trunc((i-1)/n)*n+1
d5<-(n-1)
s<-sum(response[seq(d,d+d5,1)],na.rm=T)
b<-table(response[seq(d,d+d5,1)])
names(b)<-NULL
if(s>sum(b)-s){return(1)}else
if (s<sum(b)-s){return(0)}else
{(return(NA))}}
for(i in rmo){
con<-cond(i)
response[i]<-con
  }
j<-1
k<-1
l<-(n+1)
o<-(2*n)+1
p<-(3*n)+1
r1<-4*N
response1<-numeric(r1)
for(i in 1:N)
{
response1[seq(j,j+3,1)]<-c(mean(response[seq(k,k+2,1)]),
              mean(response[seq(l,l+2,1)]),mean(response[seq(o,o+2,1)]),
                           mean(response[seq(p,p+2,1)]))
```

```
j<-j+4
k<-k+3
l<-l+3
o<-o+3
p<-p+3
}
q<-1
dose<-c(1, 2, 3, 4)
response2<-numeric(4)
estimate<-numeric(N)
for(i in 1:N)
{
             response2[1:4]<-response1[seq(q,q+3,1)]
r<-cor.test(dose,response2,method="spearman")
names(r$estimate)<-NULL
estimate[i]<-r$estimate
q<-q+4
}
obsertabar[M]<-mean(na.omit(estimate))
tabarrandom<-numeric(1000)
for(L in 1:1000) # *step 5 in order to get distribution of randomized τ bar
{
t<-1
doserandom<-numeric(4)
estimate1<-numeric(N)
response3<-numeric(4)
for(i in 1:N)
{
doserandom<-sample(1:4,4)
response3[1:4]<-response1[seq(t,t+3,1)]
r<-cor.test(doserandom,response3,method="spearman")
names(r$estimate)<-NULL
estimate1[i]<-r$estimate
t<-t+4
}
tabarrandom[L]<-mean(na.omit(estimate1))
}
#hist(tabarrandom,br = 14, col = "green", border = "black") #distribution of "τ"
 pest[M]<-quantile(tabarrandom,0.05)#due to one sided alternative hypothesis ,5%
 hist(obsertabar,br = 14, col = "green", border = "black")
 if(obsertabar[M]<pest[M]){decision[M]<-1}
 }
return(decision)
}
```