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Research Article

Application of Cluster Sampling Techniques for Analyzing the Effect of Dhatri Lauha – An Ayurvedic Formulation in Iron Deficiency Anaemia

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Abstract

The present work consists of the data of multicentric clinical study conducted on Iron Deficiency Anaemia (Pandu Roga) with an Ayurvedic formulation, Dhatri Lauha. The objective of current study was to assess the clinical safety and efficacy of Dhatri Lauha in the patients of Iron Deficiency Anaemia through measurable objective parameters. This multicentric study was conducted in 12 peripheral research institutes of Central Council for Research in Ayurvedic Sciences, Department of AYUSH, Ministry of Health & Family Welfare, Government of India, New Delhi to evaluate the safety and efficacy of Dhatri Lauha with 45 days of treatment. Total 458 Patients were enrolled in this study out of which 400 patients had successfully completed it.

Cluster Sampling techniques were used for the analysis of the data on the objective parameters such as weakness, fatigue, palpitation, breathlessness and swollen feet. There has been little work done on methods for cluster analysis of the data where the outcome measure is an event rate (per person time).

This work contributes to the application of the statistical techniques for analysis of the Bio-medical studies data.

Keywords: Cluster Analysis, Person time, Event rate, Bio-Statistics, Anaemia, Ayurveda.

Introduction

Iron deficiency anaemia (IDA) is the most common nutritional deficiency worldwide. Iron deficiency can arise either due to inadequate intake or poor bioavailability of dietary iron or due to excessive loss of iron from the body. The poor bioavailability of dietary iron is considered to be major reason for widespread iron deficiency (7). Women lose a considerable amount of iron in menstruation. Some other factors leading to anaemia are intestinal parasites (hookworm etc.) and malaria.

According to Ayurveda, Pandu is considered as a specific disease characterized by pallor of body which strikingly resembles with 'Anaemia' of modern science. Detailed description concerning the etiology, pathogenesis, classification and management of anaemia (*Pandu roga*) is available in classical literatures of Ayurveda. Correcting anaemia often requires an integrated approach due to multifactorial nature of this disease, in order to effectively combat it, the contributing factors must be identified and addressed. In settings where iron deficiency is the most frequent cause, additional iron intake is

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usually provided through iron supplements. There are many age-old remedies for the treatment of this condition in Ayurveda.

Cluster Sampling

Cluster sampling is a sampling technique in which the entire population of interest is divided into groups, or clusters, and a random sample of these clusters is selected. Each cluster must be mutually exclusive and together the clusters must include the entire population.

After clusters are selected, then all units within the clusters are selected. No units from non-selected clusters are included in the sample. This differs from stratified sampling, in which some units are selected from each group. When all the units within a cluster are selected, the technique is referred to as one-stage cluster sampling.

If a subset of units is selected randomly from each selected cluster, it is called two-stage cluster sampling. Cluster sampling can also be made in three or more stages: it is then referred to as multistage cluster sampling.

Cluster Randomized Trials

Clinical trials typically involve the randomization of individual subjects to the intervention or control groups (4). Cluster randomized trials are however, characterized by the randomization of groups or clusters of individuals (such as schools, medical practices, communities, research units) to treatment groups (6). Such designs are used increasingly frequently in trials of preventive interventions, for example of the effects of Vitamin A supplementation, Haemoglobin supplementation, etc.

Several methods have been developed for the statistical analysis of cluster randomized trials. These includes simple techniques such as the t-test, the Wilcoxon rank sum test and Fisher's permutation test, which use the proportion of individuals experiencing the event in each cluster as the observation.

There has been a little work done on methods for the analysis of cluster randomized trials where the outcome measure is an event rate (per person time) such as mortality, or incidence rate of a disease or a symptom of particular disease, rather than a proportion. The present study aims to fulfil that gap.

In the present study researcher wishes to test the efficacy of an Ayurvedic Formulation Dhatrilauha in the patients of Iron Deficiency Anemia by making use of cluster sampling techniques.

Study Design (8)

The present work consists of the data of multicentric clinical study conducted on Iron Deficiency Anaemia (Pandu Roga) with an Ayurvedic formulation Dhatri Lauha in the dose of 500 mg. (one capsule) twice daily after meal for forty five (45) days with lukewarm water. Dhatri Lauha comprises of pericarp of Amalaki, Lauha Bhasma, root of Yastimadhu and stem of Guduchi.

The objective of current study was to assess the clinical safety and efficacy of Dhatri Lauha through measurable objective parameters over a period of 45 days divided into four assessment stages after a gap of 15 days each. Hence there were four assessment stages viz; Stage I - 0 day (Recruitment day of the patient), Stage II – After 15 days, Stage III – After 30 days and Stage IV – After 45 days.

This multicentric study was conducted in 12 peripheral research institutes of Central Council for Research in Ayurveda and Siddha, Department of AYUSH, Ministry of Health & Family Welfare, Government of India. Central Council for Research in Ayurveda & Siddha has 35 units all across the country.

Before the initiation of the study it was planned to design the study as cluster randomized trial. Hence out of 35 institutes 12 institutes were selected randomly. These selected institutes formed our 12 clusters for the present study.

A target of 40 patients was fixed for each of the 12 centres. Hence a total of 480 patients was the Sample size for this study. Out of these 480 patients it was possible to achieve the target of total 458 Patients which were enrolled in this study. Out of these 458 patients 424 patients had successfully completed the study.

Material and Methods

Study Design	Open labelled trial
Sample Size	40 subjects per centre
Level of Study	OPD
Study Period	1 year

Criteria for Inclusion

1. Age between 15 to 60 years
2. Haemoglobin level between 6 to 10 gm /dl.
3. Serum iron content < 50 µg /L
4. S. Ferritin < 30 µg /L
5. MCHC < 34 g/dl
6. MCV < 80fL.
7. Peripheral smear of blood shows hypo chromic / microcytic anaemia

Criteria for Exclusion

1. Age less than 15 years and more than 60 years.
2. Pregnancy and lactation
3. Severe Renal / Hepatic/ Cardiac disease
4. Any continuing blood loss e.g. Haematemesis, Melena, bleeding piles etc.
5. Dimorphic anaemia

Statistical Methods (1, 3)

Let d_{ij} be the observed number of events (in this case presence of symptoms) and Y_{ij} the number of person time of observation in cluster j at assessment stage i, where i =1 represents the assessment stage I and i =2 represents the assessment stage II and so on. And j = 1, 2, ...12 since we have 12 clusters (5). The cluster event rates when computed by considering the mean of the cluster event rates at assessment stage i is given by

$$\bar{r}_t = \frac{1}{m_i}\sum_{j=1}^{12} r_{ij} = \frac{1}{m_i}\sum_{j=1}^{12} \frac{d_{ij}}{y_{ij}}$$

And thus an estimate of the assessment stage effect is

$$RR_M = \bar{r}_1 / \bar{r}_2$$

Confidence Intervals (CI)

A CI can be obtained for the intervention effect RR_M by using the standard deviation of the cluster event rates in each group (2, 9).

The distribution of RR_M is likely to be skewed, and so we calculate a CI on logarithmic scale. Using a Taylor series approximation the variance of log (RR_M) is estimated by

$$V_M = \hat{\text{var}}(\log \bar{r}_1) + \hat{\text{var}}(\log \bar{r}_2)$$

$$= \frac{\hat{\text{var}}(\bar{r}_1)}{\bar{r}_1^2} + \frac{\hat{\text{var}}(\bar{r}_2)}{\bar{r}_2^2}$$

$$= \frac{s_1^2}{m_1 \bar{r}_1^2} + \frac{s_2^2}{m_2 \bar{r}_2^2}$$

Where, $s_i^2 = \frac{1}{m_i - 1}\sum_j (r_{ij} - \bar{r}_i)^2$

Is the estimated variance of the cluster rates in the i^{th} group. The sampling distribution of RR_M will be asymptotically normal and a 95% CI for RR_M is given by

$$\exp[\log RR_M \pm 1.96\sqrt{V_M}]$$

Results

The Objective parameters under this study were Weakness, Fatigue, Palpitation, Effort intolerance, Breathlessness and Swollen feet.

As seen by the results there was marked improvement in all chief complaints which were present at baseline in almost all the patients. Remarkable improvement was found in weakness when compared to baseline as the percentage change was 8%, 20% and 38% respectively for 15th day, 30th day and 45th

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day respectively, for fatigue improvement percentage was 10%, 26% and 49% for 15th day, 30th day and 45th day respectively on comparing from baseline, for Palpitation improvement percentage was 22%, 49% and 69% for 15th day, 30th day and 45th day respectively on comparing from baseline, for Effort intolerance improvement percentage was 18%, 50% and 75% for 15th day, 30th day and 45th day respectively on comparing from baseline, for Breathlessness improvement percentage was 34%, 61% and 80% for 15th day, 30th day and 45th day respectively on comparing from baseline and for swollen feet improvement percentage was 93%,96% and 98% improvement in 15th Day,30th Day and 45th day respectively.

Table 1: Showing the cluster event rates of the chief complaints assessed after 15, 30 and 45 days

Chief Complaints	Assessment Stages	Mean	N	Std. Deviation
Weakness	0 day	0.9938	12	0.0154
	15 day	0.9314	12	0.0922
	30 day	0.7781	12	0.1963
	45 day	0.5967	12	0.2530
Fatigue	0 day	0.9803	12	0.0358
	15 day	0.8913	12	0.10384
	30 day	0.7088	12	0.2035
	45 day	0.4865	12	0.2700
Palpitation	0 day	0.6004	12	0.2489
	15 day	0.4655	12	0.2401
	30 day	0.2994	12	0.2346
	45 day	0.1855	12	0.1791
Effort intolerance	0 day	0.5831	12	0.3212
	15 day	0.4892	12	0.3204
	30 day	0.2986	12	0.2251
	45 day	0.1451	12	0.0986
Breathlessness	0 day	0.5267	12	0.2701
	15 day	0.3450	12	0.2390
	30 day	0.2071	12	0.1713
	45 day	0.1126	12	0.1189
Swollen feet	0 day	0.1293	12	0.0998
	15 day	0.0645	12	0.0450
	30 day	0.0328	12	0.0330
	45 day	0.0194	12	0.0222

Table 2: Showing the results of the Statistical Analysis of the cluster event rates of the chief complaints. It can be seen from the table below that the effect of the drug Dhatrilauha was significant on all the chief complaints as the p-value is less than 0.05 for all the parameters.

		Paired Differences					t-value	p-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval			
					Lower Bound	Upper Bound		
Weakness	0 day - 15 day	0.0624	0.0844	0.0243	0.0087	0.1161	2.560	0.027
	0 day - 30 day	0.2157	0.1933	0.0558	0.0928	0.3385	3.865	0.003
	0 day - 45 day	0.3971	0.2476	0.0714	0.2398	0.5544	5.556	0.000
Fatigue	0 day - 15 day	0.0889	0.1048	0.0302	0.0223	0.1555	2.939	0.013
	0 day - 30 day	0.2715	0.2048	0.0591	0.1414	0.4016	4.593	0.001
	0 day - 45 day	0.4937	0.2635	0.0760	0.3263	0.6612	6.490	0.000
Palpitation	0 day - 15 day	0.1349	0.1197	0.0345	0.0588	0.2110	3.904	0.002
	0 day - 30 day	0.3010	0.1905	0.0549	0.1799	0.4220	5.474	0.000
	0 day - 45 day	0.4149	0.2340	0.0675	0.2662	0.5636	6.141	0.000
Effort Intolerance	0 day - 15 day	0.0938	0.0824	0.0238	0.0414	0.1462	3.942	0.002
	0 day - 30 day	0.2844	0.1630	0.0470	0.1808	0.3880	6.043	0.000
	0 day - 45 day	0.4379	0.2679	0.0773	0.2677	0.6082	5.662	0.000
Breath-lessness	0 day - 15 day	0.1816	0.1829	0.0528	0.0654	0.2979	3.439	0.006
	0 day - 30 day	0.3196	0.2140	0.0617	0.1836	0.4556	5.174	0.000
	0 day - 45 day	0.4141	0.2648	0.0764	0.2458	0.5823	5.418	0.000
Swollen feet	0 day - 15 day	0.0647	0.0708	0.0204	0.0197	0.1097	3.166	0.009
	0 day - 30 day	0.0964	0.0900	0.0259	0.0392	0.1536	3.712	0.003
	0 day - 45 day	0.1098	0.0973	0.0281	0.0479	0.1716	3.909	0.002

Hence we can say that cluster analysis of the binary outcome data is really fruitful and a good choice of the analysis technique when the sample size is large as the cluster analysis of the event rates give us a chance to treat each cluster as a separate unit.

Hence we can say that the effect of the drug Dhatrilauha is really effective in treating the patients suffering from Iron Deficiency Anaemia.

Conclusion

Cluster trials are used in many area of health research and they have a particularly strong tradition behavioural and diseases prevention research. The primary feature of the cluster trial is the intra-cluster correlation that arises from assignment of treatment condition to intact groups of individuals. This means that special consideration is needed in the planning and conducting such trials which

is not required in clinical trials that randomize treatment to individuals. Because of such challenges, best plan to analyse the data is in the form of cluster trials. This is especially true for categorical outcomes and in those situations characterized by a small number of clusters, each with a large number of subjects per cluster.

In the present study we have presented some simple practical approaches for the analysis of cluster randomized trials whose out come is an event rate (appearance of chief complaints per person time).The Confidence interval that we proposed are based on large sample theory as those are given by more complex method.

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