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Clinical Research Methodology

Purvi Gandhi*

Department of Pharmacology, B. S. Patel Pharmacy College, Mehsana, India.

ABSTRACT

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Medical trials occupy a greater part of clinical trials and exceed in number over other types of clinical trials. Although clinical researches or studies other than those falling under the category of trial, lack the commercial aspect, they may contribute to the betterment of clinical practice and may constitute a base for large-scale commercial trials. Explanatory clinical studies are the most widely accepted and executed type of clinical researches/ trials. They explain not only the therapeutic aspects of a drug but also provide with a detailed description on adverse events and pharmacovigilance. Any new drug to be marketed first requires proving its safety, efficacy and the need over existing product range by passing through various phases of clinical trial that follows animal studies. For this reason, randomized controlled trials (RCTs) with placebo-control and blinding fashion is the only accepted form of clinical trials with few exceptions. Besides explanatory, descriptive clinical research is also useful for certain types of studies such as epidemiological research. While conducting RCTs on humans (either healthy volunteers or patients), various components of the trials viz. study design, patient population, control group, randomization, blinding or non-blinding, treatment considerations and outcome measures are important to strengthen the outcomes of the trial. Similarly, proper utilization of statistics, for sample-size calculation, data collection, compilation and analysis by applying proper statistical tests, signifies the outcomes of the clinical research. The presented article includes a brief, however, an informative review of literature on methods in clinical research including clinical trial.

KEY WORDS: Clinical research methods, Clinical trial methods, Statistics in clinical research

INTRODUCTION

Clinical research is a branch of medical science dealing with any research or study in living humans. 'Clinical trials' is the term interchangeably used with the terms 'clinical research' or 'clinical study'. Although there are many definitions of clinical trials, they are generally considered to be biomedical or health-related research studies in human beings that follow a pre-defined and –designed protocol. Clinical trial is defined as "a systematic study of new drug(s) in human subject(s) to generate data for discovering and/ or verifying the clinical, pharmacological (including pharmacodynamic and pharmacokinetic) and/ or adverse effects with the objective of determining safety and/ or efficacy of the new drug".[1] Clinical trial is company-sponsored, meant for a new drug or device and carried out for a specific new use of an intervention; while clinical research is meant for academic and pharmacovigilance. Large number of literature describes on clinical trial and its phases. However, it has been now started to include a detailed description on clinical research or study.

Ethics in clinical trial

Depending upon the objective, clinical trial is conducted either on healthy volunteers or on volunteer patients. Healthy volunteers are generally included in such trial that determines pharmacokinetics, tolerability, safety and even efficacy of certain types of drugs (e.g. hypoglycemic, hypnotic, diuretic etc.). Otherwise, for majority of drugs (e.g. antiepileptic, antipsychotic, anti-inflammatory, antitubercular etc.), efficacy can only be assessed in patients.[2]

The research entailing the use of human participants is considered to be absolutely essential after a due consideration of all alternatives in the light of the existing knowledge in the proposed area of research. In other words, when a new drug is with clear significant benefit at human side, human as participants for trial experimentation becomes justified. International Conference on Harmonization has provided a guideline on Good Clinical Practice (ICH GCP) as an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects.[3] World Health Organization Guidelines for good clinical practice for trials on pharmaceutical products also describe provisions and prerequisites for a clinical trial, protocol and protection of trial subjects, responsibilities of the investigator, responsibilities of the sponsor, responsibilities of the monitor, monitoring of safety, record-keeping and handling of data, statistics and calculations, handling of and accountability for pharmaceutical products, role of the drug regulatory

***Address for Correspondence:**

Purvi S. Gandhi, Department of Pharmacology, B. S. Patel Pharmacy College , Saffrony Institute of Technology Campus, Nr. Saffrony Holiday Resort, Ahmedabad –Mehsana Highway, Linch, Dist. Mehsana,Gujarat, India

E-mail: *purvi.gandhi@saffrony.ac.in*

authority, quality assurance for the conduct of a clinical trial and considerations for multicentre trials.[4] United States Food and Drug Administration Guidelines for conduct of clinical trials provide guidance for institutional review boards and clinical investigators, information for health professionals on clinical trials and human subject protection, information for clinical investigators on drugs, devices and biologic as well as policies and regulatory matters regarding human subjects research.[5] Several issues and principles have been discussed in various guidelines on conducting clinical trial (especially drug trial) which must be addressed while conducting a trial. These include the following:

1) Ethical justification and scientific validity of biomedical research involving humans

2) Ethics review board

3) Informed consent process

4) Choice of control in clinical trials

5) Research involving special group of research participants.[6]

Moreover, clinical trial in India on a new drug or device or any surgical intervention shall be initiated only after the permission has been granted by the Licensing Authority under Rule 21 (b) and the approval obtained from the respective Ethics Committee(s).[7] Subjects who are relatively or absolutely incapable of protecting their own interests i.e. "vulnerable participants" include very poor, illiterate, terminally-ill, mentally-challenged, prisoners, students or employees are examples.[8] Clinical trial must preserve their rights while being conducted in such humans. Moreover, there must be equal distribution of burdens and benefits in terms of race (in case of genetic research), economic and social level, mental-healthiness and reduced autonomy, to avoid unintentional bias and to extend the benefit of trial to maximum of communities.

Phases of clinical trial

Clinical trial of a drug is conducted through various phases. The number of phases as trial varies from literature to literature and from author to author. Most literatures describe a clinical trial/ testing of a new molecule to comprise of four phases.[9] These phases are as following:

1) Phase I : Human/ Clinical Pharmacology trial

2) Phase II : Exploratory trial

3) Phase III : Confirmatory trial

4) Phase IV: Post-Marketing Surveillance.[10]

Some literatures describe the above-mentioned first three phases as actual phases of clinical trial while others consider phase II and III as the actual clinical trial.[11,12] This may be because phase I is usually carried out on healthy volunteers except for diseases like cancer, acquired immuno-deficiency syndrome (AIDS) etc. Moreover, phase IV may not necessarily follow randomized, controlled trial (RCT) with blind fashion of the most commonly-employed type since the drug is available in market with known label-indication and it requires chronic drug administration and observation of patients.

1) Phase I: Human/ Clinical Pharmacology trial:

This phase aims to obtain the precise information on a) initial safety in terms of safe dosage range and biological effects including adverse effects; b) metabolism and kinetics and c) drug interactions.[9] The trial is carried out on healthy human volunteers (20-80 in number) except for life-saving drugs meant for treating life-threatening diseases such cancer, AIDS where actual patients only are included. The volunteers or patients are exposed to a single dose which is usually kept as $1/12^{th}$ of the effective dose found from animal studies especially from mice study.[13]

2) Phase II: Exploratory trial:

This phase is meant to find whether or not the drug possesses the actual therapeutic potential. It identifies the therapeutic efficacy of the drug with dose range, kinetics as well as metabolism.[9] It follows RCT type with blinding of treatment. It is carried out at one or few clinical centers only on small but sufficient number of patients (100-300 in number) to reach clinical significance in outcomes. It also aims to find out therapeutic index of the drug being studied. It requires to be carried out in patients meeting selection criteria of age group, sex, presence of particular disease with pre-defined and –diagnosed severity etc. It also reports the adverse effects of the drug.

3) Phase III: Confirmatory trial:

This phase applies the same study protocol designed for phase II to evaluate safety and efficacy at large.[9] It is to confirm the effectiveness of the drug or treatment, to monitor side effects, to compare it to commonly used treatments and to collect information that will allow the drug or treatment to be used safely.[14] It is simultaneously performed at a large number of clinical centers that include patients of various geographic origins with difference in responsiveness of the disease towards the drug treatment. Further, it covers large number of patients (1,000-3,000 in number) allowing the outcome to reach not only clinical significance but also statistical significance. Once the drug passes this phase successfully, it is licensed for commercial use. Thus, phase III of trial is a key study forming the primary basis for regulatory approval of an intervention and is often referred as pivotal trial. It generally

proceeds as randomized, placebo-controlled, double-blind clinical trial.[15]

4) Phase IV: Post-Marketing Surveillance:

This phase is so named because it is carried out after the drug is released in the market for therapeutic use. It is mainly to detect uncommon but significant adverse effects.[9] Once the drug enters the market, it will be utilized by many more patients having other co-morbidity and co-existing diseases in addition to the disease for which the drug is indicated and licensed. This is also conducted to provide critical information on drug-drug interactions or iatrogenic diseases. The best example is of thiazolidinedione series of drugs, which were found to deteriorate heart failure (HF) if used as antidiabetics in HF-patients. Therefore, they are now contraindicated in diabetic patients with HF.

Clinical trials are conducted with the purpose of commercialization while clinical researches/ studies do not necessarily aim at commercialization. Clinical research can be based on any of the following four concepts:

1) treatment of a disease

2) diagnosis of a disease or disorder or dysfunction

3) systematic review of several clinical studies

4) prognosis of a particular disease.[16]

Clinical research based on therapy/ treatment can be focused on any of the two areas; 1) pharmacology of a drug and 2) effects of a non-pharmacological intervention such as a surgery or a device. Clinical pharmacology is a branch of pharmacology dealing with study of a drug in humans (either healthy volunteers or patients). A large number of new drugs or molecules are synthesized in laboratories or are extracted from natural sources viz. plants or animal organs. However, only a small number enters pharmaceutical market as a successful drug for treatment of a disease. Many devices, as a disease-intervention or treatment, are also manufactured every year but only a few find a place in healthcare system. This is because of the stringent procedure for a drug or device to pass through the pre-clinical and clinical phases to prove its safety and efficacy as well. A drug can not be marketed and hence not be used for treating a disease until and unless it passes all the phases and proves its effectiveness. Further, once a drug is registered and approved for general clinical use for one indication i.e. one therapeutic use, expansion of its therapeutic-use range requires additional clinical research and trial. Similarly, for any surgical intervention to be recommended by the healthcare system, it has to pass through various stages of pre-clinical and clinical studies.

Even though most of the new drugs are either structurally similar to or are the derivatives of already-existing drug molecule and are rarely with totally new basic structure, data from extensive pre-clinical i.e. animal studies are essential to conduct clinical trials of a new drug. When a drug molecule is a new chemical entity, detailed and extensive studies in animals become essential and more stringent. Biotechnology products, mainly all recombinant drugs are considered as new drugs (irrespective of international availability of the same formulation) and hence treated accordingly. Pre-clinical studies include those carried out in lower animals as well as higher primates with special emphasis on therapeutic and adverse effects. Further, it is also required to establish minimum dose producing the desired effect (i.e. therapeutic effect) and maximum dose causing adverse effects in specific animal species. The investigator should have data on acute, sub-acute and chronic toxicity studies. The investigator can initiate clinical experiment(s) only after he/ she gets all the following information and data about a new drug from animal studies:

1) The need of the new molecule over the existing drug range- the need can be either because of its different mechanism of action and hence additional benefit or due to less adverse effect or due to its greater efficacy and less cost at least.

2) Specific pharmacological actions i.e. those with therapeutic potential for humans and general pharmacological actions i.e. those on other organs and systems, especially cardiovascular, respiratory and central nervous system.

3) Pharmacokinetic data.[17]

4) Therapeutic index (i.e. ratio of dose lethal in 50% of the study population (LD_{50}) to dose effective in 50% of the study population (ED_{50}) which is denoted as TI= LD_{50}/ED_{50}) along with the minimum dose required to produce the desired therapeutic effect and the maximum dose producing the unwanted or lethal effect.

5) Toxicity data.

6) Nature of the adverse effects of the drug along with identified signs and symptoms in order to avail the possible treatment if the same occur in humans.

Clinical research based on clinical pharmacology includes exclusively the effect(s) of a drug in human and outweighs the other types of clinical studies in number. Clinical studies are further typified and sub-typified as follows (Fig.1)[18,19]:

Clinical studies

Descriptive studies

Descriptive studies report unusual or new events such as the occurrence of sudden infant death syndrome (SIDS) in several siblings within a single family, prevalence of albinism

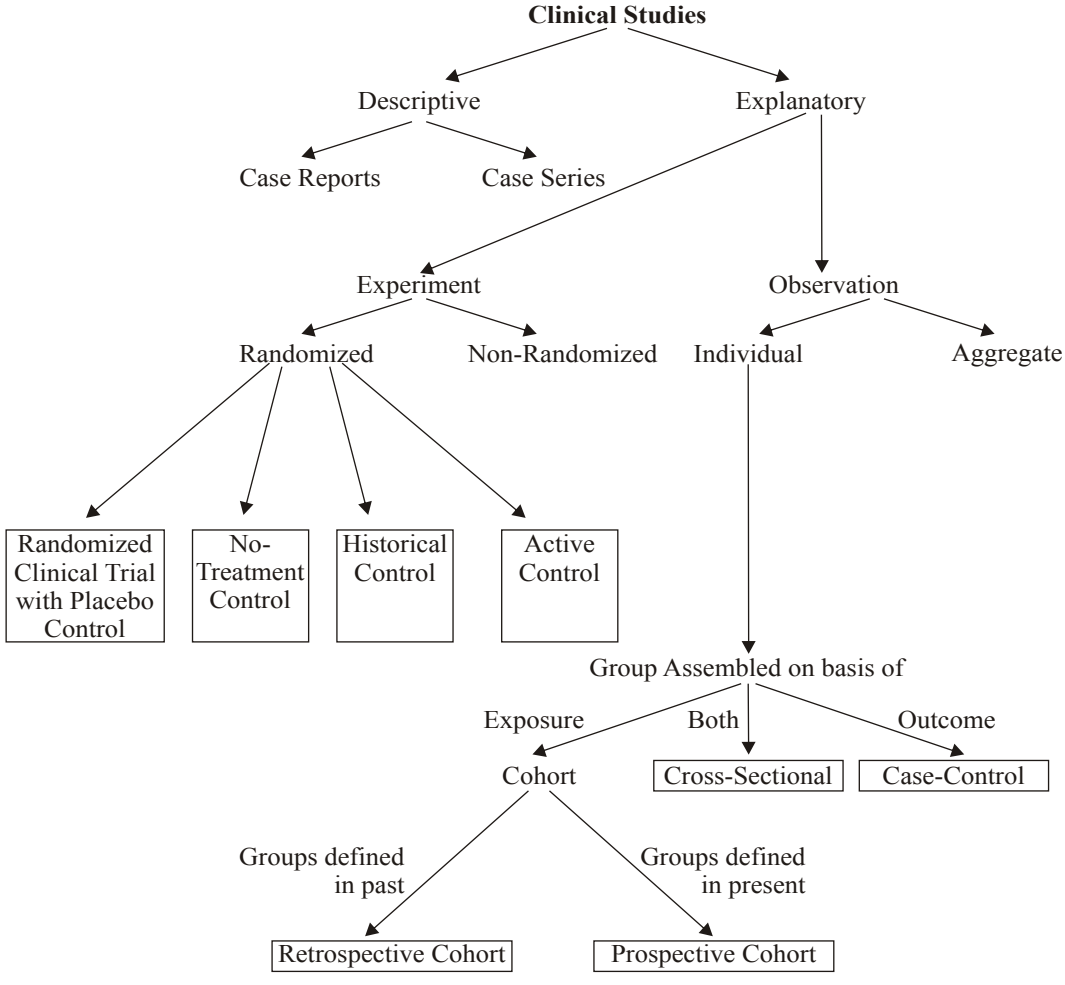
in a single family etc. The researcher simply records the observations and co-relates the events observed with possible reason. These are neither randomized nor pre-designed researches. They may be presented as case reports whereby certain individual patients with distinguished clinical characteristics are included in the study. All the baseline characteristics are recorded and the individual patient is treated as unique case with control over all the variables. The patient is observed and evaluated for the possible outcome. The results are compared with baseline values or are expressed as success or failure of the treatment given. If the treatment succeeded, a hypothesis is generated for an expanded and more rigorous study to find the relationship between the treatment and the outcome observed. In case-series, observations are documented at regular intervals from patients exposed to a particular drug or a group of drugs. They may also cover prior histories of patients with the same outcome, to find a possible cause-effect relationship if exists. These are useful in predicting the incidence of an adverse event of newly-marketed drug when reports on such events are limited.

Explanatory studies

Observation studies

In an observational study, the subject to be observed chooses whether or not to take the drug or to have the surgery being studied.[20] Errors that are likely to occur include the differences in profile of the subjects since variables such as age, family history of disease, cause and severity of disease etc. may not be defined. For example, two patients have left ventricular (LV) dysfunction, in one it is because of ischemic heart disease (IHD) and in another it is because of severe mitral valve stenosis. Thus, the therapy of both the diseases differs due to different oetioes and hence both the patients can not be compared in one study. Another example is of two patients suffering from headache, one because of migraine and the other because of common cold. These two patients can not be compared for the analgesic activity of one drug since the cause and the severity of headache and hence the analgesic activity of the drug would vary greatly. Observational studies can never be blinded. Hence, biases from patients, observer and experimenter may result into systematic and random errors.

Fig.1: Types of clinical studies



Aggregate observation studies

Pandemic and epidemic studies on communicable diseases and their treatments are generally carried out as aggregate observation studies e.g. occurrence and effective treatment of malaria and its relapse in particular geographical area.

Individual observation studies

In individual observational study, the patients/ subjects are individually observed and they are assembled in groups on the basis of outcome or exposure or both. Depending upon the basis of the grouping, the individual observational study is sub-classified as 1) Case-control; 2) Cohort and 3) Cross-sectional.[19]

1) **Case-control study**

Case-control study involves assembling of subjects in groups on the basis of the outcome found in those subjects. It compares the subjects with outcome in question (the group behaves as a case group) with the subjects without the outcome (the group acts as a control) e.g. occurrence or non-occurrence of myocardial infarction (MI) in patients with hypertension (HT). It generally follows the retrospective design and evaluates how the exposure is related to the well-defined outcome using control group.[19] However, grouping on the basis of outcome incorporates subjects with variety of distinguished characteristics. It is quick and inexpensive. Further, patients with rare outcome can be assembled in a group to study oetioes, pathophysioes and prognosis of a disease. Results are generally expressed in terms of odds ratio (OR) and risk ratio/ relative risk (RR). Although multiple exposure variables can be correlated with outcome, it does not allow the correlation of temporal sequence of cause and effect with the final outcome.

2) Cohort

It includes groups assembled on the basis of exposure. Here the exposure is well-defined but the outcome is variable. Thus, it allows study of one exposure with many more outcomes.[19] Cohort study can be retrospective wherein the groups are defined in past or it can be prospective wherein the groups are defined in present. The retrospective cohort correlates the exposure occurred in past with the outcome resulted just in recent past. Here the patients have been followed forward and hence it associates the exposure with some temporal outcomes though not all. If the patients have been treated with different treatments to control outcome-related variables, it limits the correlation between exposure and one outcome only. Like case-control study, it is also quick and inexpensive. If carried out on the basis of well-defined, -controlled exposure and followed with control over variables, retrospective cohort study suffices the requirements of prospective study with additional advantage of less time and money consumption. In prospective cohort study, the groups are observed for outcomes at particular, pre-decided time intervals. Thus, it finds firmly whether a particular exposure or sign or symptom is related with the outcomes. If the outcome is rare, the study requires inclusion of large number of patients and longer follow-up. Thus, it is expensive in terms of time and money. If the patients are not randomized and blinded, the outcomes may be influenced by bias and confounding.

3) Cross-sectional

Cross-sectional study assesses both the exposure and outcome concurrently. Generally it is survey- or review-based. Cross-sectional study is, therefore, good for prevalence research. However, it is not suitable for causal-outcome assessment.

Experimental studies

Non-randomized studies

Patients are selected on the basis of selection criteria. They are not randomized to the particular treatment(s) and are given a treatment depending upon course of disease. Generally, phase IV of clinical trial follows this way. Further, in many experimental studies in humans, randomization is not possible. Many of the surgical experiments have evolved with specific indication and application. They have a focused-patient group and therefore, randomization is not possible or is unethical. For example, patients with both the kidneys failed require undergoing kidney transplantation. Although, dialysis is an available option it is not comparable with renal transplantation and hence patients can not be randomized to such options.

Randomized controlled trials

In the studies which are randomized, controlled clinical trials (RCTs), human subjects (either healthy volunteers or patients) do not choose the therapy being studied or compared. Experimental clinical studies are generally RCTs. Randomized controlled trials are, as the name indicates, based on randomization. When a new drug successfully passes the pre-clinical studies, it is challenged to clinical experiments that follow random assignment of subjects to two or more groups one of which behaves as control group and therefore, such clinical experiments are called RCTs. The several components to be considered include 1) Study design; 2) Patient population; 3) Control group; 4) Randomization; 5) Blinding or non-blinding/ open-labeling; 6) Treatment considerations and 7) Outcome measures.

1) Study design

The common study designs employed in RCTs include parallel group design, matched pairs and cross-over designs.[21]

In parallel group design, the patients are enrolled, followed and observed for outcomes on parallel basis. Parallel group design requires large number of patients. In matched pairs, patients are matched for different variables and those matching the required variables are then randomized to various treatment groups. This type of study design overcomes the influence of variables on outcomes, although it is difficult to follow. Cross-over design is particularly used when the effect of a drug is reversible and transient. In cross-over design, the patients are given more than one treatment but in sequence i.e. one after another when the effect of previous treatment is washed out. Cross-over design, thus, requires less number of patients.

2) Patient population

As a common and required method, the RCTs are carried out on specific subject population selected on the basis of "selection criteria" which are derived in line with various fixed, independent and dependent variables. This is to overcome the misleading by variables. For example, if effects of angiotensin-converting enzyme inhibitor (ACEI) on cardiac function are to be studied in patients with LV systolic dysfunction, variables like family history of cardiac disease, presence of other cardiac diseases such as heart block or valve failure etc. should be avoided as patients with these variables are different from those not having the variables. Further, they may reveal different outcomes viz. the cardiac function and even survival. Depending upon the defined criteria, patients or healthy subjects are included in the study to randomize to various treatments for the comparison of outcomes and thus, to conclude. The criteria are namely (a) inclusion criteria; (b) exclusion criteria and (c) withdrawal criteria.

(a) Inclusion criteria: Specifications of subjects (patients or healthy volunteers) with regard to age, gender, ethnic groups, body mass index, prognostic factor, diagnostic admission criteria, should be clearly mentioned wherever relevant.

(b) Exclusion criteria: These specify the characteristics of the subjects on the basis of which they are excluded from the trial. For example, severity of the disease, concurrent medication etc.

© Withdrawal criteria: These specify the subjects on who the trial shall be terminated and mention when and how to withdraw the subjects from the study and to stop further follow-up in those.[10]

To have comparison possible between or among various treatment groups, selection of patients must be done on the basis of inclusion and exclusion criteria. This allows enrollment of subjects with corresponding clinical characteristics.[9]

3) Control group

Randomized controlled trial also includes control group (either placebo control or active control) to show the control and effect over dependent variables and to obtain clear effects of drug under consideration. Control group can be placebo control, no-treatment control, historical control or active control. The placebo means dummy to the drug under evaluation with regard to organoleptic properties but lacking any pharmacological actions. Thus, it is to overcome the psychological impact of drug administration manifested by an individual on disease progression. It allows the investigator to determine the true efficacy of the treatment being researched for a particular condition. Some studies also include no-treatment control or historical control as types of controls. In no-treatment control group, the patients do not receive the placebo even. Therefore, they know that they do not receive any treatment and hence, individual bias due to psychic factors affects the study outcomes. In other words, it is least preferred type of control. Historical control is the control group of previous study that was a different with respect to treatment group. Here control group of one study is utilized for another study and both the studies differ with regard to treatment only. This is done for studies not allowing placebo control or no-treatment control and involving high mortality disease even after availability of effective treatment e.g. studies on treatment for cancer and human immune-deficiency virus (HIV) infection.

Inclusion of placebo in drug research and sham surgery has been debated.[6] Moreover, when an effective established treatment is available, use of such placebo control group is unethical. For examples, a drug is to be assessed for its effects on cardiac function in patients with LV systolic dysfunction, as per American College of Cardiology/ American Heart Association (ACC/ AHA) guidelines all the patients would be necessarily receiving ACEI, if not contraindicated.[22] Therefore, in this type of study all the patients receive the recommended drug which has already proved its beneficial effect on cardiac function. Thus, one can not have a placebo control group but will have an active control receiving the best current therapy. It provides information about relative efficacy of the investigational drug over existing one. In the present example, the patients would be randomly assigned to a group receiving ACEI or to a group receiving ACEI in addition to the drug being evaluated- the former behaving as an active control and the later as a treatment group.

4) Randomization

Randomization is an optimal method of distributing the variables between the treatment and control groups.[9] Therefore, the bias of selecting specific treatment does not occur. Random assignment of subjects to various groups provides equal distribution of all variables in all the groups

and does not let them influence the final outcomes. Randomization techniques mainly used in RCTs are simple randomization, cluster randomization and stratified randomization. In simple randomization, patients matching the selection criteria are randomized to various treatment groups. In cluster randomization various groups of patients matching the criteria are randomized to treatment under investigation. This kind of trial is especially used to find the geographical, genetic variations. In stratified randomization technique, subjects are classified in groups i.e. strata and then within a group they are randomized to various treatment groups. In RCTs, three main methods of randomization include 1) Tables of random numbers; 2) Mathematical algorithms for pseudorandom number generators and 3) Physical randomization devices such as coins, cards or sophisticated devices such as Electronic Random Number Indicator Equipment (ERNIE).

5) Blinding

To avoid bias, trial is carried out in blind fashion. Blinding means "concealing or masking of the patients-assignment to a study group (control or treatment) from those participating in the study i.e. patients, observer and experimenter".[9] RCTs can be blinded or non-blinded. The non-blinded experiment is also called open-label study. In this type of study all three- the patient, the physician or the observer and the experimenter or the researcher, are aware of the treatment used. In many instances it is unethical to hide the treatment module from the patients especially those suffering from life-threatening disease such as cancer, AIDS, end-stage HF etc. Additionally, open-label study permits the patients to buy brand of the drug of his choice independently. However, it has the biggest disadvantage of introducing bias from any of the three components of the RCTs.

Blinding is carried out at the beginning of study. The blind RCT can be single-, double- or triple- blind. In a single-blind experiment, the participants either the patient or the healthy volunteer does not know whether he receives the test intervention or placebo. In double-blind trial, neither the patient/ subject nor the experimenter knows who belongs to the control group and who belongs to test group but the observer knows. In triple-blind RCT, none of the three components of study knows name or nature of the treatment given. Therefore, the triple-blind RCT is totally devoid of any kind of biases and allows the outcomes to be free from any such influence. In double- and triple-blind experiment the keys identifying the patients/ human subjects and the group they belonged to are preserved by a separate another party and given to the researcher only at the end of the study. Randomized controlled trial can also be conducted as PROBE. PROBE is an acronymus of Prospective, Randomized, Open-label, Blinded-End point as used earlier by Neutel and Smith (2003).[23] This type of trial is easier to carry out than a double-blinded placebo controlled design (DBPC) because it does not require the "matched placebo group" and the "open-label" allows the enrolled patients to receive a marketed preparation of the drug. However, the PROBE studies have only the end-point blinded i.e. observer is unaware of the treatment being studied while investigator and patients are aware of it. Therefore, the investigator or the patient bias may be introduced and thus, the results obtained are less reliable than those with double- or triple-blind study.

6) Treatment considerations

While conducting RCTs, the treatment (either being studied or behaving as active-control) must be considered with regard to its dosages, dosing frequency and other concurrent medication. A drug is generally available in various dosage forms viz. tablet, capsule or injectable etc. and it varies in strength. Moreover, depending upon the dosage form, the route of administration differs and hence, the amount of administration and dosing frequency also. All these factors together affect the plasma concentration of drug and thereby the effects of the drug and hence the final outcome. Therefore, except dose and frequency of drug(s), all above-mentioned factors are kept unique and constant through out the study. Whenever dose and frequency need to be changed, it is done gradually and stepwise. If two drugs are to be administered one of which is likely to interfere with the other either pharmacokinetically or pharmacodynamically, the dosage must be reconsidered to overcome the influence of such interference on study outcomes. Patient compliance is another important part of the treatment consideration. A treatment should not be non-compliant as the patient avoids or less prefers to take such medication resulting in erroneously less efficacious outcomes than those obtained with the other treatment group.

7) Outcome measures

The objective of the study determines the outcomes of interest to be measured. These measures are nothing but the points of checking and recording to accomplish the comparison. In experiments the outcomes are measured in terms of efficacy end-points i.e. primary end-points and surrogate end-points which are also called secondary end-points. For examples, in an experiment evaluating an antihypertensive agent, the clinical end-point of real interest is whether the treatment under investigation can reduce cardiovascular events; a surrogate is the ability of the treatment to reduce blood pressure.[24] The primary end-points of the study are the main measures to support or refute the hypothesis of the study. They must be defined and specified by the investigator at the beginning of the study. Secondary measures, although pre-specified before the commencement of the study, can be

further elaborated during the study. For example, when diuretics are used for treating hypertension, serum glucose level measurement can also be added though serum electrolytes are usually measured as main secondary end-point. Although various measures are determined as primary and secondary end-points, quality of life is now-a-days becoming main primary end-point.[25]

STATISTICS IN CLINICAL RESEARCH

Statistics play a crucial role in concluding a clinical research. It is applied in clinical research to analyze data and to infer the results obtained. It is important to obtain a statistically significant difference between two or more groups being compared in a clinical research, in order to make the outcomes acceptable. Statistics is also required at the beginning of the trial to calculate the sample size required to reach a statistical significance in the findings.

'P' value and level of significance

In a clinical research, a null hypothesis is stated and tested by finding the difference between/ among the results of groups involved in the research. The difference in the results obtained between/ among various groups should be of statistical significance in order to reject the null hypothesis and thus, to accept the alternative hypothesis i.e. "treatment being study as effective one". In many instances, clinical research finds the difference in the results of clinical significance but fails to attain a statistical significance and therefore, the null hypothesis is accepted. Rejection or acceptance of a null hypothesis is based on 'P' value. 'P' value is defined as "the smallest level of significance of the difference in the results that would reject the null hypothesis".[26] It tells how likely it is that the difference between/ among groups occurred by chance rather than because of an effect of treatment.[16]

Types of errors and power of study

The 'P' value is based on two types of errors that one may encounter during experiment. These two errors are designated as type I error and type II error. The former is also called alpha (α) error and the later beta (β) error. A type I error occurs if a difference is found between A and B when none actually exists.[11] Thus, α error indicates the chances of detecting a difference which does not actually exist i.e. the chances of having False Positive Difference. A type II error occurs if no difference is found though A and B do actually differ.[11] Thus, β error indicates the chances of not detecting a difference which actually exists i.e. the chances of having False Negative Difference. Alpha error indicates the level of significance of the result difference among various treatment groups. The level of significance is usually set at the traditional value of 5%. Beta error gives an idea about power (1-β) of a clinical study. Beta error is often chosen to be between 5 and 20%. Power is the ability of a statistical test to show significance if a specified difference truly exists.[27] It is essential to minimize these errors at pre-decided levels or below to draw a conclusion in a clinical study. Furthermore, results of a statistical analysis are found conclusive only when the sample size is sufficiently large. However, because of time and cost factors, it may not be possible to enroll large sample size in a study. In that case, finding power of the study may disclose and support the inability of a test not to reach a statistically significant difference between the groups, even when the clinical difference is significant.

Sample size

Sample size depends upon the design of study, nature of variable and measurement scale.[21]

Sample size formula for quantitative response

The number of patients required per group can be estimated using following formula:

$$\text{N per Group} = \frac{2\,(SD)^2}{(\text{Diff. or ?})^2} \times (Z_\alpha + Z_\beta)^2$$

Where,

SD = estimated standard deviation – known or based on some other study

Diff= ?= expected difference between two treatment considered as clinically important

Z_α = Z value from statistical tables corresponding to level of significance (α)

Z_β = Z value from statistical tables corresponding to (β)

For 2 sided tests with α= 0.05 and β= 0.20

Z_α = Corresponding to level of significance (0.05)= 1.96

Z_β = Corresponding to (β= 0.20) = 0.842

Sample size formula for qualitative data

The number of patients required per group can be calculated using following formula:

$$N = \frac{(2 \times \text{Joint Success rate} \times \text{Joint Failure rate})}{(\text{Diff})^2} \times (Z_\alpha + Z_\beta)^2$$

Confidence interval

The confidence interval (CI) gives a range. It gives a measure of reproducibility of the results within the obtained range. Expression of 'P' value along with CI is clinically more useful and acceptable by many researchers. Generally, it is kept at level of 95%. A 95% CI indicates that if the study is repeated 100 times, the study results would fall within this interval 95

times.[16] For example, if improvement in LV ejection fraction (LVEF) after revascularization in 95 patients is 6% on an average when compared with baseline with a 95% CI of 4.5 to 9% for the difference, it is concluded that the revascularization has the specificity of producing improvement in LVEF by 4.5 to 9% if this revascularization is performed in such 100 patient populations i.e. if it is repeated 100 times.

Odds ratio and relative risk

Odds ratio (OR) and relative risk (RR) both are measures of the size of an association between an exposure and a disease or death.[16] For example, association between smoking or HT and development of IHD; use of a medication and occurrence of a side effect; exposure to MI over global LV ischemia and mortality etc. are expressed in terms of OR or RR. Observational studies usually report their results as OR or RR, although experiments also include these types of measurements as safety and efficacy end-point. A RR of 1.0 indicates that the exposure does not change the risk of disease. A RR of 1.9 indicates that patients with the exposure are 1.9 times more likely to develop the disease or have a 90 percent higher risk of disease. For example, if the RR of hyperlipidemia is 1.4 for development of IHD indicates that patients with hyperlipidemia are 1.4 times more likely to develop IHD than those without hyperlipidemia or they have a 40% higher risk of developing IHD.

Odds ratio is a way to estimate relative risks in case-control studies, when the RR can not be calculated specifically. Although it is accurate when the disease is rare, the approximation is not as good when the disease is common.[16]

Data analysis

Data analysis is carried out after compiling the observations and applying appropriate statistical test. The test to be applied depends on the type of data and their distribution in the study. At large, the data are categorized as parametric or non-parametric. However, in clinical study, the data collected for analysis can alternatively be classified in four classes[28]:

1) Continuous e.g. blood pressure, blood sugar

2) Discrete, associated with numbers and ordered e.g. number of anginal episodes per week, number of MI attack in past etc.

3) Attributes: categorical, ordered e.g. degree of overweight, intensity of pain

4) Attributes: categorical, not ordered e.g. male or female, patients with diabetes mellitus or not

Data can also be typified alternatively as categorical or numerical. The categorical data can be nominal or ordinal in nature. Nominal data are expressed as proportion e.g. sex-male or female proportion in occurrence of a disease. Ordinal data are expressed as scores and ranks e.g. pain, categorized as mild, moderate and severe and can be scored as 1, 2 and 3 respectively. The numerical data are observed in form of interval measurements either continuous (e.g. blood sugar level, blood urea level) or discrete (e.g. number of patients admitted to a hospital, heart rate etc.).

Statistical tests

Application of most suitable statistical test allows analyzing the data and interpreting the results of the study. Different statistical tests are applied in a clinical research to analyze the data and to infer the results obtained depending upon the type of data and their distribution (Table No.1).[29]

Status of clinical research in india

Clinical research in form of trial is conducted not only as unicenter but also as muticenter at various clinical research centers spread over various countries including India. In India, for international collaborative study, details about foreign collaborators and documents for review of Health Ministry's Screening Committee (HMSC) or appropriate Committees under other agencies/authority like Drug Controller General of India (DCGI) are implemented and followed in line with the guidelines by Indian Council of Medical Research. The centers participating in the trial are taken care by clinical research organizations (CROs), which play a distinguished role of central facilities. With advancement and development of various guidelines to implement in such trials, more than 20 CROs have come up with many known to conduct trials at international level.[30] Though developing at full swing, further expansion of field to include research on biologics and devices is needed.

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Table No.1- Type of data and applicable statistical test.

Goal	Type of Data			
	Numerical Measurement	**Rank, Score**	**Binomial (Two Possible Outcomes)**	**Survival Time**
Describe one group	Mean, SD	Median, interquartile range	Proportion	Kaplan Meier Survival Curve
Compare one group to a hypothetical value	One-sample *t* test	Wilcoxon test	Chi-square or Binomial test	
Compare Two unpaired group	unpaired *t* test	Mann-Whitney test	Fisher's test (chi-square for large samples)	Log-rank test or Mantel-Haenszel
Compare Two paired group	paired *t* test	Wilcoxon test	McNemar's test	Conditional proportional hazards regression
Compare three or more unmatched group	One-way ANOVA	Kruskal-Wallis test	Chi-square test	Cox proportional hazards regression
Compare three or more matched group	Repeated-measures ANOVA	Friedman test	Cochrane Q	Conditional proportional hazards regression
Quantify association between two variables	Pearson correlation	Spearman correlation	Contingency coefficients	
Predict value from another measured variable	Simple linear regression or Nonlinear regression	Nonparametric regression	Simple logistic regression	Cox proportional hazards regression
Predict value from several measured or binomial variables	Multiple linear regression or Multiple nonlinear regression		Multiple logistic regression	Cox proportional hazards regression

SD, standard deviation; ANOVA, analysis of variance.

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