Methods and Materials in a Thesis

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The real purpose of scientific method is to make sure nature hasn't misled you into thinking you know something you actually don't know.—Robert M. Pirsig

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Key Points

- Clearly written methods and materials is the first step to good scientific research.
- Enough details should be provided about the cases, controls and methods to permit replication of study by other researchers.
- Adherence to ethical guidelines must be ensured in the proposed research.
- The basis of calculating the sample size and proposed analysis of results should be valid and clearly defined.

Introduction

A fundamental essential of all scientific research is that it is transparent and its results are independently verifiable. A study may claim path-breaking results, but if the methodology used to achieve those results is vague in its details, the scientific community would not readily accept it. As is usually the case with any scientific research, especially the ones that claim different outcome, peers attempt to duplicate the study to see if they can obtain similar results. In the absence of clearly described methodology, the results of the primary study may not be replicated. Thus, it is in the interest of the researcher to explain all aspects of methods and materials.

Materials include a description of human or animal subjects, instruments, chemicals, data entry sheets, computer applications and other similar aspects of the research. Methods include the details of subject selection, the tests/investigations/procedures carried out, the data collection techniques and statistical handling of the data. While the exact details of materials and methods section of any scientific research would vary, depending upon the type of study being carried out, the following paragraphs outline the general guidelines.

Components of Methods and Materials Section

This section includes the following details, usually in the order described below:

- Place or setting of study
- Period of study
- Study design
- Particulars of the subjects/materials in the study
- Details of comparison group, if any
- Sampling, randomization, allotment concealment and blinding
- Statistical basis of deciding the sample size
- Consent and ethical committee approval
- Details of intervention/procedure done
- Outcome measures
- Data management and statistical analysis

Place or Setting of Study

When writing a thesis, it is customary to mention the name and place of the institution where the research was conducted. However, when writing for a journal, the guidelines prescribed by the journal need to be followed. Some journals discourage naming the institution in this section [1], though this policy is debatable. Identifying the place of study lends credibility to the research, especially if the work has been carried out in an institution with good standing for scientific research. Naming the setting of the study also gives an idea about the likely ethnic and socio-demographic profile of the subjects, which is important when the peers wish to duplicate the research.

Period of Study

The materials and methods section should include the starting and ending dates, especially for clinical studies. The reader can interpret the results in the context of the period of study. As an example, a study done for the efficacy and side effects of a new drug soon after it has become available, may have a different outcome from a study done much later, when the drug has become established in its therapeutic use. This difference may occur because dosing and indications for the drug may change over time. Similarly, the duration is an important parameter in follow-up studies. For instance, the result of a study for efficacy of a newly introduced surgical technique for hernia repair, conducted over a period of 18 months, is likely to be viewed with skepticism. This is because the recurrences following hernia repair may occur much later in the postoperative period. The same study, when conducted over 10 years, is likely to be much more credible.

Study Design

Choosing an appropriate study design, to address a research question most effectively, is of utmost importance. However, when writing the study design in the materials and methods section, the details to be included depend upon the publication. For most journal articles, naming the study design is sufficient. For more exhaustive research publication, such as a thesis, the justification for choosing a particular study design is often included. Table 1 shows a summary of study designs and their indications.

Details of Subjects/Material Included in the Study [3]

Inclusion criteria are those characteristics which a potential subject must possess for recruitment into the study. The aim of writing the inclusion criteria is to define the study population and ensure uniformity of the subjects recruited. The subjects could

Table 1 Study designs [2]

Broad category	Characteristics	Types of study	Most suitable to
Experimental study	• Researcher manipulates exposure • Comparison group present	• RCT • Non-RCT	• Describe association • Determine causality
Observational - Analytical	• Researcher only measures exposure • Comparison group present	Cross-sectional	• Measure disease prevalence
		Case-control	• Identify multiple exposures • Describe association
		Cohort study	• Measure disease incidence • Identify multiple exposures • Identify multiple outcomes • Describe association • Determine causality
Observational - Descriptive	• Researcher only measures exposure • No comparison group	• Case reports • Case series • Surveys	• Give idea about a disease or its prevalence

be human, animal, body fluid or tissue. In human studies, the details should include age, gender, ethnicity, the population from which the subjects are selected (hospital/community-based) and the disease being studied.

It is important to define the disease condition. For instance, only mentioning 'wound infection' or 'peritonitis' as inclusion criteria are not sufficient. Wound infection could range from inflammation of wound edges to frankly purulent wound discharge. Similarly, 'peritonitis' could be interpreted differently by two clinicians. The issue is best resolved by adhering to the standard definition of diseases. If there is no unanimity in the literature regarding the precise definition of a disease condition, then the definition chosen by the researcher should be spelt out, preferably with supporting references.

Exclusion characteristics apply to those subjects who meet the criteria for inclusion. These features, if present, either interfere with the result of the research or expose the prospective subject to the likelihood of harm. For instance, subjects younger or older than a defined age may be excluded from a drug trial because of a greater risk of toxicity in these individuals. Similarly, people suffering from renal or hepatic dysfunction may be excluded from drug trials. Inability to give a valid consent may be yet another criterion for exclusion from a study. The investigator must be ready to provide a rationale for the inclusion or exclusion criteria chosen for the research.

A common mistake in listing the exclusion criteria is to name those characteristics which would have prevented the subject from being considered for inclusion in the first place. Thus, 'obesity' would not be exclusion criteria in a study of nutritional supplements in 'underweight children.' However, the presence of 'milk intolerance' could be valid exclusion criteria in this study.

Table 2 Correct and wrong grouping of inclusion criteria

Correct order	Illogical order
1. Stage I breast cancer	1. Total serum albumin ≥2.5 g/dL
2. Karnofsky performance scale = 90	2. Age ≥ 21 years
3. Age ≥ 21 years	3. Karnofsky performance scale = 90
4. Total serum bilirubin ≤1.2 mg/dL	4. Stage I breast cancer
5. Total serum albumin ≥2.5 g/dL	5. Total serum bilirubin ≤1.2 mg/dL

It is preferable to write inclusion criteria as positive statements. For instance, if the research is planned in nonpregnant women, it is advisable to write 'pregnancy' as exclusion criteria rather than writing 'non-pregnant' as inclusion criteria. When writing the inclusion criteria, the order should be logical and similar parameters should be listed together (Table 2).

Comparison Group

A control group is present in experimental studies, where it is treated differently from the intervention group. While the intervention group receives the treatment under study, the control group receives one of the following: no treatment, placebo, different dose, or different treatment. Except for this, the comparison group should match the intervention group in all the other aspects.

In observational analytical studies such as the case-control study, the control group comprises of the individuals who have had similar exposure to risk factors but do not have the disease. For example, while studying the risk factors for developing urinary bladder cancer in workers in the chemical industry, controls could be those employees who have worked for a similar period but are free from the disease.

Unlike the experimental studies, where the controls are randomly chosen from the group of individuals suffering from the disease, selecting controls for the case-control studies is more challenging and needs greater care. While population-based controls are ideal, there are logistic problems in choosing them. Instead, controls are selected from hospitalized individuals, family members or volunteers. Care is needed, when hospitalized people are taken as controls, to ensure that the concurrent condition, for which the control is hospitalized, does not confound the study. As far as possible, the control group should match the intervention group regarding age, gender, ethnicity and sociodemographic profile. The size of control and intervention groups should match.

Sampling, Randomization, Allotment Concealment and Blinding

In an ideal situation, all the subjects of the population should be included in the study to get the most accurate result. However, as this is not practical, a sample is chosen from the population for the conduct of the research. The study sample is selected from the population by different sampling methods. The sampling method

could be random or non-random. In random sampling, each subject in the study population has an equal chance of getting selected. Such a sampling could be done using random number tables. Random sampling methods are suitable for community-based research, where the study population is present in a defined area at a given time. On the other hand, for most of the hospital-based clinical studies non-random sampling methods are more suitable, as all the subjects are not available at the same time. They are recruited over a period, as and when they present themselves to the hospital for treatment. Table 3 gives the features of these two sampling methods and their subtypes.

An experimental study, such as a drug trial, randomizes the subjects into treatment and control groups. This randomization could be done using a random number table or a randomizer application. The entire study population could be randomized at once (simple randomization) or could be divided into smaller blocks (block randomization). Thus, the cases and controls at any point in time are relatively equal in number. This method is especially useful if the condition is uncommon, the study population is small, or the study is prematurely terminated.

While randomization of subjects into intervention and control groups is essential, it is equally important to conceal from the researcher, the study group allocated to a subject. This may be done using opaque and sealed envelopes, or allocation by a person independent from the study. In the absence of allocation concealment, the researcher may knowingly or otherwise influence the selection of subjects into intervention or control group.

Just as randomization and allocation concealment minimize the bias at the time of treatment allocation, blinding reduces the bias while recording the results and

Table 3 Sampling methods [2]

Broad category	Sub-types	Features
Random (probability) sampling	Simple	• Subjects are selected one at a time, using draw of lots or random number table
	Systematic	• Sampling technique is devised in a way that all the subjects are randomly selected at one go
	Cluster	• The population is divided into homogeneous groups. The study population is selected from any one group using simple or systematic sampling technique
	Stratified	• The study population is stratified based on characteristics such as age, gender or severity of the disease. Subjects are randomly selected from each stratum
Non-random (non-probability) sampling	Convenience	• Subjects selected according to researcher's convenience • No specific selection criteria
	Purposive	• Subjects selected according to researcher's convenience • Selection based on a list of selection criteria
	Quota	• Sampling stops once a certain number of subjects are recruited • Sampling done in a non-random fashion

evaluating the outcome. Depending upon the feasibility, the subjects could be unaware of their study group (single blinding) or both the subject and the researcher may be unaware whether the subject is allocated intervention or control group (double blinding). It is imperative to mention the details of the techniques of sampling, randomization, allocation concealment and blinding, when applicable.

Deciding the Sample Size

The outcome of a research study and its extrapolation to the general population is predominantly based on statistical methods. In a well-done study, the results are fairly representative and applicable to the larger population. The materials and methods section should define the basis for arriving at a given sample size for the proposed study.

The prior information needed to calculate the sample size includes the study design, nature of variable under evaluation, an estimate of the expected outcome and the desired level of precision. There are computer applications available which calculate the sample size, once the required values are put in the program. The sample size needs to be an adjustment for dropouts or covariates. Some of this information is based on previous similar studies, which should be cited at this stage. For pilot studies, which are novel and for which no prior data is available, sample size calculation is not necessary [2, 4–6].

Since most of the theses for a postgraduate medical course are completed within a limited timeframe, it may not be feasible to recruit the calculated number of subjects during the available time. Under such circumstances, the researcher must first calculate the sample size and then explain why a lesser number of subjects are proposed to be included in the study. It should be understood that the outcome of such a study is not valid and generalizable.

Ethical Considerations and Informed Consent

It is mandatory for all human and animal research to conform to guidelines issued by the regulatory bodies. Templates for informed consent are available at the World Health Organization web page [7]. Indian Council of Medical Research has published a document which describes the ethical considerations relating to human research [8].

In the materials and methods section, it only needs a mention that the subjects gave informed consent to participate in the study and the institutional committee has cleared the research proposal. Some journals compulsorily require registration of the research project with the clinical trial registry. For clinical trials in India, the plan requires registration at the website of Clinical Trials Registry—India (CTRI). [www.ctri.nic.in/] The consent certificate, patient information sheet, and institutional ethics committee certificate should be included in the annexures.

The consent form suggested by the WHO has two parts: participant information sheet and consent document. The patient information sheet broadly consists of the following details:

- Name, contact information and institutional affiliation of the principal investigator.
- Details about the research proposal in language easily understood by the participant.
- The reason for selecting the participant.
- Possible risks, benefits, and compensation for the participant.
- Permission to use and share results without breaching confidentiality.
- The option of withdrawing from the study at any point.

Some of the general principles in biomedical research include:

- The need to conduct human clinical trial is essential as there is no other option.
- The participants should have given a voluntary informed consent.
- There is non-exploitation of the participants due to any reason.
- Ensuring the privacy and confidentiality of the participants at all times.
- Due care and precaution must be maintained at all times to minimize the risks to the participant.
- Preserving and dissemination the records relating to the research in the public domain, for the benefit of all.
- The researcher must take full responsibility for the conduct of the study according to the guidelines.

Details of the Intervention/Procedure Done

After having described the subjects, the next step is to give details of the data collection, intervention and follow-up, if any. The data collected could be in the form of demographic details, clinical history and examination, questionnaire, laboratory investigations, radiological tests, molecular biology techniques and others. For using a standard data collection procedure, giving reference to the source of information is sufficient. However, a modification of a technique or a new method should be described in detail, to enable the readers to duplicate it.

In experimental studies, the exact particulars of the intervention should be described. The units of measurement, evaluation scales, laboratory kits and instruments used should be mentioned. In follow up studies, the frequency of follow up and data collection should be described. This information is often best given in the form of a flowchart. At times, it may be advisable to provide the details of the procedure as an annexure.

Outcome Measures

All medical research starts with a research question and a hypothesis. The study is designed in a way to answer the research question most effectively. Depending upon

the objective of the study, the research question may be about the efficacy of a treatment modality, risk factors or causation of a disease, sensitivity, and specificity of a diagnostic modality or prevalence or incidence of a disease condition.

The researcher must identify at the outset, the one result of the study which will answer the research question. For instance, in a study of the effectiveness of a new antibiotic in surgical prophylaxis, the incidence of postoperative wound infection would be the primary outcome measure. Similarly, in a case-control study of the association of pesticide exposure to breast cancer, serum levels of pesticide in cases and controls would be the primary outcome measure.

A study may aim to explore more than one parameter. For example, in the previously mentioned study of the effectiveness of a new antibiotic for surgical prophylaxis, the researcher may also plan to explore the cost and the safety of the medicine. The latter goals of the study are called secondary outcome measures. Identification of the primary outcome measure is necessary because it is used for calculation of the sample size. While a study may have many secondary outcomes, it is preferable to have a single primary outcome.

Data Management and Statistical Analysis

The researcher must describe the type of data expected at the end of the study period. The proposed statistical tests to be applied and the possible computer application to be used for the statistical analysis must also be mentioned at this stage. It is possible that the data obtained at the end of the study period may turn out to be different from the one expected at the beginning. For instance, the distribution of the data may be skewed, while the tests were proposed for normally distributed data. Under such circumstances, the researcher may apply the appropriate statistical tests, which may be different from those planned at the start of the study. The reason for such a change must be explained, especially in a research thesis. Figure 1 shows a plan for the research methodology.

General Guidelines for Writing the Materials and Methods Section

Following are the broad guidelines to be followed while writing this section:

- This section is written in future tense at the proposal stage. On completion, at the time of reporting, either as a journal article or as a thesis, past tense is used.
- Enough details should be provided, preferably with references, to enable other researchers to replicate the study.
- All the data proposed to be collected in this section should be presented in the results section. It is important that the results must not give data for which there is no proposal in the materials and methods section. Similarly, it is to be ensured that no data proposed in materials and methods are missing from the results

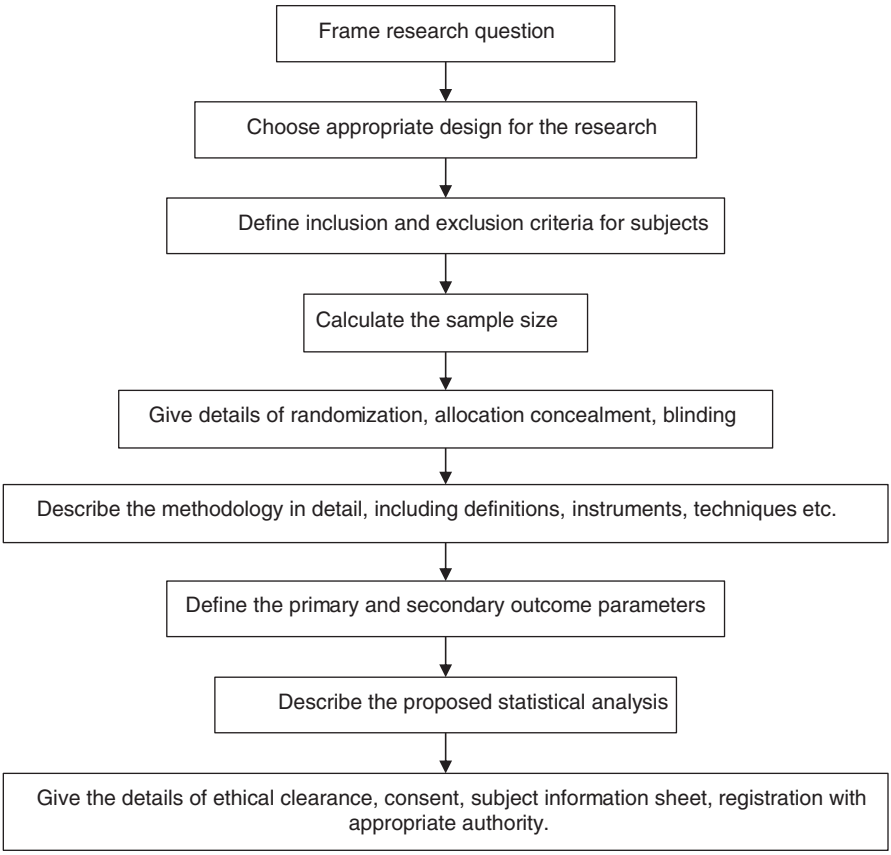

Fig. 1 Flowchart for research methodology

section. It may occasionally happen that the researcher may not be able to carry out all the components of proposed study due to unforeseen events, such as non-availability of some equipment or laboratory test. Under these circumstances, the reason for deviation from the proposal must be explained.

In conclusion, materials and methods section lays down the framework for conduct of research, not only for the peers but also for the researcher as well. Precisely written methodology ensures reproducible results and valid outcome.

Case Scenarios

1. What is wrong with the following inclusion and exclusion criteria?
   "The inclusion criteria were patients with painless hematuria, histologically proven grade 1 transitional cell carcinoma of urinary bladder and normal renal functions. Patients with painful hematuria, grade 2 and 3 transitional cell carcinoma and deranged renal function were excluded from the study."

2. Which of the following study designs needs the smallest sample size?
   (a) Descriptive study.
   (b) Experimental study with crossover design.
   (c) Experimental design with a control group.
   (d) Experimental study in a single group with pre and post type of design.

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