Chapter 3
MATERIALS AND METHODS

3.0 Introduction

Research methodology is a way to systematically solve the research problem. The scope of research methodology is wider than that of research methods. Thus, when we talk of research methodology, we not only talk of the research methods but also consider the logic behind the methods we use in the context of our research study and explain why we are using a particular method/technique and why we are not using others. So that research results are capable of being evaluated either by the researcher himself/herself or by others. Why a researcher study has been undertaken, how the research problem has been defined, in what way and why the hypothesis has been formulated, what data has been collected and what particular method has been adopted, why particular technique of analyzing data has been used and similar other questions are usually asked when we talk of research methodology concerning a research study by Kothari (2007).

Research work cannot be carried out successfully without perfect materials and methodology, for the present study, scientific methodology has been used to reduce the possibility of any type of errors. This chapter has been presented under following headings:

1. Objectives
2. Hypothesis
3. Research Design
4. The sample
5. Sampling Techniques
6. Tools and Techniques
3.1. Objectives

The objectives of the present study have been formulated to explore and investigate a new era for “Impact of Indigenous foods MKK (Methi Dana, Kale Til & Kali Jiri) on Blood Glucose level of middle aged NIDDM patients. The objectives are as follows:

1. To compare the effect of MKK and MKK + hypoglycemic drugs on blood glucose level of NIDDM patients.
2. To compare the change in weight of NIDDM patients taking MKK and MKK + hypoglycemic drugs.
3. To study the Dietary pattern of NIDDM patients taking MKK and MKK + hypoglycemic drugs.
4. To study the Nutrients intake of NIDDM patients taking MKK and MKK + hypoglycemic drugs.

3.2 Hypothesis

According to David Nachmias et.al (1981) “Hypothesis are tentative answers to research problems. They are expressed in the form of a relation between independent and dependent variables, Hypothesis are tentative conjectures because their veracity can be evaluated only after they have been tested empirically. When a researcher suggests a hypothesis, he or she has no assurance that it will be verified. A hypothesis is constructed and if it is rejected, another one is put forward; if it is accepted, it is incorporated into the scientific body of knowledge.”

Hypothesis of the present study are:

1. There shall be no significant difference in mean of fasting and postprandial blood glucose levels of NIDDM patient taking MKK and MKK + Oral Hypoglycemic drugs.
2. There shall be no significant difference in change in mean body weight of NIDDM patient taking MKK and MKK + Oral hypoglycemic drugs.
3. There shall be no significant difference in dietary patterns of NIDDM patient taking MKK and MKK + Oral hypoglycemic drugs.
4. There shall be no significant difference in mean nutrient intake of NIDDM patient taking MKK and MKK + Oral hypoglycemic drugs.
3.3 Research Design

All items in any field of Inquiry constitute a ‘Universe’ or ‘Population’. When the field of inquiry is large it is difficult to undertake a complete enumeration of all items in the ‘population’. Thus when filed studies are undertaken in practical life, consideration of time and cost almost invariably lead to a selection of respondents i.e. selection of only a few items. The selected respondents constitute a “Sample”.

Research design of experiment provides a valuable source of help in handling the valuable material for reasonable conclusion.

The statistical aspects of the design of an experiment are
(a) Selecting the treatment (MKK) whose effects are to be studied.
(b) Specifying a layout for the experimental limits (NIDDM Patients of age 40-50 years) to which the treatments are to be applied.
(c) Providing rules according to which the treatments are to be distributed among the experimental limits.
(d) Specifying what measurements (10 gm MKK powder twice a day before meal) are to be made for each experimental unit.

All these things must be accomplished in such a way that the techniques to be used in the analysis of the results should be clear prior to the conducting of an experiment. In present study experimental design used was completely randomized design.

3.4 Sample and Sampling Technique

a. The Sample

When a small group is taken as the representative of the whole, the study is called sampling study. The whole group from which the sampling has been drawn is technically known as universe or population and the group actually, selected for study is known as sample. Bajpai (1989) A sample as the name implies is a smaller representation of a large whole Goode (1952). According to young (1954) “Sample is a miniature picture of the universe”.
b. The Purpose of Sampling

It is more or less impossible to study every single person in a target population so researcher selects a sample or sub-group of the population that is likely to be representative of the target population.

c. Criterion for the Study

Criterion for the Study was as follows

- NIDDM patients; age 40 to 50 years, were selected belonging to any socioeconomic status, cast, religion and sex. This was because type 2 Diabetes usually develops after the age of 40 and diabetes is not affected by above particulars.
- NIDDM patients who were willing to take indigenous foods (MKK) ingredient for the experiment (Group I).
- NIDDM patients who were on oral hypoglycemic drugs and willing to take indigenous food (MKK) ingredients with regular medication (Group II).

d. Sampling unit and Sourcing of Samples

Samples were collected from local clinic, social clubs, diagnostic centers and personal contacts from Indore. Here sampling unit is NIDDM diabetic patients aged between 40 to 50 years, staying at Indore city, Madhya Pradesh, India.

e. Sample size

As per the recommendation of RDC committee 100 samples were selected for this study. Samples were divided into two groups.

1. Group I (sample size 50) NIDDM patients willing to take MKK powder and not taking any other medicine.
2. Group II (sample size 50) NIDDM patients already on hypoglycemic drugs but willing to take MKK powder.
f. Sampling Technique
Sampling is the process of selecting a sufficient number of elements from the population. There are various types of sampling method. In the present study random sampling method was used. Non-insulin dependent diabetic patients were selected randomly. It is the simplest possible design and its procedure of analysis is also easier.

3.5 Tools and Techniques
In this study following tools were implemented for data collection

- Interview schedule
- Observation

3.5.1. Interview Schedule: According to Goode et. al., (1952), interview schedule is the name usually applied to a set of questions which are asked and filled by an interviewer in a face to face situation with another person. An interview schedule was used to collect the data regarding the age, address, phone number; information about the occupation, history of disease in the family, income group, dietary assessment, outside eating habits, intake of artificial sweetener, alcoholic/nonalcoholic, smokers/nonsmokers, vegetarian /non vegetarian, and other health problems etc. (refer Annexture no. 1)
According to Floyd J., *et. al.*, An interview schedule is the guide an interviewer uses when conducting a structured interview. It has two components: a set of questions designed to be asked exactly as worded, and instructions to the interviewer about how to proceed through the questions. The questions appear in the order in which they are to be asked. The questions are designed so they can be administered, exactly as they are written. The questions need to be communicated about what information is being asked from the respondents.

Following information’s was collected through this Interview schedule:

**a. General Information:** General information like name, sex, age, education, occupation, family history of diabetic patients was collected by using interview schedule. (Refer Annexure 1)

*Age:* - Age was calculated according to the date of birth mentioned by the sample and checked in their medical report or any valid documents issued by Central/State Govt.

*Educational qualification* was categorized into 4 levels
Under 12\(^{th}\) / Graduate / Postgraduate and any other professional degree

*Occupational group* was divided into 3 classes for Women and 2 classes for Men.
For Women: 1Service 2, Business 3. Housewife
For Men: 1 Service 2, Business

*Family History-* Diabetic history about family members has been taken by asking questions to the samples.

**b. Food Habits and Consumption of food groups of samples:** 24 hours dietary recall method was used to collect information related to food habits and consumption of food group. Daily food intake of each sample was analyzed on the basis of food groups like cereals, pulses and legumes, fats and oils, sugar, milk and milk product, fruits and vegetables.(Refer annexure 2a ,2b)

1. *Food Consumption:* - Food consumption or dietary assessment is a comprehensive evaluation of a person’s food intake. It is one of the four parts of a nutrition assessment done in a clinical setting. According to W.A. Grante (1999), there are many ways to
document dietary intake, the accuracy of the data is frequently challenged, however, since both questioning and observing can impact the actual intake. During nutrition interview the practitioner may ask what the individual ate during the previous twenty-four hours, beginning with the last item eaten prior to the interview. Practitioners can train individuals on maintaining a food diary, and they can request that the record be kept for either three days or one week. Documentation should include portion sizes and how the food was prepared. Estimating portion sizes is difficult therefore food models and photographs of foods are used to assist in recalling the portion size of the food.

At first, study participants were asked about various food habits and consumption related queries like vegetarian (diet that excludes meat, eggs, fish, and food derived from animal other than a diary products) or non vegetarian (diet frequently consist of meat, fish and egg), taking some special foods prescribed by the doctor, liking and disliking of food etc. during interview session to get actual details of food habits. Food habits related information helped us a lot for proper dietary assessment of study participants.

Information collected and recorded regarding meal pattern of 24 hours (one day meal details) with details of raw ingredients used in preparations of food. The amount of raw and cooked preparation was recorded with the help of household weighing balance/ measuring cup/ measuring glass/ measuring spoons/ standardized kotaris/cardboard chappatis.(Annexure 3 and 4). Information related to food group intake was collected before treatment schedule started.

2. Dietary Pattern

Food frequency questionnaires are used to gather information on how often a specific food or category of food is eaten. The food guide suggests portion sizes and the number of servings from each food group to be consumed on a daily basis, and can also be used as a reference to evaluate dietary intake. During the nutrition interview, data collection included questions about the individual’s related to:

- Different types and amount of cereals, grains, pulses, legumes and their products
- Amount and types of vegetables, fruits, milk and milk products
- Amount of sugar, oil and ghee
• Outside eating habits and their frequency

For collecting dietary data 24 hours dietary recall method was used. To begin with standardization of food was done. The subject was asked to recall all food items consumed either in home or outside. As foods commonly used were taken in utensils of different size (like katori can be small medium and large size), standardized katories, cups, glasses, spoons, serving spoon to estimate the quantity of food consumed (B. Shrilaxmi, 2000). This amount was cooked and weighed again and as per serving calculated. Then samples were asked about the amount of food they consumed in relation with the size of the utensils.

In order to increase accuracy of quantity of wheat flour consumed in chapattis, subject was shown the cardboard chapattis different size and thickness were shown for further accuracy. Consistency of dal, amount of sugar in tea and milk, quantities of oil or fat in vegetables etc. were also checked. Subjects were asked about specific food which was consumed outside and also for the food items which were consumed thrice, twice or once a day/week, rarely or never used. Quantity of beverages was recorded in size and number of glasses consumed. Probing questing was asked to help the subjects remember all that has been consumed. Questions were regarding the method of preparation of meal, portion size, amount of oil/ghee being used in the meal, as well as approximate sizes of the meal.

c. Nutrient Intake: To calculate nutrient intake in this study, the daily food intake by each sample was split into their ingredients. The standardized utensils were used to convert cooked food into raw ingredients. The nutritive value of these ingredients in terms of energy, carbohydrates, proteins and fats were calculated by using the food composition table of the book by C. Gopalan, et al., 2004.

3.5.2. Observation: Observation is way of gathering data by watching behavior, events, or noting physical characteristics in their natural setting. Observations can be overt or direct (everyone knows they are being observed) or covert or indirect (no one knows they are being observed and the observer is concealed). The benefit of covert observation is that people are more likely to behave naturally if they do not know they are being
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observed. In this the information was collected by observation without interviewing the respondents in the following heads:

a. Anthropometric Measurement:

(i) Height: Standard height measuring instrument (company: Prestige, India) was used for height measurement.

**Height procedure for the present study**

Samples were asked to remove their shoes and hair ornaments. Samples were kept straight without jumping. The back of the head, back, buttocks, Calve and heels should be touching the upright, feet together. The headpiece of the stadio meter or the sliding part of the measuring rod lid lowered so that the hair (if present) is pressed flat. Height was measured in cms.

(ii) Weight: Weight machine (Crown Victoria DX,) manufactured by Ramon Surgical, Delhi was used for weight measurement. Body weight was measured for each sample before schedule started and after schedule was over.

**Weighing procedure for the present study**

Weights of the samples were taken in kgs, by using spring weighing machine. Samples were asked to wear minimum clothes; the heavy outer garments and shoes were being asked to remove. The samples were asked to empty their pockets. Samples were asked to stand barefooted on weighing machine. They were asked to stand in the center of the machine, weight distributed evenly on both feet. Samples were kept straight without jumping, leaning or holding anything, while the weight is recorded. The zero error of the weighing scale was checked before taking the weight.

b. Blood Sugar Level (Biochemical Analysis): In the development of any disease biochemical changes can be expected to occur prior to clinical manifestations (Mehtab, S. Bamji. 1996). Biochemical tests related to fasting and postprandial blood sugar was taken into account before and after one month. The laboratory procedures applied for the analysis of fasting and postprandial blood glucose was GOD-POD method.The blood
sample was collected through syringe by pathologist and transferred into test tube and tested through GOD-POD method in pathology lab. Fasting blood glucose level was measured in the morning or minimum after 8 hours of fasting condition and postprandial blood glucose level was measured exactly after 2 hours of meal.

**GOD-POD Method**

Glucose is the reducing monosaccharide that serves as the principal source of cellular energy in the body. It enters into the cell under the influence of insulin and undergoes a series of chemical reactions to produce energy. Lack of insulin or resistance to its action at the cellular level causes diabetes. Therefore, in diabetes mellitus the blood glucose level are very high. However, high blood glucose level is also observed in the pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease whereas low glucose level is associated with starvation, hyperinsulinaemia, neoplasms or insulin induced hypoglycemia.

**Principle**

Glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4 amino- antipyrene(4-AAP) and phenol in the presence of peroxidase(POD) to yield a red quinoeimine dye that is measured at 505nm. The absorbance at 505 nm is proportional to concentration of glucose in the sample.

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\text{Glucose} + 2\text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Gluconate} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-AAP} + \text{Phenol} \rightarrow \text{Quinoeimine Dye}
\]

Absorbance of the colored solution is directly proportional to the glucose concentration, when measured at 505nm.

**Experimental Schedule**

Total 100 samples were selected and divided into two groups.

3. Group I NIDDM patients willing to take MKK powder and not taking any other medicine.

4. Group II NIDDM patients already on hypoglycemic drugs but willing to take MKK powder.
Control group was not formed because it is risky for NIDDM Patients to live without any medication. Group II was also treated with M KK along with hypoglycemic drug which is a common controllable factor.

### 3.6 Preparation of M KK Powder (Indigenous food)

As per the guidance and suggestions of Ayurvedic doctor equal proportion of M KK can be beneficial for the treatment of NIDDM patients. Thus 100 gm Methidana, 100 gm Kaletil, 100 gm Kaliziri were taken, cleaned, mixed and grinded in the form of fine powder. The feeding program was conducted on the selected samples by giving M KK powder.

- **Amount of feed** - 10 grams at a time (twice a day)
- **Timing** - 15 min. before the meal
- **Duration** - one month

### 3.7 Ethical Issue

As per the consultance from ayurvedic doctor information and relevant documents on all ingredients (M KK) of indigenous food were collected and M KK powder was prepared under their guidance. The methidana seeds are found all over India and are usually used as one of the major constituent of Indian spices. It has long been used as a spice and herbal remedy in India. People harvest and roast dried seeds of the plants for food flavoring and medicinal purpose. Similarly for thousands of year’s kale tils have been a source of food and oil in Indian diet. Kaliziri is widely used in the Ayurvedic system of medicine preparation and distributed widely in India. The review showed, there was no risk of mental and physical injury, disability and/ death by using these ingredients. Voluntary concern of the study samples was considered as a must before selecting them.

### 3.8 Health Problems

Information related to other health problems (like Blood pressure, Heart disease, Kidney disease, Vision problem, joint problem etc) was collected by studying clinical symptoms and/ by biochemical and biophysical reports and by asking during interview session.(Refer Annexure- 1)
3.9 Data Analysis

Analysis of data requires a number of closely related operations like editing, coding, classification, tabulation and then drawing statistical inferences. Consultations were made with statisticians and then statistical package of social science SPSS 17 was used for whole data analysis process. The responses of the samples were expressed in frequency and percentage. The collected data was analyzed and statistical evaluation of hypothesis was done for statistical significance by applying t test and chi test using SPSS 17.

The t-test is used for testing differences between two means. In order to use a t-test, the same variable must be measured in different groups, at different times, or in comparison to a known population mean.

Chi-square is a versatile statistical test used to examine the significance of relationships between two (or more) nominal-level variables.

For the statistical evaluation of hypothesis $H_0$ No. 1 t test was used to test for significant difference between mean of two experimental groups to compare the effect of MKK powder on fasting and postprandial blood glucose level of group I and group II.

For the statistical evaluation of hypothesis $H_0$ No.2 t test was used to test for significant difference between mean weights of two experimental groups to compare the effect of MKK powder on weights of group I and group II.

For the statistical evaluation of hypothesis $H_0$ No.3 t test was used to test for significant difference between mean consumption of food groups (Cereals, Pulses, Milk & Milk product, fruits, vegetables, fats, oil and sugar) of NIDDM patients of both the groups (I & II). Chi square test was applied for studying food habits and the dietary pattern of group I and group II.

For the statistical evaluation of hypothesis $H_0$ No.4 t test was used to study significant difference between mean of calorie and nutrient intake of group I and group II.