This chapter presents the research methodology adopted to investigate the “Effect of Barley Consumption on Cardiovascular Risk Factors among patients with Metabolic Syndrome” Research methodology is the backbone of all scientific research and it is an attempt to minimize the influence of the researcher’s bias on the outcomes. It includes research design, setting, outcome measures, data collection technique, plan for data collection, intervention strategy and statistical analysis. For reporting the methodology of the present study, the (Consort 2010) flow diagram was used. The methodology used for the present study described below:

3.1. Study Area: This study was performed at the Sir Sundar Lal Hospital Banaras Hindu University Varanasi, which is situated in Uttar Pradesh India. Sir Sundar Lal Hospital attached to Institute of Medical Sciences; Banaras Hindu University Varanasi an apex tertiary care hospital primarily for teaching and training. The study was undertaken as an interdepartmental collaborative research between department of Community Medicine and Department of Cardiology.

3.1.1 Geo-Social Characteristics of the Study Centre

About District

Varanasi, or Benaras, (also known as Kashi) is a city on the banks of the Ganges in the Uttar Pradesh State of North India, and is also one of 72 districts in the Uttar Pradesh. The Kashi Naresh (Maharaja of Kashi) is the chief cultural patron
of Varanasi, and an essential part of all religious celebrations. Varanasi is home to the Kashi Vishwanath Temple, one of the most important places of worship in the country, considered to be one of the twelve Jyotirlingas of Lord Shiva, holding great significance in the spiritual history of India.

Mark Twain, the English author and literature, who was enthralled by the legend and sanctity of Benaras, once wrote:

“Benaras is older than history, older than tradition, older even than legend and looks twice as old as all of them put together”

3.1.2 Geography

Varanasi is located at an elevation of 80.71 metres (264.8 ft) in the centre of the Ganges valley of North India, in the Eastern part of the state of Uttar Pradesh, along the left crescent-shaped bank of the Ganges, averaging between 15 metres (50 ft) and 21 metres (70 ft) above the river. The "Varanasi Urban Agglomeration" - an agglomeration of seven urban sub-units - covers an area of 112.26 square km. The urban agglomeration is stretched between 82° 56’E - 83° 03’E and 25° 14’N - 25° 23.5’N. Varanasi is often said to be located between two confluences: one of the Ganges and Varuna, and other of the Ganges and Assi.

3.1.3 Climate

Varanasi experiences a humid subtropical climate with large variations between summer and winter temperatures. The dry summer starts in April and lasts until June, followed by the monsoon season from July to October. The temperature ranges between 22 and 46 °C (72 and 115 °F) in the summers. Winters in Varanasi
see very large diurnal variations, with warm days and downright cold nights. Cold waves from the Himalayan region cause temperatures to dip across the city in the winter from December to February and temperatures below 5 °C (41 °F) are not uncommon. The average annual rainfall is 1,110 mm (44 in). Fog is common in the winters, while hot dry winds, called loo, blow in the summers. In recent years, the water level of the Ganges has decreased significantly; upstream dams, unregulated water extraction, and dwindling glacial sources due to global warming may be to blame.

**Table No. 3.1.1 : Demographic profile of Varanasi district**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Population</td>
<td>3,676,841</td>
</tr>
<tr>
<td>Male</td>
<td>1,921,857</td>
</tr>
<tr>
<td>Female</td>
<td>1,754,984</td>
</tr>
<tr>
<td>Population Growth Rate</td>
<td>17.32%</td>
</tr>
<tr>
<td>Area</td>
<td>1,535 Sq. Km</td>
</tr>
<tr>
<td>Density (per km²)</td>
<td>2,399</td>
</tr>
<tr>
<td>Proportion to Uttar Pradesh Population</td>
<td>1.84%</td>
</tr>
<tr>
<td>Sex Ratio (Per 1000)</td>
<td>909</td>
</tr>
<tr>
<td>Literacy</td>
<td>67.09%</td>
</tr>
<tr>
<td>Male Literacy</td>
<td>83.66%</td>
</tr>
<tr>
<td>Female Literacy</td>
<td>48.59%</td>
</tr>
</tbody>
</table>

*(Source: Census India 2011)*
3.1.4 Educational Facilities

Varanasi is one of the most ancient cities of learning. This was a place where hundreds of enlightened beings lived at a time. In every street, you had an enlightened being to meet.”

– Sadhguru

Historically, Varanasi has been a centre for education in India, attracting students and scholars from across the country. It is home to a number of colleges and universities. Most notably, it is the site of Banaras Hindu University (BHU), which is one of the largest residential universities in Asia with over 29,000 students. The Indian Institute of Technology, BHU is designated an Institute of National Importance and is one of 16 Indian Institutes of Technology. Other colleges and universities in Varanasi include Imania Arabic College, the Institute of Integrated Management and Technology (IIMT), Mahatma Gandhi Kashi Vidyapith, Nav Sadhana Kala Kendra, Sampurnanand Sanskrit University, Sri Agrasen Kanya P.G. College, and Udai Pratap Autonomous College. Institute of Medical Sciences, Banaras Hindu University, is the premier medical institution of this country. Over the last decade, it has been consistently ranked amongst the top 20 Medical Colleges of the country and in 2012 was rated as the 6th Best Medical College.

3.1.5 Health Services

Health services in Varanasi district are provided through number of hospitals and clinics. A brief list is given below in table 3.1.2:
### Table: 3.1.2 : Health services in Varanasi district

<table>
<thead>
<tr>
<th>Hospitals in Varanasi</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Homeopathic</td>
<td>13</td>
</tr>
<tr>
<td>Allopathic</td>
<td>80</td>
</tr>
<tr>
<td>Ayurvedic</td>
<td>28</td>
</tr>
<tr>
<td>Unani</td>
<td>1</td>
</tr>
<tr>
<td>Community Health Centre</td>
<td>6</td>
</tr>
<tr>
<td>Primary Health Centre</td>
<td>30</td>
</tr>
<tr>
<td>Family welfare Centre</td>
<td>41</td>
</tr>
<tr>
<td>Family welfare sub-centre</td>
<td>305</td>
</tr>
<tr>
<td>T.B. Centre</td>
<td>2</td>
</tr>
</tbody>
</table>

(Source: District Statistical Magazine 2007)

Varanasi has several hospitals, including Heritage Hospital, Marwari Hospital, Pitambari Hospital, Mata Anand Mai Hospital, Rajkiya Hospital, Ram Krishna Mission Hospital, Shiv Prasad Gupta Hospital, Pandit Deen Dayal Upadhyay hospital (managed by state govt) etc.

The largest & super speciality hospital is **Sir Sundar Lal Hospital (University Hospital)** a premier tertiary care referral center catering to the medical needs of an approximate population of 15 crores of the states of Uttar Pradesh, Bihar, Jharkhand, Madhya Pradesh, Chattisgarh, and neighbouring countries of Nepal and Bangladesh. The SS Hospital provides expert facilities to patients in this region of our country. The hospital is well equipped with state-of-the-art modern facilities under the care of a dedicated competent faculty overall.
3.2 Study Population

This study was performed at the Department of Cardiology, Sir Sundar Lal Hospital Banaras Hindu University Varanasi on an outdoor patient basis from February 2014 to June 2015. Participants were men and women aged 30-70 years who were visiting the OPD and had a confirmed diagnosis of Metabolic Syndrome. The patients who came for the first time (old or new cases) at OPD of cardiology department were considered for this study.

3.2.1 Study Period: February 2014 to June 2015

3.3 Sampling Technique

3.3.1 Inclusion Criteria: The criteria for identifying the patients of metabolic syndrome was depending on the definition given by the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP ATP III) (modified 2004). The inclusion criteria were as follows:

1. Waist circumference (males: ≥90 cm and for females: ≥80 cm),
2. Triglycerides ≥150 mg/dl (1.7 mmol/l),
3. Low HDL (Males <40 mg/dl (1 mmol/l) and or females <50 mg/dl (1.3 mmol/l)),
4. Systolic blood pressure ≥130 mmHg and/or Diastolic blood pressure ≥85 mmHg.
5. Elevated blood sugar (fasting blood sugar ≥100 mg/ dl (5.6 mmol/l).

To be enrolled in the study, patients had to have ≥3 of the above-mentioned criteria to be classified as having metabolic syndrome.
3.3.2 Exclusion criteria

The exclusion criteria were as follows:

1. Subjects were excluded if they were diagnosed with the following diseases such as thyroid disease or diabetes mellitus, cardiovascular diseases, chronic liver or kidney disease, advanced cancer or any other chronic disease.
2. Subjects were excluded if they were already on a specific diet and exercise recommendations.
3. If they had a history of eating disorder or substance abuse, severe mental illness, unstable medical status.
4. Fasting blood glucose > 126 mg/dl.
5. If they were not willing to adhere to the prescribed diet.

3.3.3 Ethical Consideration: The present study was approved by the institutional ethics committee on biomedical research in humans of institute of medical sciences Banaras Hindu University Varanasi (Annexure I).

3.3.4 Sample size determination

The following formula used to calculate the sample size for this study was:

\[ N = 2 \left( Z_\alpha + Z_\beta \right)^2 \left( S_1^2 + S_2^2 \right) / \left( m_1 - m_2 \right)^2 \]

- \( Z_\alpha = 1.64 \)
- \( Z_\beta = 0.84 \) (at 80% power)
- \( M_1 \) & \( M_2 \) = the mean of triglycerides in the intervention and control group at the end of trial
- $S_1$ & $S_2$ = the SD in the intervention and control groups respectively.
- There are five components of metabolic syndrome but for the sample size calculation, triglycerides was considered because other parameters were not change much between the point of intervention and final measurement.
- We did not come across any study in which barley was given to reduce the risk factors. Therefore, first 20 study subjects from the intervention and control group each were selected to measure the mean and SD of triglycerides at the end of trial.

$$N = 2 \times (1.64+0.84)^2 \times \frac{(42.1)^2 + (45.06)^2}{(176.1-160.6)^2}$$

$$= 12.3 \times \frac{(1772.41+2030.40)/(15.5)^2}{240.2}$$

$$= 12.3 \times 15.82$$

$$= 194.5$$

$N=195$

The trial size for this study was calculated to be 195 (98 each for both arms) for both arms. Considering loss of about 32% in all follow-ups (after 12-week) we have started this study with 258 subjects.

3.4 Study Design

This study was based on a randomized, controlled, parallel group design:
Figure 3.4.1: Flow diagrams showing the time-flow of participants recruited, randomized and followed-up. (CONSORT 2010)
The whole study was conducted in three phases:

Phase I: Screening for eligibility and selection of subjects.

Phase II. Stabilization Phase.

Phase III. Randomization & 3 month Intervention.

Phase I: Screening for eligibility and selection of subjects.

A metabolic screening was carried out for all the cases (n=1020) who came for the first time to cardiology OPD (old & new cases) who were suspected to have metabolic syndrome by the physicians during the study period for eligibility in the study. Among them 742 were excluded because they did not meet the inclusion criteria or they were declined to participate. Overall, two hundred seventy eight men and women aged 30 to 70 year, with metabolic syndrome were recruited for this study (Figure 3.4.1). The diagnosis of metabolic syndrome was based on the National Cholesterol Education Program Adult Treatment Panel III criteria. Details of the inclusion & exclusion criteria are previously described. During screening, health status and medical history, of the participants were examined by interview. Written informed consent was obtained from all potential participants at the time of screening visit.

Phase II. Stabilization Phase

Participants meeting all inclusion criteria as determined at initial screening visit were eligible to enter in the diet stabilization phase. During the stabilization phase, all participants were instructed to eat a “background diet (control)” that was patterned according to the NCEP (ATP III) Step -1 diet (≤30% of total energy as fat, ≤10% of energy as SFA, and ≤300 mg dietary cholesterol/d) for 4 weeks. Detailed
descriptions of the recommended diet during stabilization phase are given below (Table 3.4.3). During this period all the participants were given individualized healthy nutrition counselling on the basis of their dietary history and lifestyle habits. The dietary intervention was designed to be practical and realistic for free-living individuals to achieve. At the end of this phase, a 24-hour dietary recall was conducted, a physical activity questionnaire was filled out, and all anthropometry and biochemical variables were carried out. Patients who were taking medicines were not excluded and were allowed to continue taking their prescribed dosage.

Table 3.4.3 : Daily nutritional profiles recommended to each intervention group.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>NHLBI (NCEP STEP-I Diet) (Control Group)</th>
<th>(NCEP STEP-I Diet)+ Barley (intervention Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>55–60% of total energy intake, mainly (complex carbohydrates)</td>
<td>55-60% of total energy intake, mainly (complex carbohydrates)</td>
</tr>
<tr>
<td>Protein</td>
<td>10–15% of total energy</td>
<td>10–15% of total energy</td>
</tr>
<tr>
<td>Fat</td>
<td>&lt; 30% of total energy</td>
<td>&lt; 30% of total energy</td>
</tr>
<tr>
<td>Saturated fats</td>
<td>8–10% of total energy</td>
<td>8–10% of total energy</td>
</tr>
<tr>
<td>PUFA</td>
<td>Up to 10 % of energy</td>
<td>Up to 10 % of energy</td>
</tr>
<tr>
<td>MUFA</td>
<td>Up to 15% of energy</td>
<td>Up to 15% of energy</td>
</tr>
</tbody>
</table>

Phase III. Randomization & 3 month Intervention.

During the third phase of the study, participants who still met all inclusion criteria after the stabilization phase were randomly allocated to intervention or control group for a 12-week of intervention period as described during sample size calculation.

1. Intervention group: (addition of barley foods containing soluble fibre (β-glucan) to the background diet menus.)
2. **Control group:** (continuation of the background (control) diet NCEP-STEP I diet).

3.4.1 Randomization

Simple randomization method was used for randomization of respondents to each group. A table of random numbers were used for group assignment to minimize the differences between the intervention and control groups for all variables. Randomization is an optimal method of distributing the variables between the intervention and control groups. 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 does not let them influence the final outcomes.

3.4.2 Blinding

This study was a single blinded parallel group trial. To avoid bias, trial was 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” RCTs can be blinded or non-blinded. In the non-blinded experiment all three- the patient, the physician or the observer and the experimenter or the researcher, are aware of the treatment used. Blinding was carried out at the beginning of study. The blind RCT can be single, double or triple blind. In this study, Because of the nature of the intervention blinding participants and the researcher were not possible while the physicians who treated the patients and laboratory personnel who analyzed all the biochemical variables were blinded to the group assignment.
3.4.3 Dietary Intervention Strategy

Background diet (Control diet) for all the Respondents (n=278)

The background diet was designed to be identical in both arms of this study to ensure that the only nutrient that differed between the groups was β-glucan (soluble fiber present in barley). At screening, all subjects received general information from the physicians, who gave advice according to their usual clinical practice but no written information or recommendations was given by the physicians. In addition to the physician’s advice, they were provided with detailed verbal and written recommendations emphasizing the importance of a healthy lifestyle with details of the recommended and non-recommended foods by the researcher. All the participants were provided with different eating plans which was based on the National Cholesterol Education Program Step I guidelines (total fat less than 30%, saturated fat 8-10%, protein approximately 10-15%, carbohydrate 55-60% of total calories and cholesterol< 200 mg/day) (Table 3.4.3). Different eating plans with different energy levels were used, depending on the energy requirements of each participant. Methods used to calculate the energy requirement for 24 hour is described below. There was no attempt to limit energy intake or to maintain iso-caloric intake for each participants. They were advised to avoid sodium-rich food, limit the intake of food with moderate amounts of sodium, and eat no more than one-fourth a teaspoon of salt per day. They were also suggested to increase the fruits, vegetable consumption and limit animal foods such as meat, chicken, beef, pork etc but fish should be eaten three-four times a month and eggs no more than two or three weekly. Skimmed dairy products and vegetable oils were recommended whereas animal fats (butter, cream, lard, etc.) sugar, honey, jam, chocolate and other sweets were restricted. Alcohol was to be
avoided and low-fat cooking methods (steaming, non-stick pans, etc.) were strongly suggested (Annexure- IV).

3.4.4 Calculation of Energy Requirements

Energy requirement for each participants were estimated on basis of resting metabolic rate (RMR) and physical activity level. RMR was calculated with the help of Bioelectrical Impedance technique by using Body fat Analyzer (OMRON HEF-200) model and Physical activity level was determined on the basis of their occupation and physical activity.

The following activity factors were used to calculate the total calorie requirements.

Table 3.4.4 : Harris Benedict Equations for estimation of total calories needs/day (Revised 2004).

<table>
<thead>
<tr>
<th>Activity Level</th>
<th>Equation</th>
<th>Daily Calories Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary: Little to no exercise</td>
<td>Daily calories needed = BMR x 1.2</td>
<td></td>
</tr>
<tr>
<td>Mild activity level: Intensive exercise for at least 20 minutes 1 to 3 times per week. This may include such things as bicycling, jogging, basketball, swimming, skating, etc. If you do not exercise regularly, but you maintain a busy life style that requires you to walk frequently for long periods, you meet the requirements of this level.</td>
<td>Daily calories needed = BMR x 1.3 - 1.375</td>
<td></td>
</tr>
<tr>
<td>Moderate activity level: Intensive exercise for at least 30 to 60 minutes 3 to 4 times per week. Any of the activities listed above will qualify.</td>
<td>Daily calories needed = BMR x 1.5 - 1.55</td>
<td></td>
</tr>
<tr>
<td>Heavy or (Labor-intensive) activity level: Intensive exercise for 60 minutes or greater 5 to 7 days per week (see sample activities above). Labor-intensive occupations also qualify for this level. Labor-intensive occupations include construction work (brick laying, carpentry, general labor, etc.). Also farming, landscape worker or similar occupations.</td>
<td>Daily calories needed = BMR x 1.7</td>
<td></td>
</tr>
<tr>
<td>Extreme level: Exceedingly active and/or very demanding activities: Examples include: athlete with an almost unstoppable training schedule with multiple training sessions throughout the day or a very demanding job, such as shoveling coal or working long hours on an assembly line. Generally, this level of activity is very difficult to achieve.</td>
<td>Daily calories needed = BMR x 1.9</td>
<td></td>
</tr>
</tbody>
</table>
**Total energy requirement/24 hours**

Total energy requirements were calculated by multiplying the activity factor with the resting metabolic rate with activity factor.

**For ex.** If a person are sedentary worker and having resting metabolic rate (1302 kcal), than the energy requirement for that person was $1302 \times 1.85 = 2410 \text{ kcal/day}$

**3.4.5 Diet for Intervention Arm**

The menu for intervention group was prepared by replacing 20% of total energy from the background diet (STEP-I) with barley foods. This was achieved by making proportional reduction to all the foods mainly wheat & rice in the background diet menus to accommodate the energy supplied by the barley foods containing soluble fibre. The emphasis of the intervention diet was to consume soluble fibre (beta-glucan) as achieved primarily through consumption of barley. Participants were recommended to eat barley foods either in the form of chapatti, sattu, dalia, or in the roasted form. They were also provided the booklet containing details about barley, importance of barley, barley dishes and their preparation methods. (Annexure-V). The only difference between both the groups was inclusion of barley food products as the main carbohydrate source in the intervention group. In terms of nutrients almost all the major and minor nutrients were similar in both the groups except total dietary fiber and soluble dietary fiber ($\beta$-glucan). Details about the recommended amount of barley in the intervention group are given below (Table 3.4.4).
### Materials & Methods

#### Table: 3.4.5 Recommended mean intake of barley in the intervention group.

<table>
<thead>
<tr>
<th>Recommended Calorie</th>
<th>No. of patients</th>
<th>% of Respondents</th>
<th>Recommended Barley intake (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200-1500</td>
<td>4</td>
<td>3.8</td>
<td>82.49±5.3</td>
</tr>
<tr>
<td>1501-1800</td>
<td>29</td>
<td>27.9</td>
<td>98.89±5.2</td>
</tr>
<tr>
<td>1801-2100</td>
<td>25</td>
<td>24.0</td>
<td>117.92±4.5</td>
</tr>
<tr>
<td>2101-2400</td>
<td>25</td>
<td>24.0</td>
<td>132.33±4.8</td>
</tr>
<tr>
<td>2401-2700</td>
<td>14</td>
<td>13.5</td>
<td>150.97±6.4</td>
</tr>
<tr>
<td>2701-3000</td>
<td>7</td>
<td>6.7</td>
<td>172.62±6.8</td>
</tr>
<tr>
<td><strong>Mean ± SD : 2063 ± 395</strong></td>
<td><strong>N=104</strong></td>
<td><strong>100%</strong></td>
<td><strong>Mean ± SD : 122.8 ± 23.5</strong></td>
</tr>
</tbody>
</table>

#### 3.4.6 Control arm:  
During third phase, the participants in the control group were recommended to consume the same diet as in the second stage (background diet).

#### 3.4.7 Dietary Compliance

To ensure adequate compliance with dietary recommendations, assessment of dietary intake was performed at each visit (at inclusion and thereafter once a month) during the whole intervention period for a period of three months. On these occasions, a 24 hour recalls were conducted followed by individual meetings to discuss any deviations from the prescribed diet. In addition, participants were also followed at every 14-15 days by phone calls and unscheduled 24-hour recalls were obtained without prior knowledge of the subjects. Participants were also provided the researcher phone number and advised them to call at any time if they have any query and any suggestions required.

#### 3.4.8 Physical Activity Protocol

Physical activity of the participants were also assessed by pretested interview schedule and it was based on the patients self-report technique. There was no strict
protocol for the physical activity recommendation to any groups. In addition to the physicians advice they were only instructed to participate in light or moderate intensity exercise such as brisk walking, at least 30 -40 minutes 3-5 times per week.

3.4.9 Experimental Method : (Nutrition Education)

Nutrition and health education is defined as a planned effort to improve nutrition and health status by bringing about changes in behaviour of people. It is a process by which people gain knowledge and develop confidence and skills needed for establishing good dietary and health practices. Most people have some knowledge about nutrition but few of them have correct concepts of the nutrition hence there seems a need for nutrition education for both literate and illiterate.

**According to Geoffrey (2002) the stages of nutrition education are as follows:**

1. **Sender (health promoter)**
2. Message is seen/heard by receiver
3. Receiver understands and correctly interprets the message
4. Message is accepted, believed and learned
5. Behaviour change occurs
6. Improvement in health
Methods Used for Nutrition education in this study: (Individual counselling)

Systematic individual counselling method was adopted for the experimental purpose. It was a two-way method and is one of the best ways to modify behaviour. The intervention program consisted primarily of dietary counselling, physical activity & general lifestyle recommendations in order to aid patient’s adherence to a healthy life plan during the intervention.

Orientation & brief introduction: After recruitment, first of all greeting of the participants was done. All the respondents were provided with the brief introduction about the researcher, importance of the study followed by general information of the participants to develop a rapport and to convince them to participate in the intervention program.

Teaching aids used: Education material (colorful booklet) was prepared in Hindi language with all the recommended and non-recommended foods, benefits, cooking methods and lifestyle suggestions for distribution among the respondents (Annexure-IV & V). The education material contained all the points discussed during the session.

Refreshment: In this study there was no monetary or material gifts would be given to the participants while taking the consent for participating in this study.

Informed Consent: Each selected respondents were explained about the purpose and possible benefits of this study and assurance of the confidentiality. Written informed consent was obtained from each subject after an oral explanation of the study as per the WHO guidelines.
3.7.2 Contents of the Nutrition Education/ Intervention

First Individual Session (After recruitment) (Average duration of 50-60 minutes)

Counselling session was undertaken individually of each respondent for at least 60 minutes focusing on dietary recommendations and lifestyle tips. Following components covered during the interventional education session they were also given in the booklet form.

Each Participant Received:

- Knowledge regarding the lifestyle diseases, its causes, symptoms, and preventive measures.
- General dietary recommendations (about cooking, lowering fat intake, reducing salt intake, reducing beverages and food with added sugars, and counting calories from alcoholic beverages).
- Suggestion about food composition, how to identify total/saturated fat and high calorie density foods, how to choose high-fiber foods, low calorie, low fat alternatives and related behavioral counseling.
- Provided knowledge about weight management technique, identification of common dietary mistakes, suggestion, meal planning, food shopping and preparation, portion control.
- A brief written guide for selection of foods. A copy of the food pyramid. (USFDA 2010)
Materials & Methods

- Explanations about benefits of diet and exercise in controlling metabolic abnormalities and how to increase daily exercise and to include physical movement into habitual activities.

After completion of the session, the respondents were asked to put on their queries if any. Phone no. of the researcher was given to the respondents for any future queries.

Subsequent 4 another Interactive Sessions (at every month): At baseline (after stabilization) participants in the intervention group were provided the detailed oral and written instructions about the importance of barley, nutrient content, and preparation method and portion size. During these sessions all queries & complaints of the participants were cleared and discussions were also made with the subjects. Personal contacts with the respondent were maintained during the whole intervention period.

A colourful booklet for the intervention group covering all aspects about barley, benefits, how to consume barley, barley dishes, and methods to prepare was also prepared and given to all the subjects to facilitate the intervention programme (Annexure-IV &V).

3.4.10 Measurements & follow-up protocols

The follow-up protocols were identical for both arms. Table 3.4.5 displays all the measurements which were measured at every month. The study consisted of five visits including screening visit during the whole intervention period. At the screening, the participant’s who satisfied inclusion criteria were interviewed and their socio demographic characteristics, medical history, life style & dietary assessment were
performed for each participant. During each subsequent visits eg. at baseline (end of stabilization) 4, 8 and 12 weeks all the participants underwent clinical & biochemical investigations including blood pressure, anthropometry, and fasting blood profile. Participants were considered as completers of the study if they made their last visit at the end of 4, 8, or 12 week.

**Table 3.4.6 : Clinical examinations and biochemical investigations conducted at baseline, 4, 8 & 12-weeks for each groups.**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Fasting Examinations Intervention &amp; Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical examinations</strong> *</td>
<td>Systolic arterial blood pressure</td>
</tr>
<tr>
<td></td>
<td>Diastolic arterial blood pressure</td>
</tr>
<tr>
<td><strong>Biochemical markers</strong> *</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
</tr>
<tr>
<td></td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td></td>
<td>Low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td></td>
<td>Very Low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td></td>
<td>Fasting blood Glucose</td>
</tr>
<tr>
<td><strong>Anthropometric &amp; body composition assessments</strong> *</td>
<td>Height</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
</tr>
<tr>
<td></td>
<td>Body mass index</td>
</tr>
<tr>
<td></td>
<td>Waist</td>
</tr>
<tr>
<td></td>
<td>Hip</td>
</tr>
<tr>
<td></td>
<td>Waist/hip ratio</td>
</tr>
<tr>
<td></td>
<td>Total body fat %</td>
</tr>
<tr>
<td></td>
<td>Visceral fat %</td>
</tr>
<tr>
<td></td>
<td>Resting Metabolic rate (RMR)</td>
</tr>
<tr>
<td><strong>Dietary Assessment</strong></td>
<td>24-hour dietary recall*</td>
</tr>
<tr>
<td></td>
<td>Food consumption frequency</td>
</tr>
<tr>
<td><strong>Lifestyle Factors Assessments</strong> *</td>
<td>Addiction habits (smoking, tobacco, alcohol and others)</td>
</tr>
<tr>
<td></td>
<td>Physical activity pattern</td>
</tr>
</tbody>
</table>

*Analysis repeated at every month including before stabilization (screening), after stabilization (baseline 0-week), and at every month 4, 8 and 12-week. Analysis repeated at screening & at the end of intervention
3.4.10 Endpoints

Primary outcomes were the changes in the clinical and biochemical risk factors such as blood pressure, fasting lipid profile after 3 month of dietary intervention. The secondary outcomes were the body composition, BMI, waist circumference and intake of all the major and minor nutrients.

3.4.11 Premature Withdrawal and Discontinuation Criteria

"Drop-outs" were defined as those participants who discontinued the trial for reasons not related to the treatment. "Withdrawals" were defined as those subjects who discontinued the trial due to treatment-related reasons. In case a participant was prematurely withdrawn from the study, date of the last visit and reason for discontinuation was recorded for all drop-outs and withdrawals. Nevertheless, all random dropouts and withdrawals were replaced with other participants to ensure that sufficient data was collected from the pre-set number of participants.

3.5 Data Collection Procedure

Collection of data is most important step in any research work. An interview schedule was developed with the consultations of supervisor, co-supervisor, biochemist and statistician to collect the relevant information related to the research topic.

Research Tools & Techniques: Following tools were used in this study:

1. Interview and examination schedule: The primary tool in this study was a pre-designed and pretested interview and examination schedule (Annexure-III) for recording of information pertaining to study objectives.
This interview schedule had following sections:

Section 1: Socio-Demographic Profile of Respondents  
Section 2: Medical History & Treatment Profile.  
Section 3: Lifestyle Assessment  
Section 4: Assessment of Nutritional status

2. Testing of Interview Schedule

To get relevant and reliable data from respondents, standardization of interview schedule, which is a crucial step, was done. The schedule was pretested (through the pilot testing) on 20 subjects. The questions which were found ambiguous were eliminated and necessary modifications were made in the light of experience of pretesting of schedule. This pre-testing system helped in the physical layout of final interview schedule.

Table 3.5.1: Phase and objective wise Tools and Techniques adopted for the study.

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Tools</th>
<th>Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. To assess the baseline characteristics and disease profile of the study subjects.</td>
<td>Pre-designed, Pre-tested, Interview Schedule</td>
<td>Interview of respondents. With the help of patients report.</td>
</tr>
<tr>
<td>2. To assess the differential pattern of food &amp; nutrient intake at baseline&amp; throughout the trial.</td>
<td>24-hour recall &amp; Validated (Food frequency Questionnaire) computerized nutrition database</td>
<td>By interviewing and by showing the standardized utensils</td>
</tr>
<tr>
<td>3. To assess the lifestyle &amp; behavioural determinants of study subjects at baseline and during of the trial.</td>
<td>Pre-designed, Pre-tested, Interview Schedule</td>
<td>On the basis of patients response (by interview)</td>
</tr>
<tr>
<td>4. To examine the effect of barley consumption on body composition, blood lipid profile and glucose metabolism of the study subjects.</td>
<td>Data collection sheets</td>
<td>Body composition analyser OMRON(HEF-200)&amp; Standard method of fasting assessment</td>
</tr>
</tbody>
</table>
General Characteristics

Section 1: Socio-Demographic Profile.

General information viz. gender, age, religion, caste, education, occupation, type of family & family income etc. were obtained from respondents were elicited from study subjects as by interview technique.

i. **Age:** Age was asked to the study subjects and ascertained using life events method.

ii. **Caste:** The caste was asked directly and responses were stratified into General/Others, Other Backward Class (OBC), Schedule Caste (SC) and Schedule Tribe (ST) groups.

iii. **Educational status:** Respondents were asked about their education level. They were categorized as illiterate, just literate, primary and middle, high school, intermediate, graduate and post graduate and above.

iv. **Occupation status:** Respondents were asked about the work status of their family members and were categorized as self employed, government or private services, skilled and unskilled labourers, and unemployed.

v. **Type of Family:** For the purpose of present study a family was a unit comprising of two or more persons related by blood, marriage or adoption and residing together in the same dwelling unit with a common kitchen. A family was considered nuclear when it consisted of husband, wife and their dependent children, and joint family if it included close relatives like sons, daughter who are not dependent, father, uncle, brother etc.
vi. **Total family income:** Data for total family income was calculated by interviewing the respondents. If family had more than one income source, all sources were pooled together to get total family income. Separate procedures were adopted to estimate activities and the income from the other sources.

vii. **Socioeconomic status/social class**

For monthly income of the family statements were recorded from the study subjects. The social class of the subject was determined by modified BG Prasad classification for the year 2014. Almost all community-based studies focus on socioeconomic stratification, which is the key parameter for proper understanding the affordability of the community of health services, amenities and their purchasing capacity. Prasad's classification (1961) based on the per capita monthly income has been widely in use in India. It is computed as:

\[
\text{Per capita monthly income} = \frac{\text{Total monthly income of the family}}{\text{Total members of family}}.
\]

**Table 3.5.2: Revised modified BG Prasad socioeconomic classification scale, January 2014**

<table>
<thead>
<tr>
<th>Socio Economic class</th>
<th>Per Capita Monthly Income limits (Rupees)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1961 (Base Year)</td>
</tr>
<tr>
<td></td>
<td>Revised income categories for all India (IW) 2014*</td>
</tr>
<tr>
<td>Upper (Class I)</td>
<td>100 &amp; above</td>
</tr>
<tr>
<td>Upper Middle (Class II)</td>
<td>50-99</td>
</tr>
<tr>
<td>Middle (Class III)</td>
<td>30-49</td>
</tr>
<tr>
<td>Lower Middle (Class IV)</td>
<td>15-29</td>
</tr>
<tr>
<td>Lower (Class V)</td>
<td>Below 15</td>
</tr>
</tbody>
</table>
Section 2: Medical History & Treatment Profile

Getting accurate medical histories is vitally important to deliver proper care. A good history can help speed the diagnosis of a current complaint. A medical history is a record of a patient’s condition, past and present. It documents:

- Physical or mental conditions of which the patient is aware.
- Major illnesses the patient may have suffered.
- Surgeries the patient may have undergone.

i. History of Present Illness: This section was filled out with the help of physicians. Duration of disease was defined as the period of time from the date of first clinical diagnosis of hypertension.

ii. Past Medical History (Surgical History): In this section the participants were asked about the major illnesses, surgeries, significant injuries, allergies and any severe complications from which they had suffered in the past.

iii. Family History: This component was used to extract information regarding the family history of heart disease and related metabolic diseases such as hypertension and diabetes among their first-degree relative (parent or sibling) which was self-reported by the study subjects. In medicine, a family history consists of information about disorders from which the direct blood relatives of the patient have suffered. Accurate knowledge of a patient's family history may identify a predisposition to developing certain illnesses, which can inform clinical decisions and allow effective management or even prevention of conditions.
iv. **Treatment History & Medicine Intake:** The respondents were asked about whether they were taking any prescription medicines? If so, what is the dose and frequency? This section is also filled out with the help of physicians.

**Section 3 : Lifestyle Assessment**

Interview method was used for life style assessment. The lifestyle pattern such as physical activity pattern, smoking and alcohol consumption was also collected by using a questionnaire.

1. **Physical Activity Pattern**

   Physical activity was assessed with the help of pretested interview schedule. Questions related to the physical activity focused on patient’s lifestyle which ranged from sedentary to hard or strenuous physical activity. This measure was selected to assess the degree to which participants acceded to the requirement.

   i. **Type of Exercise :** Type of physical activity was defined as any type of non-occupational physical exercise, at least once a week and was graded in qualitative terms such as none, walking, yoga, running, swimming and outdoor games.

   ii. **Frequency and Duration of Exercise :** This section also included the frequency or duration of physical activity they performed in the days per week. Respondents were asked to report the total time spent on physical activity during a typical week, the number of days as well as, the intensity of physical activity was recorded.

2. **Substance Abuse & Social History of Participants**

   Substance abuse such as smoking, alcohol, tobacco and pan, gutka etc. was also addressed in the questionnaire. Smoking status at the time of screening was
categorized as current, former, and never smoker. For former smokers respondents were also asked about years since quitting. In case of yes, it was asked to specify the type, frequency consumption in terms of days, per week and quantity in terms of numbers/grams/ml/day.

Section 4 : Assessment of Dietary Intake

Compliance with the experimental diets was assessed using two methods: **24-h diet recall and food frequency questionnaire** method during the run-in period, before starting the intervention and at the 4th, 8th and 12th week of intervention. In addition, compliance was also monitored by phone calls. Dietary information was collected on meal pattern, food habits, food consumption pattern, type of oil used for cooking and knowledge about cholesterol lowering foods.

i. 24-Hour Dietary Recall: A 24-hour dietary recall method was used to collect the information pertaining to dietary intake. This method is based on the process of recall of food consumption over a specified period of time (24 hours), prior to the survey. As a retrospective method it relies on an accurate memory of intake, reliability of the respondent and an ability to estimate portion size.

Procedure

The participants were asked to report everything that they had eaten or drunk during the previous day over the past 24-hour period. In order to obtain accurate information, the subjects were asked how often they usually ate during the day, what foods were consumed, how the food was prepared, what the serving size was & special mention was made for salt, sugar and cooking fat. To estimate portion size of
food consumed, standardized set of cups and spoons with varying capacities (volumes) were displayed to estimate the exact amount of cooked food eaten in each meal (NIN 2010).

Participants were also prompted to remember eating and drinking episodes by time periods (e.g. starting on awaking), or linking to day time activities (e.g. arriving at work) and to estimate the portion sizes of the items consumed. From the size and volume of food consumption obtained by this method, the quantities consumed by the participants were converted in to exchanges and the equivalent weight of raw food in terms of gram or millilitres was calculated using a conversion table for Indian foods formulated at the National Institute of Nutrition ICMR (2010). In case of chapattis, the amount of flour used in the preparation of one chapatti was calculated by dividing the total amount of flour used by number of chapatis prepared; this value was then multiplied by the number of chapatis consumed by the patients to find out the raw amount of flour consumed by the patients.

Some points were particularly considered while taking information regarding the dietary intake, which were as follows:

- Sizes and volumes of some foods like size of chapatti.
- Amount of dal katori size and consistency of dal.
- Amount of sugar in beverages, plate size, glass and cup size etc.
- Information regarding use of oil or fat in vegetable preparations, with chapatti, parathas and in rice and pulao making.
ii. Food Frequency Questionnaire: Food Frequency Questionnaire (FFQ) is the standard method to collect dietary data in studies of chronic disease all over the world. First step of this method is to identify and organize the list of most commonly consumed foods of the study population and to assess the frequency of intake of foods. Participants were asked to report their frequency of consumption of each food from a list of foods during the last six month on a daily (e.g. bread), weekly (e.g. rice, meat) or monthly (e.g. fish) basis. For each food item, participants were asked how frequently (never, seldom, once a month, once a week, two-three times a week, daily) they consumed the food, followed by a question on amount of consumption in household measurement scale such as fists, bowl and plates. Data were recorded manually on a paper form at the end of the health interview by the study interviewer. It is an accepted method to estimate usual food habits of the respondents. (Thompson & Byers 1994). In addition, the appropriate quantity eaten (standard cups) was also asked and recorded.

3.6 Nutritional Analysis

Average daily energy intakes were quantified from the data collected in the 24-hour recalls, using a standard exchange list based on the recommendation of the Indian council of medical research 2010. All food records were analyzed by a specially designed computerized program using the food database of Nutritive value of Indian foods (ICMR 2010). This software contains a set of foods with an extensive and complete set of nutritive value. There is wide range of food ingredients (approx. 700) which includes all the vegetarian and non-vegetarian foods including herbs, spices and fast foods. Some foods which were not listed in the database, data from the
standard sources were used and added to the programme. The main nutrients of interest were energy, protein, carbohydrates, fat (saturated, monounsaturated and polyunsaturated) total dietary fiber(soluble, insoluble & crude) and micronutrients such as Na, K, Mg, Ca, Ph, zinc manganese, vit A, C iron and folic acids. The above nutrients documented as these have been identified as having a direct relationship to cardiovascular disease. Data obtained from the 24- hour food records were processed and converted to the gram equivalents by using the Indian system of food equivalents. Each food and beverage was then coded according to the software and entered into a computerized nutrition database which contains the nutritional values of all Indian foods, for analysis.

3.7 Measurements of Anthropometry and Body Composition

In this study, all the anthropometric measurements including Height, Weight, Waist circumference, Hip circumference and Waist hip ratio were recorded using the standard protocol according to the standardization reference manual. 10.

i. **Weight**: Body weight was measured while the subjects were minimally clothed without shoes by using the Omron Body Fat Analyzer (HEF-200).

ii. **Height**: Height was measured in a standing position, without shoes, by using a tape meter while the shoulders were in a normal state. Height is a measure of linear growth of the body and degree of skeletal development. It is primarily a reflection of cumulative or past nutritional status (Jelliffe, 1966).

iii. **Waist and Hip Circumference**: Waist and hip circumference is a measurement at the level of navel, when the patient breathes quietly. Waist girth was measured as the
narrowest circumference between the bottom of the rib cage and the iliac crest by using a un-stretched tape measure without any pressure to body surface. Whereas; hip circumference was measured at the intertchantric level (Despres 1991) with the help of non-stretchable tape.

iv. Waist to Hip Ratio: Waist to hip ratio is a measurement of visceral obesity and is a strong indicator of risk of hypertension, cardiovascular disease and some other diseases like cancer etc (Rockville 1993). It was calculated as:

\[
\text{Waist to Hip ratio} = \frac{\text{Waist circumference (cm)}}{\text{Hip circumference (cm)}}
\]

v. Body composition: Body composition such as body fat %, resting metabolic rate, body mass index, visceral fat % and body weight was measured by bioelectrical impedance analysis (BIA) using the Body Composition Analyzer (OMRAN HEM-903). Same instrument were used in both groups at all the visits in order to avoid inter-instrument variation in all measurements.

3.9 Clinical parameters and Biochemical estimations

All the clinical and biochemical assessment was performed by the hospital staff by using the standard procedure.

i. Blood pressure: Blood pressure was measured after 5 minute of rest in the sitting position on the right arm using a standard mercury sphygmomanometer.

ii. Biochemical Analysis: A serum chemistry value was estimated in the hospital routine clinical laboratory by personnel blinded to the group assignment. Each blood
sample was collected after 12 hours of fasting and serum samples were maintained at 28°C until they were used for analysis. Fasting blood samples were analyzed for plasma concentrations of glucose, triacylglycerol, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol.

**Assessment of Biochemical Parameters:** All the biochemical measurements was carried out by the routine clinical staff Sir Sundar Lal Hospital Institute of Medical Sciences Banaras Hindu University, Varanasi, following standard procedure.

- Fastnig plasma glucose was measured by using the (GOD-POD method).
- Total cholesterol was measured enzymetically by using the (CHOD-POD) method.
- Fasting triacylglycerides concentration was measured by using the (GPO-POD) method.
- Low-density lipoprotein cholesterol was measured by using LDL Cholesterol Kit by direct method for quantitative measurement of LDL cholesterol.
- High-density lipoprotein cholesterol was also measured with the help of kit.

### 3.10 Statistical Analysis

- All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 21st trial version).
- Continuous variables were expressed as mean ± standard deviation, and categorical variables (dichotomous variables) were expressed as frequencies and proportions (percent).
Materials & Methods

- All the continuous variables were assessed using the Kolmogorov-Smirnov Z test to examine the distribution type; if the data did not exhibit a normal distribution, either they were logarithmically transformed prior to analysis or the non-parametric tests were applied. The advantage of non-parametric methods over asymptotic methods is that they remain valid for very small sample size, and it is a distribution-free method because they do not assume an underlying parametric distribution for the data.

- For the normally distributed data, descriptive statistics & Independent-sample t-test with 95% confidence intervals was used for each measure to establish that there were no significant baseline differences between groups (Intervention vs. Control) and to determine whether the changes associated with the intervention diet were greater than those associated with the control diet at the endpoint.

- Chi-Square was used to assess the baseline differences between the Intervention and control group for nominal or ordinal variables.

- In addition, to analyze the differences within groups from baseline to 3 month (12-weeks) paired comparison 't' tests were calculated. And to compare the changes from baseline to 3 month between groups for categorical variables, McNemar’s test was used for dichotomy variables and Friedman’s test was used for variables of more than two categories. P<0.05 was considered statistically significant.

- Repeated data were analysed by using General linear model (two-way repeated measures ANOVA) by using intervention& control group as a between subject factor and repeated data as a (within subject factor). If the
main effect was found to be significant Sidak multiple comparison test was applied to know where the differences was actually occurring.

- The level of significance was set at 0.05 for testing the main effects of diet and time and the interaction effect.
- Percentage change for each variables were also calculated by the formula

\[ \frac{(E - B)}{B} \times 100, \]

where \( E \) is the end of treatment value and \( B \) is the baseline value.

***