Methodology
3. Methodology

The methodology of the study on "Baseline risk factors for coronary heart diseases in Kochi" consisted of the following steps:

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3.2. Selection of Sample

3.2.1. Cases (CHD group)

3.2.2. Controls (Non CHD group)

3.3. Selection of Tools and Techniques of data collection

3.3.1. Socio-economic background and lifestyle

3.3.2. Anthropometric measurements

3.3.3. Clinical and Bio-chemical status

3.3.4. Diet Survey- Dietary habits and Food consumption pattern

3.3. Analysis of data

3.1. Selection of Area

The area selected for the present study was Kochi, the district centrally located in the State of Kerala. Kochi is a cosmopolitan city often referred as the industrial capital of Kerala. This city is being urbanised faster than any
other region in Kerala. Improved rail and road connectivity makes the city rapidly accessible to the people from the hilly east and coastal west of the city, as also from the plains of North and South. This gives an impetus to the rapid urbanization, which is an independent risk factor of coronary heart disease.

Urbanization according to Mahan and Stump, (2004) and Singh et al. (1999), is usually related to major changes in diet, physical activity, and socioeconomic status as well as increased obesity. Hence degree of urbanization seemed to have a positive correlation to incidence of CHD (Rissam et al., 2001). Moreover, Kochi has good health care infrastructure, including many hospitals with state-of-the-art Intensive Coronary Care Units (ICCU) facility. Therefore availability and accessibility to hospitals also factored in the selection of Kochi as the area of study. Location map of Kochi, Kerala, the study area is given in figure 2.

3.2. Selection of Sample

According to Gupta (2003) sampling is simply the process of learning about population on the basis of a sample drawn from it. Under this, small group of the universe is taken as the representative of the whole mass and the results are drawn.

In the present study out of six hospitals with Cardiology units in and around Kochi, a cluster of three hospital - Lissie Hospital, Lourdes Hospital and Indira Gandhi Co-operative Hospital - were selected, based on the availability of sample population. The willingness of the cardiologists, hospital
authorities, in-patients, and their family members to cooperate with the study was also taken into account during sample selection.

Case-control studies are commonly used to assess factors associated with a disease (Luepker et al., 2001). This method was adopted by Sheehan et al. (2005) in Ireland (Cork coronary care case-control study), Lipoeto et al. (2004) in Indonesia, Erkens et al. (2002) in Netherlands, Suh et al. (2001) in Korea. In India, Rastogi et al. (2004), Patil et al. (2004), Gupta et al. (2000), Zodpey et al. (1998), Kodali et al. (1999) and Chacko (1998) used this method to find out the difference in dietary patterns and CHD risks between the coronary heart disease cases and their sex matched healthy individuals serving as the controls.

In a Case-control study (Luepker et al., 2001), cases are compared with controls to determine whether the exposure of interest is more or less common in the cases. Cases are individuals who, according to a strict definition, have a specified illness or condition; they are chosen to be as alike as possible in their disease status. Two groups of sample, case (CHD subjects) and control (NonCHD subjects) groups were thus selected for the study. These two groups were further decided based on the following inclusion and exclusion criteria.
Fig. 2
Location map of Kochi, Kerala
3.2.1. Cases (CHD group)

Inclusion criteria: Incident of CHD as defined by Hoffmann et al. (2004) is the first acute myocardial infarction or a first episode of angina. The sample included in the study was 350 patients who had experienced a first event of acute myocardial infarction and unstable angina and admitted in the selected hospitals during the year 2004-2005. They were in the age group of 25 to 79 years.

Exclusion criteria: Patients were excluded if they had a history of myocardial infarction or unstable angina in the past, with or without any clinical symptoms or suspected coronary artery disease in their medical history. Those who were reluctant to co-operate were also excluded.

3.2.2. Controls (Non CHD group)

Inclusion criteria: Controls are individuals without the disease (CHD) but with the same background characteristics as the patients with disease (Luepker, 2001). The whole population is exhaustive, so that only a random sample of 100 Non CHD subjects (50 male and 50 female) in the age group 25 to 79 years were selected for the purpose of studying the relative risk of CHD subjects. The controls were selected from patients who got admitted in the hospital during the same period of study and the ones who came for health checkup.

Exclusion criteria: Subjects were excluded if they had a history of diabetes mellitus, hypertension, myocardial infarction or unstable angina in the past.
3.3. **Tools and Techniques of data collection**

Tools and techniques used for the collection of research data should be appropriate and accurate for ensuring credibility of information.

The interview method of collecting data involves presentation of oral verbal stimuli and reply in terms of oral verbal responses. This can be used through personal interviews and also can be carried out in structured way (Kothary, 2003). According to Gupta (2003) interview facilitates interstimulation between the interviewer and the interviewee and helps to secure data, not obtainable by methods that do not involve any interpersonal relationship. Interview method is suitable way to collect the data as it proceeds systematically and enables to record the information quickly (Kothari, 2001). The information obtained by this method is likely to be more accurate because the interviewer can clear up doubts of informants and thus obtain correct information (Singh, 1997). Therefore, in the present study the direct interview method was adopted to procure the relevant information.

According to Thanulingam (2000) interview schedule is a proforma containing a set of questions and are very useful in gathering information. It is generally filled by the researchers or the ones who are specially appointed for the purpose. A survey with the help of a structured interview schedule which was pre tested on a comparable sample, was conducted to get information on the following lines.
3.3.1. Socio-economic background and life style

Socioeconomic classification is important because rates of ill-health display marked social gradients in most societies. Understanding the causes of these gradients is a key area of research into the epidemiology and control of CHD, opined Kaplan and Keil (1993). According to Luepker et al. (2001) high level of formal education is an excellent indicator of socio-economic status and is easily and reliably collected. It is strongly associated with occupational status and income. In some surveys, it proved as a better predictor of CHD risk.

A well-structured interview schedule, which was pre-tested on a group of coronary heart disease patients, who were not included in the study population, was used for the purpose. The variables which are suggestive of risk factors of CHD by many authors like age (Sadikot, 2006; Krummel, 2004 and AHA, 1999), sex (NCEP, 2001 and McGill and Stern, 1979), religion (Gupta et al., 2000 and Gopinath et al., 1995), educational level (Gupta et al., 2003 and WHO, 1994), income (Gupta et al., 2002 and Davey, 1997), occupational status (Gafarov et al., 2003 and Singh et al., 1999), size of the family and marital status of the sample were included in the schedule. The survey was conducted among both cases as well as control groups.

Appropriate questions to elicit information on life style and personal habits of the sample prior to the onset of the disease (CHD) in cases, and pre-interview period in the control group were also formed part of the schedule. The details on these factors such as smoking (Pais et al., 2001 and Kumar,
2000), alcoholism (Rehm et al., 2004 and Gaziano et al., 2000), physical inactivity (Singh and Sen, 2003) and stress (Uppaluri et al., 2002) reported to have an influence on incidence of CHD as evidenced from literature also included in the schedule. The sample schedule used to procure socioeconomic and lifestyle of the sample is given in Appendix I.

3.3.2. Anthropometric Measurements

Nutritional anthropometry is measurement of human body at various ages and levels of nutritional status and it is based on the concept that appropriate measurements should reflect any morphological variation occurring due to a significant functional physiological change (Rao and Vijayaraghavan, 2003).

According to Luepker et al. (2001) anthropometry in cardiovascular surveys has three main uses: to standardise for body size, to estimate body composition as defined by percentage of body fat, and to measure the distribution of body fat. To standardise body size, body mass index is used. Waist girth correlates well with the intra-abdominal (visceral) fat mass measured by computed tomography (CT). Waist: hip ratio standardises for body size, and takes into account gluteal fat deposit.

The anthropometric measurements considered in the present study included height, weight, body mass index, waist and hip measurements (Appendix I).
**Height:**

The height of the individual is influenced both by genetic (hereditary) and environmental factors. The maximum growth potential of an individual is decided by hereditary factors, while the environmental factors, the most important being nutrition and morbidity, determine the extent of that genetic potential (Rao and Vijayaraghavan, 2003). Jalali *et al.* (2005) reported that height had an independent relationship with myocardial infarction in men and in younger subjects.

Height was determined by using a nonstretchable measuring tape fixed on a wall with a precision of 0.5 cm. The subject's height was measured observing the points suggested by Jelliffe (1966) to ensure accuracy of measurement. After removing the shoes, the subject was asked to stand on a flat floor against the measuring tape with feet parallel and with heels, buttocks, shoulders and back of head touching the upright. The head was in a comfortably erect position, with the lower border of the orbit in the same horizontal plane, as the external auditory meatus and the arms should be hanging at the sides in a natural manner. A headpiece, a flat metal bar was placed gently on the head, and the point of contact with the top of the head was marked against the wall and heights were recorded.

**Weight:**

Weight is the key anthropometric measurement (Jelliffe 1966). Body weight, according to Venkatalakshmi and Peramma (2000) is a sensitive
indicator of obesity. Importance and reliability of weight as a measure to assess nutritional status was also emphasized by Bamji et al. (2003) and Rolfes and Whitney (2002).

The body weight of the subjects was measured using a portable bathroom weighing scale with a sensitivity of 500 grams, calibrated against a lever balance. The subjects were asked to stand erect; barefooted on the weighing scale with minimum clothing worn and the body weight was measured.

**Body Mass Index:**

Body Mass Index provides reasonable indication of the nutritional status (Hubbard, 2000). Body Mass Index was calculated using the formula given below.

\[
\text{Body Mass Index (BMI)} = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2}
\]

The clinical guidelines given by National Institute of Health (NIH, 1998) and Indian Conasensus Group (1998) on the identification, evaluation, and treatment of over weight and obesity in adults, operationally defined over weight as a BMI of 25 to 29.9 and obesity as a BMI of at least 30.

But WHO Regional Report (2000) has recommended different ranges for classifying overweight and obesity for population in the Asia Pacific Region. This is on the basis of the fact that increase in health related risk factors and co morbidities associated with obesity occur at a lower BMI in
Asian population than in other ethnic groups. So, the lower cut off points for
over weight and obesity for Asians were identified as BMI greater than 23 and
obesity BMI greater than 25 respectively.

The cut off points as suggested by WHO Regional Report (2000) was
used in the present study and it is presented in Table1.

<table>
<thead>
<tr>
<th>Table 1 BMI cut off values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI Range</strong></td>
</tr>
<tr>
<td>Less than 18.5</td>
</tr>
<tr>
<td>18.5 - 20.0</td>
</tr>
<tr>
<td>20.0 - 23.0</td>
</tr>
<tr>
<td>23.0 - 25.0</td>
</tr>
<tr>
<td>Greater than 25.0</td>
</tr>
</tbody>
</table>

**Waist to Hip Ratio:**

The girth measurement technique was adopted from WHO report
(Helsing, 1988). Waist girth was measured using a non-stretchable measuring
tape at a level halfway between the iliac crest and the costal margin in the
mid-axillary line, with the subject in the standing position. Hip girth was
measured with the subject in the standing position, with both feet together at
the level of the greater trochanters. When the greater trochanters are not
palpable, then the measurement was taken at the level of the largest horizontal girth around the buttocks.

Waist to hip ratio was calculated using the formula given below:

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

The waist circumference recommended by WHO (James, 2005) for Asians was less than 90 cm for men and less than 80 cm for women and waist to hip ratio (WHR), as suggested by Willett et al. (1999) was less than 0.95 in males and less than 0.80 in females. These measurements were used in the present study as standards of comparison of data.

3.3.3 Clinical and Bio-chemical status

Clinical features:

Clinical screening of all the subjects, both cases (n=350) and controls (n=100) was done with the help of a schedule developed for the purpose. The schedule included related factors, which are reported to predispose CHD by many authors. Assessment schedule started with an appropriation of the case-history, diagnosis of incidents of CHD, signs and symptoms. It also had subject's own medical history and associated morbidity conditions like diabetes (Sadikot, 2006; Grundy et al., 1999 and Enas et al., 1998), hypertension (Mc Gill and Mc Mahan, 2005; Mahan and Stump, 2004 and Gafoorunissa and Krishnaswamy, 2000) and chronic obstructive pulmonary disease, postmenopausal status (Srinivasan and Sathyamoorthy, 2002) and
also family history of CHD, diabetes and hypertension (Sadikot, 2006 and Srinivasn and Sathyamoorthy, 2002).

After developing the schedule, it was subjected to screening by a panel of cardiologists. Necessary modifications as suggested by the panel were made and it was pre-tested on a comparable group of subjects prior to actual data collection (Appendix I).

For diagnostic purposes the standard values and references were made use. For hypertension a systolic blood pressure greater than 140 mm of Hg and / or a diastolic blood pressure greater than 90 mm of Hg or that the individual was being treated with anti hypertensive drugs were taken into account in accordance with the Sixth Joint National Committee (JNC VI, 1997) recommendation. Recording of systolic and diastolic blood pressure was also done using sphygmanometer.

Diabetes was diagnosed if any one reported to have diabetes with evidence of medical treatment, and fasting plasma glucose level of greater than 126 mg/dl or the two hour blood sugar was 200 mg /dl, as suggested by WHO (1999).

The postmenopausal state of women was ascertained if they had no regular monthly menstruation for more than one year as stated by Hoffmann et al. (2004).

Regarding the family history, it was considered to be positive when myocardial infarction or sudden death occurs before the age of 55 years in a
male first degree relative or before the age of 65 years in a female first degree relative (Krummel, 2004). Further the first-degree relative considered in this study included parents, siblings or offspring who were ever diagnosed with heart disease, diabetes or hypertension as suggested by Luepker et al. (2001).

**Bio-chemical parameters:**

Bio-chemical tests which can be conducted on easily accessible body fluids such as blood and urine, can help to diagnose disease at the subclinical stage, and confirm clinical diagnosis at the disease stage (Bamji, 2003).

For the last 50 years, a strong relationship has been recognized between the level of total cholesterol in the blood and risk of CHD. This relationship is seen at all levels of CHD mortality (Keys et al., 1980). Most international studies like MRFIT Study group (MacDonald and Joffies, 1992); Framingham Study (Levy and Kannel, 1988) and Seven Countries Study (Keys et al., 1986) emphasized the importance of elevated total cholesterol and LDL in the development of CHD. Also appropriate biochemical tests, as Jelliffe (1966) stressed will have to be selected for the particular survey contemplated.

The biochemical parameters like serum cholesterol (measured by a CHOD-PAP method), triglyceride (by a GPO-PAP method) and HDL
cholesterol (phosphotungstate/Mg) were studied on all samples (CHD and non CHD). The procedure is given in Appendix III.

LDL was calculated by using Friedewald formula (Friedewald et al., 1972):

\[
LDL \text{ cholesterol} = (\text{total cholesterol}) - (\text{HDL} \text{ cholesterol}) \times \left( \frac{\text{triglycerides}}{5} \right)
\]

\[VLDL = \frac{\text{triglycerides}}{5}\]

Total cholesterol to HDL cholesterol ratio was also calculated.

Standards for detecting hypercholesterolemia, and low HDLc level, were obtained from guidelines of Adult Treatment Panel III, National Cholesterol Education Programme, (2002) which is shown in the Table 2.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Desirable</th>
<th>Borderline high</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>&lt;200</td>
<td>200-240</td>
<td>&gt;240</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>&lt;130</td>
<td>130-160</td>
<td>&gt;160</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>&gt;40</td>
<td>&lt;40</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>&lt;150</td>
<td>150-200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>


Kang et al. (1992) defined hyperhomocystenemia when the plasma total homocysteine level is more than 15 micro mol per litre. The serum homocysteine level of a sub-sample of 30 CHD subjects were also tested to study its association with CHD. Patient's blood samples were analysed with
the assay kit (Diayme Labs, Canada). The procedure for the estimation of homocysteine is given in Appendix III.

3.3.4. Diet survey- dietary habits and food consumption pattern

Diet surveys constitute an essential part of any complete study of nutritional status of individuals or groups, providing essential information on nutrient intake levels, sources of nutrients, food habits and attitudes (Swaminathan, 2004). In the present investigation the association between diet and cardiovascular diseases which, has been indisputably shown in numerous studies (WHO, 2003; Jacobs and Steffen, 2003 and Singh et al., 1998) adds special significance to dietary inquiry.

As Thimmayamma and Rao, (2003) pointed out precise information on food consumption pattern of people through application of appropriate methodology is often needed not only for assessing the nutritional status of people but also for elucidating the relationship of nutrient intakes, their surplus or deficiency with degenerative diseases. Association of certain diseases including obesity, diabetes, hypertension and atheroma, with dietary patterns characterised by high intakes of calories, fat and cane sugar, has been stressed by Jelliffe, (1966), although other factors undoubtedly come into play, such as genetic constitution, the physiological stress of urban life, amount of exercise, etc.
The methods of diet survey adopted were:

- 24-hour diet recall method
- Food frequency questionnaire method

Dietary habits before the onset of the disease for the cases (n=350) and the dietary pattern of the control group (n=100) were elicited with the help of a pre tested open-ended structured interview schedule (Appendix I).

24-hour dietary recall method:

Twenty-four hour dietary recall on a large group of participants is an efficient way to measure the average dietary intake of a group (Patterson et al., 2004; Willett, 1998 and Thimmayamma, 1987). According to Garrow (2000) in diet recall the respondent is asked to recall the actual food and drink consumed on specified days, usually the immediate past 24 hours (24 hour recall).

The food intake of all cases (prior to hospitalization) and controls were recorded by 24hour diet recall method. The subjects were asked to recall a days food intake in terms of simple household measures. During the interview, food models and reference standard measuring cups and spoons were shown to the subjects so that they could give the portion sizes accurately.

Food items available in natural units (eg. a slice of bread, one egg, one fruit) add clarity to the question (Singhal et al., 1998). Values of house hold
measures, eg. cups, spoons were converted into raw equivalents and the nutrient intake was calculated using the food composition table (Gopalan et al., 2004). Nutritive value for fish was calculated using biochemical composition of Indian food fish by ICAR (Gopakumar, 1997) and cholesterol content of Indian fish and shellfish by Mathew et al. (1999). The mean food and nutrient intake of the sample were also calculated and compared with RDA given by ICMR (Pasricha and Thymmayamma, 2005 and Gopalan et al., 2004).

**Food Frequency questionnaire method:**

Garrow (2000) stated that in food frequency (and amount) questionnaires the respondent is presented with a list of foods and is required to say how often each item is consumed, in broad terms as X times per day / per week / per month etc. Foods listed are usually chosen for the specific purposes of a study and may not assess total diet. The food frequency questionnaire may be interviewer administered or self-completed.

A food frequency questionnaire including the list of foods commonly consumed by the people in Kerala was developed (Appendix IV) and administered to sub sample of 110 cases. Each subject was asked to report the usual frequency of food consumption and the usual portion size consumed during last one month. The frequency of consumption was measured on a seven-grade scale: Never, once in a month, twice per month, once in a week, two to three times per week, four to six times per week and daily. The questionnaire also included specific question like the type of fat used for
cooking. The cases (with CHD) were interviewed within a week of admission to the hospital and were asked to describe their usual dietary pattern before diagnosis of any known coronary artery disease. A modified version of food frequency questionnaire developed by Singhal et al. (1998) was used for the purpose.

The daily per capita nutrient intake was arrived at by multiplying the nutrient content of the specified portion of each food item by the frequency of it's daily consumption and summing over all items. The dietary information thus collected included each person's usual daily intake of energy, fat, protein, carbohydrate, saturated fatty acids, mono unsaturated fatty acids, polyunsaturated fatty acids, cholesterol, vitamin A, vitamin C, sodium, potassium and iron. The food frequency questionnaire was compared with a 24-hour diet recall of the same sample.

3.4. Analysis of data

The data collected by administering the pre-tested schedules and standardized tests described, were scored, tabulated, and analysed using SPSS package (version 15).

> 't' test

't' test was made use of to examine whether there was significant differences between the cases (CHD) and control (Non CHD) subjects with respect to the parameters like anthropometry, biochemical profile, food and nutrient intake.
Pearson chi square

Pearson chi square was used to find out the degree of association between selected variables and CHD.

Kendall’s coefficient of concordance

Kendall’s coefficient of concordance was computed for the various parameters like stress and emotions for the case and control subjects to have a mean rank of order of importance for the different variants in the parameter.

Canonical Discriminant function analysis

Canonical discriminant analysis was carried out to discriminate between the case and control sample based on the nutrient intake of both the sex.

Binary logistic regression

Binary logistic regression was used in the present study to estimate the relative risk of CHD based on the quantity of food consumption. According to Patterson et al (2004) relative risk is the ratio of the incidence in the exposed to the incidence in the unexposed group.

Correlation analysis

Correlation analysis was carried out to measure the degree of association between the incidence of CHD and various causative factors.
Multiple regression

Multiple regression is the procedure for quantifying the relationship of one variable with two or more variables. It was used in the present study to access the relationship between the total score (Y) on scores of the variables such as age, sex, income, education, smoking, alcohol consumption, exercise, work status, family history of CHD, blood pressure, comorbidities, lipid profile and anthropometric measurements. And also to assess the relative importance of each parameter on total score of patients suffering from CHD.