Studies on Prevalence of Cardio metabolic Risk Factors and Anaemia in Relation to Antioxidant Status of Postmenopausal Women from Different Ethnic Communities.

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Certificate

This is to certify that this thesis entitled ***" STUDIES ON PREVALENCE OF CARDIO METABOLIC RISK FACTORS AND ANAEMIA IN RELATION TO ANTIOXIDANT STATUS OF POST MENOPAUSAL WOMEN FROM DIFFERENT ETHNIC COMMUNITIES"-*** is a bonafied work of Mrs. Soma Chakraborti and was carried out under our supervision and guidance.

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I declare that the thesis entitled ***" STUDIES ON PREVALENCE OF CARDIO METABOLIC RISK FACTORS AND ANAEMIA IN RELATION TO ANTIOXIDANT STATUS OF POSTMENOPAUSAL WOMEN FROM DIFFERENT ETHNIC COMMUNITIES"*** has been prepared by me under the guidance of Dr. Manjunatha R. Aithala, Professor & Head, Department of Physiology, BLDE University's Shri B.M. Patil Medical College, Hospital and Research Centre, Vijaypur, Karnataka, India and co-guidance of Dr. Sankar Roy, Associate Professor of Department of Biochemistry, Tripura Medical College, Agartala, Tripura. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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DEDICATION

I dedicate this thesis to

The loving memory

of

MY BELOVED PARENTS

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Soma Chakraborti

ABSTRACT

BACKGROUND :

Cardio metabolic risk is a constellation of physical conditions and metabolic abnormalities occurring together that increases an individual's risk for the development of cardiovascular diseases and type II diabetes mellitus. It has been recognized over past few years that women are prone to various cardio metabolic risk. Menopausal changes are thought to increase cardio metabolic risk in women. Evidences show that sociodemographic and ethnic differences play important roles in cardio metabolic risk profile of subjects. Anaemia is a common finding in women. There are probabilities that both the conditions may coexist in women. Evidences suggest that increased oxidative stress to adipocytes is central to the pathogenesis of cardio metabolic disorders in subjects.

OBJECTIVES:

The main objective of the study was to assess the cardio metabolic risk profile of both premenopausal and postmenopausal women from different ethnic and non-ethnic communities of India and to evaluate association of traditional cardio metabolic risk factors with obesity and atherogenicity markers, anaemia status and status of various oxidative stress markers in women.

METHODS:

The present thesis is part of a hospital based observational study on cardio metabolic risk profile of women from Vijaypur, Karnataka and Agartala, Tripura. Four hundred and fifty (450) female subjects (age: 25-65 years) were evaluated for presence of various cardio metabolic risk factors. The study protocol included calculation of Body Mass Index(BMI), measurement of Waist Circumference (WC), Waist-Hip Ratio (WHR) and Blood Pressure (BP). Haemoglobin concentration (Hb%), Fasting blood glucose (FBG) level and lipid profile were measured in an auto analyser by using commercial kits. Plasma concentration of lipid peroxidation product Malondialdehyde (MDA) was estimated. Antioxidant enzymes Erythrocyte catalase (CAT), Erythrocyte superoxide dismutase (SOD), Erythrocyte Glutathione peroxidase (GPx) were assayed colorimetrically. Blood levels of antioxidant vitamins- Vitamin C (VIT-C) and Vitamin E (VIT-E) were estimated. Cardio metabolic risk profile of the subjects was evaluated according to consensus statement for diagnosis of general obesity, abdominal obesity and metabolic syndrome for Asian Indians. The association among menopausal status, various obesity and atherogenicity markers, anaemia, sociodemographic status, ethnicity and antioxidant status with cardio metabolic risk profile of the subjects were evaluated.

RESULTS:

26.60% of premenopausal subjects and 49.24% of postmenopausal subjects were found to have profound cardio metabolic risk which increases with age. Central obesity appears to be the most important cardio metabolic risk component in both pre and post menopausal groups. Triglyceride and LDL levels of the subjects were correlated with BMI, WC, WHtR and TG:HDL-C ratio. Systolic and diastolic blood pressures were correlated with TG:HDL-C ratio and WHR. Fasting blood glucose was correlated with WHR and WHtR. 44.71% of subjects with anaemia showed co-existence with profound cardio metabolic risk in them. Subjects with higher educational level and less family income were found to have lesser cardio metabolic risk. There was no significant difference in occurrence of cardio metabolic risk factors in women from different ethnic groups. Level of lipid peroxidation product MDA was significantly high in subjects with profound cardio metabolic risk. Levels of antioxidant enzymes CAT, SOD and GPx were found to be significantly less in these subjects. Levels of antioxidant vitamins - Vitamin C and Vitamin E did not show significant difference between subjects with profound cardio metabolic risk and those without cardio metabolic risk.

CONCLUSION:

The findings suggested that postmenopausal women had significantly more cardio metabolic risk than premenopausal women. In all groups of women, central obesity was the main component associated with cardio metabolic risk. There was a high co-existence of anaemia and cardio metabolic risk in subjects. Level of education and family income influenced cardio metabolic risk of the subject. Antioxidant status was associated with presence of cardio metabolic risk factors in women.

ABBREVIATIONS

1. apoA – Apoprotein A.
2. apoB – Apoprotein B
3. AT1R – Angiotensin II Type 1 Receptor
4. BMI – Body Mass Index.
5. CAT - Catalase
6. CHD – Coronary Heart Disease.
7. CHOD-PAP- Cholesterol oxidase - phenol + aminophenazone
8. CRP – C Reactive Protein.
9. DBP – Diastolic Blood Pressure.
10. DNPH - Dinitrophenylhydrazine
11. EGIR – European Group for the study of Insulin Resistance.
12. EDTA - Ethylenediaminetetraacetic acid.
13. eNOS – Endothelium-derived Nitric Oxide Synthase.
14. FBS – Fasting Blood Sugar
15. FFAs – Free Fatty Acids.
16. FRAP – Ferric-reducing ability of plasma.
17. $GLUT_4$ – Glucose Transporter-4.
18. GPx – Glutathione Peroxidase
19. GPO-PAP – Glycerol-3-phosphate oxidase- phenol + aminophenazone
20. GSH - Glutathione
21. H_MS – A Harmonized Definition.
22. H_2O_2 – Hydrogen Peroxide.
23. HC – Hip Circumference.
24. HDL – High Density Lipoprotein
25. HDL_C – High Density Lipoprotein Cholesterol.
26. HERS – Heart and Estrogen/Progestin Replacement Study.
27. ICMR- Indian Council of Medical Research
28. IDF – International Diabetic Federation..
29. IL-6 – Interleukin 6.
30. IR – Insulin Receptor.
31. IRS – Insulin Receptor Substrate.
32. LDL – Low Density Lipoprotein.

33. MAP Kinase – Mitogen Activated Protein Kinase.
34. MDA – Malondialdehyde.
35. MI – Myocardial Infarction.
36. NADP - Nicotinamide adenine dinucleotide phosphate
37. NADPH - Nicotinamide adenine dinucleotide phosphate reduced form
38. NCEP ATP III – National Cholesterol Education Programme-Third Adult Treatment Panel.
39. NHANES – National Health And Nutrition Examination Survey.
40. NHLBI – National Heart, Lung, Blood Institute.
41. NO – Nitric Oxide.
42. OxLDL – Oxidised Low Density Lipoprotein.
43. PAI-1 – Plasminogen Activator Inhibitor-1.
44. PP – Post Prandial
45. ROS – Reactive Oxygen Species.
46. SAT – Superficial Adipose Tissue.
47. SBP – Systolic Blood Pressure.
48. SdLDL – Small dense Low Density Lipoprotein.
49. SHEP – Systolic Hypertension in the Elderly Programme.
50. SNPs – Single Nucleotide Polymorphisms.
51. SOD – Superoxide Dismutase.
52. TBA – Thiobarbituric acid
53. TCA - Trichloroacetic acid
54. TC – Total Cholesterol.
55. TGs – Triglycerides.
56. TNFα – Tumour Necrosis Factor alpha.
57. VAT – Visceral Adipose Tissue.
58. VA-HIT – Veterans Affairs Cooperative studies Program High Density Lipoprotein Cholesterol Intervention Trial.
59. WC – Waist Circumference.
60. WHO – World Health Organization.
61. WHI – Woman's Health Initiative.
62. WHR – Waist - Hip Ratio.
63. WHtR – Waist-Height Ratio.

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LIST OF PUBLICATIONS RELATED TO PhD THESIS:

Full Papers :

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CHAPTER 1:

PURPOSE OF THE STUDY

1.1 INTRODUCTION:

A global transition in disease pattern has been observed during recent years, where the relative impact of infectious diseases are decreasing while chronic diseases like cardiovascular diseases (CVD) and diabetes mellitus are increasingly dominating the disease pattern[1]. Epidemiologists in India along with experts in WHO have been sounding an alarm on rapidly rising burden of CVDs for past 15 years. It is estimated that by 2020, CVD will be the largest cause of disability and death in India with 2.6 million Indians predicted to die due to CVDs[2].

Various chronic non-communicable diseases in particular cardiovascular diseases, anaemia, diabetes mellitus and cancer are increasingly becoming a significant cause of morbidity and mortality in developing countries[3].World Health Organisation (WHO) projected, by the end of the year 2015, non-communicable diseases will account for over 70% of all deaths with 80% of death occurring in developing countries[4].Rapid urbanization, changing life style, sociocultural factors, poverty and poor maternal, foetal and infant nutrition form the basis of the development of different forms of non-communicable diseases[5].

Cardio metabolic disorders can be predicted by presence of various risk factors in subjects that are represented as a constellation of interconnected physiological, biochemical, clinical and metabolic risk factors including hypertension, dyslipidaemia, central obesity, glucose intolerance, pro-inflammatory and pro-thrombotic states, which reflects an underlying insulin resistance[6]. It is a modern day epidemic which predicts total and CVD mortality, the incidence and progression of carotid atherosclerosis and sudden death, independent of other CVD risk[7]. Subjects with predominance of such risk factors have three fold probability of suffering from heart attacks or stroke, two fold probability of developing CVD or dying from such events, and five-fold greater probability of developing type 2 diabetes mellitus in both sexes when compared to normal individuals[8-10].

Women share many risk factors for cardiovascular disorders as men and in both the risk are associated with age[11].However, sex difference in occurrence of various cardio metabolic risk factors exists, suggesting a sex-specific difference in the physiological mechanisms and risk factors for disease occurrence[12]. During the last decade, researchers all over the world including India, have shown the interest on assessment of burden of cardio metabolic risk in different groups of women.

Menopause is thought to be one of the major contributors for increasing burden of cardio metabolic risk in women[13]. The biological plausibility of such relationship depends on the fact that menopause brings in various physiological and hormonal changes including increased adiposity, hyperglycaemia, hyperinsulinism and dyslipidaemia which contribute to predominance of such risk in menopausal women[14]. Limited information is available on cardio metabolic status of pre and postmenopausal Indian women. Moreover, the association among various obesity and atherogenicity markers and traditional cardio metabolic risk factors is not evaluated in this group.

Anaemia is one of the most common types of nutritional problems in women worldwide[15]. A World Health Organisation estimate suggested that globally up to 500 million women during their reproductive age suffered from anaemia[16]. As both anaemia and various cardio metabolic risk factors are associated with inflammatory state in the subjects, there is a theoretical possibility that both these conditions are interconnected. Anaemia is common in patients with heart failure[17]. There has been increasing appreciation of the significance of anaemia in the pathophysiology, treatment and prognosis of heart failure. Once considered a downstream complication of heart failure, anaemia is now emerging as a crucial and potentially modifiable factor in the overall treatment strategy for patients with chronic heart failure[18].There are very few reported studies on co-existence of anaemia and cardio metabolic risk factors. It is, therefore, important to assess the prevalence and possible co-existence of the two conditions in a population who are prone to develop above said conditions.

Studies all over the world have shown significant role of ethnicity, life style, socioeconomic and nutritional factors in pathogenesis of many diseases[19].According to the United Nations geographical region classifications, India falls under the region-South Asia along with Afghanistan, Bangladesh, Bhutan, Iran, Maldives, Nepal, Pakistan and Srilanka[20]. Ethnic distribution of South Asian population is complex as it has been invaded and settled by many ethnic groups over the centuries including various Dravidian, Indo-Aryan and

Iranian groups and amalgamation of Dravidian, Indo-Aryan and native societies has produced a composite culture[21]. Nationally, representative studies comprising of subjects from diverse ethnic and socioeconomic background are generally not available from any South Asian country[22]. Considering India as a multi-ethnic, multi-racial and multi-cultural country, it is, therefore, pertinent to include subjects from different ethnic and cultural backgrounds to draw a meaningful conclusion from such studies[23,24].

Recent evidences, especially from animal models, suggested role of antioxidant defence mechanism in pathogenesis of different diseases[25,26]. In recent years, number of studies confirmed that oxidative stress, chronic inflammation and angiogenesis all play important role in the pathogenesis of cardio metabolic disorders[27]. A growing body of evidence now suggests that increased oxidative stress to adipocytes is central to the pathogenesis of different cardio metabolic disorders[28]. Chronic hyperglycaemia causes oxidative stress in tissues prone to complications in patients with diabetes mellitus[29]. Oxidative stress occurs in a cellular system when the production of free radical moieties exceeds the antioxidant capacity of that system. If cellular antioxidants do not remove free radicals, radicals attack and damage proteins, lipids and nucleic acids. The oxidized or nitrosilated products of free radical attack have decreased biological activity leading to loss of energy metabolism, cell signalling, transport and other major functions. These altered products are also targeted for proteosome degradation, further decreasing cellular function. Accumulation of such injury ultimately leads a cell to die through necrotic or apoptotic mechanisms. A puzzle of many pieces of evidence suggests that free radical over generation may be considered the key in the generation of insulin resistance, diabetes mellitus and cardiovascular diseases [30]. However, evidences linking these factors with pathogenesis of cardio metabolic disorders and anaemia in human, especially in women during postmenopausal stage of their life is sparse.

Studies conducted over last decade revealed that burden of cardio metabolic risk is gradually increasing among different populations. Women, especially the menopausal women are found to be more susceptible to such risk. There is considerable biological and epidemiological evidence that suggested presence of different cardio metabolic risk factors increased the probability of future development of cardiovascular diseases and diabetes mellitus. Early detection of such risk might help in planning early intervention through life style modification which might prevent these diseases .

In view of the above fact, the present study is designed to evaluate cardio metabolic risk profile in a representative sample of postmenopausal Indian women consisting of subjects from two different ethnic and non-ethnic groups from two different regions of India and to compare the parameters with premenopausal women from same regions.

The health status, especially of women of the two ethnic groups chosen for the study is still remained unexplored. Reports suggested women from such groups are at the highest risk of developing various kinds of diseases which might be related to their life style and socioeconomic status.

Therefore, this study which aimed to relate the health status of these women with their life style, socioeconomic status and ethnicity is having a great scientific relevance .

The National Health Policy of the country aims to achieve the goal of Health for all by the year 2020. The present study might contribute in achieving the goal by providing a background knowledge regarding the health status of the targeted group to the policy makers.

The present study is designed to investigate the relationship between anaemia and cardio metabolic risk in women. There are very few reports on this approach of simultaneous investigation on both these conditions in women, who are particularly prone to both the conditions.

Moreover, the study aimed to relate oxidative stress and status of some non-enzymatic antioxidants in postmenopausal women. This noble approach will provide insight into the understanding of pathophysiological change associated with various cardio metabolic risk profile in women.

There are limited published data on cardio metabolic risk profile and the effects of different confounding factors on these factors in the targeted study group. It is, therefore, justifiable to conduct the study that describes the cardio metabolic risk profile in a representative sample of Indian postmenopausal women with studies on status of various confounding factors like anaemia and antioxidant status in them.

The study findings might form the basis for future research on cardio metabolic risk among this population.

1.2. HYPOTHESIS:

Hypothesis I: The cardio metabolic risk in women is influenced by their menopausal status.
Hypothesis II: Various obesity and atherogenicity markers present in the subjects influence traditional cardio metabolic risk factors of the subjects.
Hypothesis III: There is relationship between anaemia status and cardio metabolic status of the subjects.
Hypothesis IV: Occurrence of cardio metabolic risk in the subjects is influenced by their socio-demographic status and ethnicity.
Hypothesis V: The cardio metabolic risk in women is influenced by oxidative stress and antioxidant defence mechanism of the subjects.

1.3. OBJECTIVES OF THE STUDY:

Broad Objective:

With above background, the present study aims to evaluate the cardio metabolic risk profile in Indian women comprising of subjects from two different ethnic and non-ethnic communities in relation to their menopausal status, anaemia and status of their antioxidant defence system.

Specific objectives:

i) To evaluate cardio metabolic risk profile in both pre and postmenopausal Indian women.

ii) To evaluate association of traditional cardio metabolic risk factors with various obesity markers and atherogenicity markers in the subjects.

iii)To evaluate association of various haematological parameters, specially haemoglobin concentration with various markers of cardio metabolic risk in subjects.

iv)To evaluate role of nutritional status, socio-demographic characteristics and ethnicity on cardio metabolic risk and anaemia in the subjects.

v) To evaluate association between various enzymatic and non-enzymatic antioxidants and cardio metabolic risk profile and anaemia in subjects.

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CHAPTER 2:

REVIEW OF LITERATURE

2.1. INTRODUCTION:

Researchers began to document regarding clustering of various elements of cardio metabolic risk in subjects in early 1960s and 1970s.The combination of cardiovascular and metabolic disturbances was first described as the clustering of hypertension, hyperglycaemia and gout by Kylin in 1923[1].Later, it was established that insulin resistance is the key abnormality associated with atherogenic, prothrombotic and inflammatory states relative to cardiovascular risk. Two decades later, Vague, in 1947 noted that upper body obesity known as android or male-type obesity was the type most often associated with the metabolic abnormalities seen with diabetes and cardiovascular diseases [2].

In 1977, Haller used the term "Metabolic Syndrome" for association of obesity, diabetes mellitus, hyperlipoproteinaemia, hyperuricaemia and steatosis hepatitis when relating the additive effects of risk factors on atherosclerosis[3]. In the same year, Singer used the term for association of obesity, gout, diabetes mellitus and hypertension with hyperlipoproteinaemia (Singer, 1977)[4]. Gerald B. Phillips, in 1978, developed the concept that risk factors for myocardial infarction form a "constellation of abnormalities" including glucose intolerance, hyperinsulinaemia, hyperlipidaemia, hypercholesterolemia, hypertriglyceridemia and hypertension. There is association not only with heart disease, but also with aging, obesity and other clinical states. He suggested there must be an underlying linking factor, the identification of which could lead to the prevention of cardiovascular diseases. He hypothesized that this factor was sex hormone[5].

In 1988, Gerald M. Reaven proposed insulin resistance as the fundamental factor and named the constellation of abnormalities as " Syndrome X"[6]. Unfortunately, Reaven did not include abdominal obesity, which has also been put forward as the underlying factor, as part of the condition today. After several name changes over the past two decades including the term diabesity used in lay publications, name became "Metabolic Syndrome". The terms "Metabolic Syndrome," "Insulin Resistance Syndrome" and "Syndrome X" are now used exclusively to define a constellation of abnormalities that is associated with increased risk for the development of type 2 diabetes and atherosclerotic vascular disease.

A number of expert groups have attempted to develop a unifying definition for the metabolic syndrome. The most widely accepted definitions have been produced by the World Health Organization (WHO)[7]. European Group for the Study of Insulin Resistance (EGIR)[8]

and National Cholesterol Education Program-Third Adult Treatment Panel (NCEP ATP III) [9]. All groups agreed on the core components of the metabolic syndrome: obesity, insulin resistance, dyslipidaemia and hypertension. However, they provide different clinical criteria to identify such a cluster**.** For example, unlike the other two definitions, NCEP ATP III definition does not obligatorily require impaired glucose regulation or insulin resistance as an essential component. In addition, the levels set for each component and the combination of components required to identify subjects with potential cardio metabolic risk are slightly different in these three recommendations[10]. Recently, a harmonized definition (H_MS) on cardio metabolic risk factors was released by an expert group from the International Diabetic Federation (IDF), National Heart, Lung, Blood Institute (NHLBI), World Health Federation and other international associations [11.] The harmonized definition uses uniform cut-off points for all the risk factors and recommended that individuals with any three of the following components should be considered at potential cardio metabolic risk : Increased waist circumference (population specific) plus any 2 of the following : i) Blood pressure - 130 mmHg systolic or ≥85 mmHg diastolic or on treatment for hypertension ; ii) Blood glucose - ≥100 mg/dL or on antihypertensive treatment ; iii) Lipid profile - TGs ≥150mg/dL or on TGs lowering drug ; HDL-C <40mg/dL in men or <50mg/dL in women or on HDL-C lowering drug. The population and gender specific cut-off for waist circumference recommended on the basis of various epidemiological studies are as follows [12].

Country/ethnic group	Waist circumference cut-off Male (cm) Female (cm)
Europids In USA, ATPIII values (102 cm for males; 88 cm for females) are likely to continue to be used for clinical purposes.	≥94 ≥80
South Asians Based on a Chinese, Malay and Asian-Indian population.	≥90 ≥80
Chinese	≥90 ≥80
Japanese	≥90 ≥80
Ethnic South and Central Americans	Use South Asians recommendations until more specific data are available.
Sub-Saharan Africans	Use European data until more specific data are available.
Eastern Mediterranean and Middle East (Arabs) population	Use European data until more specific data are available.

Table I : Country-wise cut off for waist circumference [12].

2.2 PATHOPHYSIOLOGICAL BASIS OF VARIOUS CARDIO METABOLIC RISK FACTORS IN SUBJECTS :

The pathophysiological basis of various cardio metabolic risk factors in subjects are not yet fully understood. These are affected by complex genetic and environmental factors and their interactions. The most accepted and unifying hypothesis to describe the pathophysiological basis of various cardio metabolic disorders are insulin resistance and abdominal obesity [13].

Insulin resistance is a condition in which there is an insufficient insulin action in liver, skeletal muscle and adipose tissue. Insulin resistance induces increased gluconeogenesis in the liver, decreased glucose disposal in the muscle, endothelial dysfunction in the arteries and increased release of free fatty acids (FFAs) from the adipose tissue [14]. Elevated levels of circulating FFAs contribute to the development of insulin resistance by inhibiting insulin signalling. Plasma FFAs are derived mainly from adipose tissue by the action of lipases. Insulin inhibits lipolysis in adipose tissue and glucose production in the liver. Thus, when insulin resistance develops, the inhibitory effect of insulin on lipolysis is suppressed. The increased amount of lipolysis in adipose tissue produces more FFAs, which could further inhibit the antilipolytic effect of insulin, creating additional lipolysis. In addition, increased FFAs may result in ectopic lipid formation in the liver. Ectopic lipid accumulation in liver, muscle and pancreas further increases insulin resistance in these sites [15].

In the case of obesity, particularly abdominal obesity, the release of FFAs is increased. In addition, there is an increased production of several inflammatory cytokines and reduced production of anti-inflammatory adipokines [16]. This imbalance in the production of inflammatory cytokines favours not only the inflammatory state associated with obesity but also induces insulin resistance by impairing insulin signalling transduction[17]. Adipose tissue is a heterogeneous mix of adipocytes, stromal pre adipocytes, immune cells and endothelium, and it can respond rapidly and dynamically to alterations in nutrient excess through hypertrophy and hyperplasia [18]. With obesity and progressive adipocytes enlargement, blood supply of adipocytes may be reduced with consequent hypoxia [19].

Hypoxia has been proposed to be an inciting etiology of necrosis and macrophage infiltration into adipose tissue that leads to an overproduction of biologically active metabolites known as adipocytokines which include glycerol, free fatty acids (FFA), pro-inflammatory mediators (tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6)),

plasminogen activator inhibitor-1 (PAI-1) and C-reactive protein (CRP) [20].This results in a localized inflammation in adipose tissue that propagates an overall systemic inflammation associated with the development of obesity related co-morbidities [21]. Adipocytokines integrate the endocrine, autocrine and paracrine signals to mediate the multiple processes including insulin sensitivity, oxidant stress, energy metabolism, blood coagulation and inflammatory responses which are thought to accelerate atherosclerosis, plaque rupture and atherothrombosis[22,23]. This shows that the adipose tissue is not only specialized in the storage and mobilization of lipids but it is also a remarkable endocrine organ releasing the numerous cytokines associated with pathogenesis of cardio metabolic risk in subjects.

In the case of insulin resistance, increased flux of FFAs to the liver increases the hepatic production of apoB containing triglyceride rich VLDL particles. ApoB serves as a structural protein for cholesterol and triglyceride containing lipoproteins that are carried from the liver to the site of use, whereas apoprotein A1-containing particles mediate the reverse transport from the peripheral tissue to the liver [24]. Several studies have recently identified hepatic VLDL overproduction as a critical underlying factor in the development of metabolic dyslipidemia [25]. The presence of hypertriglyceridemia induces changes in lipoprotein composition and reduction of HDL cholesterol.

The composition of LDL is modified producing small dense LDL (SdLDL). Potential atherogenic mechanisms of SdLDL particles is low affinity to the LDL receptor and long retention time in the circulation [26]. In addition, SdLDL contains relatively smaller amounts of antioxidants compared to LDL cholesterol and therefore is more prone to oxidation forming oxidized LDL (OxLDL) particles. Recent studies have suggested that OxLDL may have an important role in the pathogenesis of obesity and insulin resistance [27].

Hypertension has been suggested to relate to insulin resistance in several mechanisms. First, in the presence of insulin resistance the vasodilatory effect of insulin in the endothelium may be suppressed resulting vasoconstriction [28]. Compensatory hyperinsulinemia increases the activity of the sympathetic nervous system, where the effect on insulin action is preserved[29]. Renal Sodium reabsorption in the kidney is increased directly by adipose tissue and via increased sympathetic nervous activation. In addition, FFAs produced by adipose tissue may directly mediate vasoconstriction [30]. Adipocytes also produce a variety of vasoactive peptides, which may impair the vasodilatory effect of insulin. Indeed, the relation between insulin resistance and hypertension is more evident in the case of obesity, suggesting that the effect may be mediated by adipose tissue. In addition, it has been suggested that LDL

and triglycerides may damage the arterial epithelium, impair Nitric Oxide release and cause endothelial dysfunction. Therefore, dyslipidemia characterized by elevated levels of apoB containing lipoproteins could cause hypertension by mechanisms only partly related to obesity and insulin resistance [31].

Results from multiple genome-wide studies have shown genetic basis for the individual components of the cardio metabolic risk factors in individuals like obesity, hypertension, dyslipidemia, hyperglycemia[32]. Such associations might facilitate or enable the development of the cardio metabolic risk in subject. In addition, some candidate genes have been suggested to affect more than one risk component. Although genetic contribution on the development of cardio metabolic disorders exists , the proportion of variance explained has been low [33,34]. No genetic test is available that may be used in the diagnosis of such disorders. The lack of association is likely due to the complex interplay between gene and environment necessary for expression of this phenotype. Genetics of cardio metabolic risk involves a large number of genes having weak effects but they may interact with each other and work synergistically with environmental factors like diet, physical activity, alcohol intake, smoking etc. in the pathogenesis of the cardio metabolic risk in subjects [35].

2.3 GENDER DIFFERENCES IN CARDIO METABOLIC RISK FACTORS:

Men were traditionally considered to be at higher risk for cardio metabolic disorders than women. This was mainly due to misperception that females are naturally protected against cardiovascular diseases. However, the scenario started changing during 1990s. Since then, an increasing number of studies have been reported on status of cardiovascular health in women. Accumulating and emerging data demonstrated that a significant heterogeneity exists between men and women regarding cardio metabolic risk[36].

Recent data from the National Health and Nutrition Examination Surveys (NHANES) have shown that over the past two decades the prevalence of myocardial infarctions has increased in midlife (35 to 54 years) women, while declining in similarly aged men. Women with clinical manifestation of coronary heart diseases are in general older than men with higher expression of cardiovascular risk factors [37].

Although most of the cardio metabolic risk factors contribute to the health outcome in both men and women, the relative impact of individual risk factor might be different. Key sex differences in risk factors include distinctions in (a) glycemic indices, (b) body fat distribution, (c) adipocyte size and function, (d) hormonal regulation of body weight and adiposity and (e) the influence of estrogen decline on risk factor clustering[38].

Obesity is an independent cardio metabolic risk factor in women as well as in men. Willet and colleagues [39] from the Nurses' Health Study showed that even women with a modestly increased body mass index (<25 and >29 kg/m^2) had twice the risk of coronary heart diseases as the leanest women (body mass index >21 kg/m^2). Independent of overall obesity, the distribution of body fat is the most important determinant of cardiovascular risk. It has been shown that truncal obesity, the so-called android habitus, confers a far higher risk than the peripheral or 'gynecoid' body fat distribution [40]. Both waist-hip ratio and waist circumference are highly correlated to the risk of coronary heart disease.

Men and women display a conspicuous sex dimorphism in body fat distribution with substantial variation that may be exclusive to our species. In his seminal observations,
Vague referred to android and gynoid obesities when describing adipose tissue accrual in the upper body (trunk and abdomen) in men and lower body (hips and thighs) in women, respectively. The teleological explanation for differential fat partitioning is presumably due to evolutionary and sexual selection pressures which favour storage of excess calories in different depots. However, the precise biologic mediators leading to topographical

differences in body fat distribution remain to be fully elucidated[41].

With regard to sex differences in central obesity as shown by computed tomographic measurements, the amount of VAT is up to 2-fold higher in men than in premenopausal women [42]. In men, VAT accrual generally increases with the amount of total body fat, whereas in women, VAT accumulation is less a function of total adiposity. It has been convincingly demonstrated that even after accounting for total body fat mass, premenopausal women have a lower ratio of VAT to total body fat than men. Women had less visceral fat despite having a higher total body fat, BMI and abdominal SAT. Premenopausal women therefore appear to accumulate a substantial amount of total body fat before increases in visceral fat are observed. Moreover, it has been demonstrated that for the same waist circumference, men have more VAT than women [43]. Thus, a large waistline alone, although a convenient measure, may not be an accurate indicator of visceral obesity. Data from corroborative findings from a more recent meta-analysis suggested that men experience greater reductions in visceral fat and potentially greater improvements in metabolic profile than women despite similar levels of weight loss. The sex differences in distribution and impact of visceral adipose tissue factors responsible for better metabolic profile in men require further study[44].

Total cholesterol and low-density lipoprotein cholesterol (LDL) levels in men and women are similar up to 20 years of age. In the third and fourth decades, cholesterol levels increase more sharply in men than in women. HDL levels are higher in women than in men from young adulthood onwards. Some studies have described a decrease in HDL levels following the menopause. The loss of protection from HDL is considered to be a major factor for the increased coronary risk in postmenopausal women. It has been suggested that low levels of HDL are more predictive of coronary artery disease in women than in men. Because of the higher level of HDL in women, a modification of the current National Cholesterol Education Program (NCEP) guidelines has been proposed with more aggressive targets for HDL in women. The Veterans Affairs Cooperative Studies Program High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) showed that a modest increase in HDL levels in men with coronary heart disease and normal LDL levels resulted in a significant reduction in the risk of major cardiovascular events. So far, similar data for women are not available[36].

Elevated levels of triglycerides have been associated with an increased risk of CHD in men and women. However, the role of plasma triglycerides as an independent risk factor is still elusive. First, there are methodological difficulties in interpreting triglyceride. Second, strong interactions exist between triglycerides and other lipid factors. Elevated triglycerides are often seen with lower HDL levels and this combination has been associated with increased cardio metabolic risk [45]. A meta-analysis including more than 46000 men and nearly 11000 women showed for men and women respectively, a 32% and 76% increase in cardiovascular risk associated with a 1μmmol/ l increase in triglycerides. After adjustment for HDL and other risk factors, these risks were decreased to 14% in men and 37% in women, but this remained statistically significant for both genders. It seems that elevated triglycerides increase cardiovascular risk more in women than in men, implying a gender difference in the role of triglycerides in atherosclerosis. Therefore, analogous to the gender-specific approach for HDL, the latest NCEP guidelines have suggested that the optimal levels for triglycerides may be lower for women [46].

Diabetes mellitus is a powerful cardio metabolic risk factor. Up to 75–80% of adult diabetic patients die of cardiovascular diseases and 75% of these deaths are caused by coronary heart disease (CHD). Compared to diabetic men, who have a two-fold to three-fold increased risk of CHD, diabetic women are reported to have a three-fold to seven-fold increased risk. Thus, diabetes seems to eliminate the premenopausal 'female advantage' in the prevalence of cardio metabolic disorders [47]. Mortality from myocardial infarction is significantly higher in diabetic women than in non-diabetic women and in men with or without diabetes mellitus. Lipid abnormalities frequently found in patients with diabetes type II are elevated triglycerides, low HDL levels and small dense LDL [48]. Decreased HDL and very low-density lipoprotein levels predict CHD mortality in diabetic women but not in non-diabetic women or diabetic and non-diabetic men. Because of the poor prognosis for women with diabetes, aggressive treatment of cardiovascular risk factors, such as dyslipidemia, should be a high priority[49].

Isolated systolic hypertension is a common finding in elderly women with a prevalence of 30% in women over 65 years of age. The Systolic Hypertension in the Elderly Program (SHEP) has shown that both men and women with isolated systolic hypertension benefit from blood pressure control. However, large long-term clinical trials have included both men and women and a meta-analysis of these studies did not show significant gender differences in blood pressure and clinical outcome [50].

Analysis of the published reports on the role of a number of risk factors with emphasis on possible differences between men and women revealed, except for female hormonal status, no risk factor has been recognized as acting on one gender but not on the other. These findings indicated that the pathogenesis of coronary heart disease is very similar for men and women. Yet, in individuals with diabetes mellitus, HDL and triglycerides levels have been found to have a greater impact on CHD risk in women compared to men. In addition, there are indications that risk factors such as smoking, family history and inflammation characterized as C-reactive protein, have a more negative influence on CHD in women than in men. On the other hand, the evidence showed that lipoprotein (a) is a stronger cardiovascular risk factor in men than in women [51]. Therefore, for optimal treatment and prevention of cardio metabolic disorders, it is necessary to acknowledge that women and men did not show a similar response to risk factors or to treatment. Therefore, it is essential that studies present results according to gender, in order to comprehend to what extent preventive measures of cardio metabolic risk are similar for men and women.

2.4 CARDIO METABOLIC RISK PROFILE IN WOMEN - ROLE OF MENOPAUSE:

The hypothesis that menopause and consequent biological modifications are related to cardio metabolic risk in women is derived from the observation that incidence and mortality rates for coronary heart disease in women below menopause are substantially lower than in men but tend to rise approaching those of men at older ages [52]. However, it is difficult to disentangle the effect of age from that of menopause on cardio metabolic risk, because the two variables are strongly related and an apparent higher risk from menopause may simply be due to the rise of coronary heart disease incidence and mortality with increasing age[53].

The overall epidemiological evidence on the relationship between menopause and age and cardio metabolic risk is still controversial. Most information is derived from different cohort studies and case–control studies. As for the cohort studies, the 20-year follow-up of the Framingham Study showed a 2-fold increase in relative risk in postmenopausal versus premenopausal women; in the 24-year follow-up of the same cohort, based on 43 cases of fatal and non-fatal MI, the MI incidence rate was 1.4 in premenopausal and 3.9 in post-menopausal women. In a cohort of Swedish women, based on 25 cases of MI, the relative risk were 2.0(95% CI: 0.2–19.1) for women aged more than 40 years at menopause, 2.2 and 1.4 for women aged more than 45 years and more than 50 years, compared with premenopausal women. In 6-year follow-up of the American Nurses' Health Study, after strict allowance for age, compared with premenopausal women, never HRT users with natural menopause had a relative risk of 1.1, and those with surgical menopause had a relative risk of 1.7 [54].

In a Dutch study of 12195 women including 824 deaths from CVD, the overall relative risk was 0.982 per year delay of menopause and the inverse relation was greater at younger age. In a study from Norway, including 2767 cases of coronary heart disease, the relative risk was 0.84 for women aged more than 53 years at menopause compared with those aged more than 40 years. In the US National Health and Examination Survey (NHANES) I Study, based on 84 cases of fatal acute MI, a moderate and not significant association was observed with age at menopause [55].

The relative risk of cardiovascular disease was found to be 3.2 in women with natural menopause and of 2.7 in those with surgical menopause at age more than 45 years, compared with women with natural menopause when aged more than 51 years. Study found increased risks of coronary heart disease in women with menopause either at young (35–40 years) or

later age(56–60 years), the association being stronger in non-HRT users[56]. Thus, there is some suggestion that after allowing for age, postmenopausal women are at higher risk of coronary heart disease, although there is substantial heterogeneity in the results across various studies on menopause, and age at menopause and coronary heart disease. This is not easily explained by the different type of studies (cohort or case–control), the inclusion of fatal or non-fatal diseases, the inclusion of hormone therapy users, the cut-points selected for age at menopause and other identified factors. Part of these discrepancies may depend on difficulties in the collection and analysis of epidemiological data on menopause. Besides uncertainties in the definition of the peri-menopausal period, age at menopause is difficult to establish in women after hysterectomy and in those using HRT. Moreover, similar to any other time factor, it is important to make an extremely strict age-adjustment to obtain an unbiased quantification of risk [57].

Although the epidemiological studies do not show a large immediate effect of menopause on coronary heart disease events, ovarian hormone deprivation after menopause is associated with an increase in CVD risk factors. Estrogens improve endothelial function and vascular reactivity and reduce the progression of coronary atherosclerosis both in animals and in early post-menopausal women. When administered in combination with estrogens, progestogens may, in some instances, interfere with the endothelial effect of estrogens. In post-menopausal women, data on the anti-atherogenetic effect of progestogens are scanty and mainly limited to medroxyprogesterone acetate and gestodene. Combining more androgenic progestogens with estrogens also negatively affects peripheral vascular resistance and vascular reactivity. Lipid, glucose and insulin metabolism are improved by estrogen replacement therapy, but this effect may be reversed by the combination of estrogen with androgenic progestogens, whereas combination with non-androgenic progestogens has a more favourable metabolic profile[58,59].

It is well known that endogenous and exogenous estrogens stimulate hepatic synthesis of angiotensin that in turn increases plasma levels of aldosterone through an activation of the rennin angiotensin system. Therefore, in predisposed women estrogens may cause sodium and water retention. Progestogens have different effects on sodium metabolism that may range from extreme sodium retention to sodium excretion. Synthetic progestogens cause an increase of hepatic angiotensin and plasma angiotensin, thereby enhancing Sodium retention. Progesterone competes with aldosterone at kidney level causing a dose-dependent natriuretic effect. Similar effect on Renal excretion of Na+ is shared by dydrogesterone, whereas a

newer progestogen, drospirenone, have a more complex effect on sodium balance having direct anti-aldosterone activity. Drospirenone has a powerful antimineralocorticoid effect that is effective in counter balancing the increase of aldosterone that may be induced by estrogen administration especially in predisposed women and in those predisposed to develop arterial hypertension. Furthermore, in hypertensive women drospirenone is effective in reducing blood pressure either alone or in combination with other anti-hypertensive agents[60].

Some cardiovascular effects of estrogen may be counteracted by progestogens. Progesterone receptors are present in the arterial wall and there is evidence that the arterial effects of progestogens are mediated through progesterone receptors as well as through down-regulation of E2 receptors. Progestogen therapy can stabilize arteries in a state of vasomotor instability but may also induce vasoconstriction of estrogenized vessels and precipitate arrhythmia. According to their chemical structure, progestogens have different metabolic and vascular effects that may enhance or abolish the effects induced by estrogen therapy on cardiovascular risk factors and on vascular functions[61].

Therefore, the overall effect of hormone replacement therapy on blood pressure is related, on one hand, to the individual response to the activation of the renin angiotensin system and on the other hand, to the dose and type of molecules used. Higher doses of estrogens may induce sodium retention as do synthetic and androgenic progestogens. Micronized progesterone, dydrogesterone and drospirenone have an antimineralocorticoid effect (that is higher for drospirenone) and therefore antagonize the sodium retention effect of estrogens . These progestogens should be preferred in women with borderline hypertension, in those with arterial hypertension well controlled by anti-hypertensive therapy and in women with a tendency to sodium retention[62].

Evidence from many observational studies suggests that estrogen replacement therapy after menopause can provide protection against heart disease. However, the results of randomized studies using estrogen and progestogens in women averaging >60 years of age failed to confirm the results of the observational studies. Moreover, awareness and knowledge regarding the cardiovascular risk in women, vis-a-vis, their menopausal status is suboptimal. Therefore, further studies are necessary to better evaluate various cardio metabolic risk factors in women in relation to their menopausal status to understand risk/benefit ratio of various therapies available. Until then, prevention of events due to atherosclerosis should rely on diet and fitness and low-dose aspirin therapy[63].

2.5. EPIDEMIOLOGY OF CARDIO METABOLIC RISK FACTORS:

Cardiovascular diseases (CVD) are the number one reason of death globally and are predictable to remain the leading cause of deaths. An approximate 17.5 million people died from cardiovascular diseases in 2005, signifying 30 % of all global deaths. Of these deaths, 7.6 million were due to heart attacks and 5.7 million were due to stroke. Around 80% of these deaths occurred in low and middle-income countries (LMIC). An approximate 20 million people will die from CVD every year, mainly from heart attacks and strokes if right action is not taken, by 2015 [64,65].

Women are supposed to experience excessively high mortality from CVD. By 2040, women in countries like Russia, Brazil, India, China, and South Africa will represent a higher proportion of CVD deaths in comparison to men. It was projected that by 2040, women population in China will become 49.5 per cent of the total population and they will account for 54.6 percent of CVD deaths. The increase of CVD deaths in Brazil and China among working-aged women between 2000 and 2040 will be higher than that for men. Projections suggested that coronary heart disease (CHD) mortality for all developing countries will increase by 120 percent for women and 137 percent for men. Estimations for the next two decades enclose tripling of CHD and stroke mortality in Latin America, the Middle East, and even sub-Saharan Africa. The proportion of increase will surpass the increase in other regions, except Asian and Pacific Island countries. The increase in more-developed nations, largely attributable to a growth of the population of older people at risk [66]. Data available from the World Health Organization MONICA Project indicated that the coronary event rate in men was highest in Finland (North Karelia, 835) and lowest in China (Beijing, 81). For women, the maximum occurrence rate was in the UK (Glasgow, Scotland, 265) and the lowest in Spain (Catalonia, 35) and China (Beijing, 35). These data revealed results from 35 MONICA Project populations collected during the mid- 1980s until the mid-1990s [67].

Data from the INTERHEART study showed that rates of CVD have risen greatly in low-income and middle-income countries. Nine potentially modifiable risk factors associated with myocardial infarction (MI) were identified. These varied by populations. The effect of the risk factors is remarkably noticeable in young men and women , demonstrating that most premature MI is avoidable. Two-thirds of an acute MI worldwide is related to smoking and abnormal lipids. High blood cholesterol is predicted to cause about 4.4 million deaths which sum up to 18 percent of strokes and 56 percent of global CHD. A blood cholesterol level of

less than 5.0 millimoles per liter (mmol/L) is recommended for both primary and secondary prevention of CHD [68,69].

Approximately 66 percent of men and women in the UK have blood cholesterol levels of 5.0mmol/L and above. According to data published by World Health Organisation, approximately 600 million people with high blood pressure are at risk of heart attack, stroke and cardiac failure, . In African-Americans, hypertension develops at much earlier age compared to whites. The cause for this difference may be due to a complex interchange between environmental response to diet, stress and a potential genetic/physiological difference in Sodium/Potassium excretion. A study of hypertension in Canada, United States and in six European countries : Germany, Finland, Sweden, England, Spain and Italy showed the average blood pressure was 136/83 mmHg in the European countries and 127/77 mmHg in Canada and the United States, among men and women ages 35–74 years. Blood pressure measurements for all age groups were highest in Germany and lowest in the United States. The measurement of blood pressure in England revealed that 34 percent of men and 30 percent of women have high blood pressure or are being treated for hypertension. The number of men and women not being treated for high blood pressure are 67 % and 78%, respectively. In Asia, a steep increase in stroke mortality goes together with a rapid rise in the prevalence of hypertension. Projections suggest that in China, hypertension will increase from 18.6 percent to 25 percent between 1995 and 2025. In India, the equivalent figures are 16.3 percent to 19.4 percent [70].

Coronary heart disease (CHD) mortality is at least 40% higher in UK Indian Asians compared with European whites. Traditional coronary risk factors including smoking, hypercholesterolemia, and hypertension do not clarify their increased CHD risk compared with whites. Diabetes mellitus and insulin resistance are more common among Indian Asians The exact mechanisms underlying the increased CHD mortality in Indian Asians are not known. Myocardial infarction (MI) was seen to occur at a lower age in Indian population compared with the group population of countries to which they have migrated and mortality from MI was ten times higher . Although insulin resistance may be involved in the early event of risk factors and CHD in Indians, there is some data to suggest that other aspects of the lipid profile such as the lipoprotein (a) [Lp(a)] level, affect risk in Indian patients. Lp(a) was an independent risk factor for CHD in type 2 diabetic patients in South India [72].

Epidemiological studies have shown that South Asians also are more likely to have central obesity, increased waist/hip ratio (WHR) and glucose intolerance compared with

Caucasians [73]. Studies observed a strong tendency for insulin resistance in lean Asian Indians. The latter were much more insulin resistant than lean Caucasians. The curve of insulin sensitivity against percent body fat was relatively steep in Caucasians. This was not the case in Asian Indians. In the latter group increasing adiposity was accompanied by some decrease in insulin sensitivity. The mechanisms underlying the low insulin sensitivity in Asian Indians, whether due to physical inactivity, dietary differences, or hereditary factors, still continue to be evaluated[74].

Asian Indian women had a higher rate of CHD than do other ethnic groups, despite similar conventional risk factors and lipid profiles. It may partly be explained by the differences in the prevalence of atherogenic HDL-C and low-density lipoprotein cholesterol LDL-C sizes and their subclass concentrations among Asian Indian women compared with Caucasian women[75]. In a study conducted by Ranjith *et al.*, that examined differences in major cardiovascular risk factors and clinical outcome in South African Asian Indians of different age groups and gender, who presented with acute coronary syndromes, it was observed that diabetes mellitus and hypertension were less frequent in young male patients. Total cholesterol was elevated in 65 to 70% of all patients while high-density lipoprotein (HDL) levels were significantly lower in men compared with women for all age groups[76].

In the study by Chambers *et al.*, they investigated CRP concentrations in a representative sample of Indian Asian and European white men living in West London, UK. They found that CRP levels were elevated in Indian Asians and were closely associated with increased central adiposity and markers of insulin resistance in Asians compared with Europeans[77].

The defined reasons underlying increased central obesity among Indian Asians compared with European whites are not known. The genetic factors in the first-degree relatives of Indian Asian CHD patients may play a major role in explanation of increased abdominal obesity in this racial group [78]. That is in agreement with the data that suggested that CRP concentrations may be influenced by genetic factors, although the molecular basis remains to be identified [79]. On the basis of the reported relationship between CRP and risk of CHD, it was observed that increased CRP concentrations and/or the processes underlying elevated CRP are associated with an increase in population CHD risk among Indian Asians compared with European whites. The extent of this effect on CHD risk is comparable to a rise in diastolic blood pressure or an increase in total cholesterol. The studies suggested that

inflammation or enhanced cytokine production and/or their acute phase consequences may contribute significantly to the increased CHD mortality in Indian Asians [80,81].

Studies demonstrated that CRP concentrations were also closely associated with levels of HDL cholesterol, triglycerides, glucose, blood pressure, and a composite insulin resistance score in different racial groups. The similar data was reported in North American and European populations, which have additionally shown that CRP concentrations and other inflammatory markers, including white cell count and fibrinogen, are strongly correlated with plasma insulin and insulin-mediated glucose uptake[82].

In a study by Mohan *et al.*, it was established that CRP showed a strong association with coronary artery disease (CAD) and diabetes mellitus, even after adjusting for age and gender in an urban south Indian population. The association of body fat with diabetes mellitus seems to take place through hs-CRP. However, CRP didn't appear to mediate the relationship between body fat and CAD. The relationship between CRP and dietary nutrients was investigated in young Asian Indians residing in a major metropolitan city in north India. Raised CRP levels (>3 mg/L) were noted in 9% study subjects (8.6% males and 12.8% females). Saturated fat appear to be the single most important nutrient contributing to increase in serum CRP levels after adjustment for other covariates. The probability of having a raised CRP level in subjects eat more than 10% energy as saturated dietary fat were twice that compared to subjects having a normal saturated fat intake . Elevated CRP levels in adolescents and young adults in Asian Indians in north India were observed in 21.8% of the overweight subjects and 24.5% of the subjects with high (>85th percentile) percentage body fat (%BF). Levels of CRP correlated significantly with body fat (%), WHR, biceps skinfold and triceps skinfolds for males only. The findings of significant prevalence of elevated CRP levels in adolescents and young adults having increased generalized and abdominal adiposity may be important for the development of cardio metabolic risk in Asian Indian adults[83,84].

2.6. CARDIO METABOLIC RISK AND ANAEMIA:

Using the historical definition by the World Health Organization, anaemia is defined when Hb concentration is less than 13 g/dL for men or less than 12 g/dL for women [85]. Anaemia is prevalent in patients with CHF but the exact rates vary widely. A recent meta-analysis of 1,53,180 patients with CHF, reported in 34 published studies from 2001 to 2007, estimated the prevalence of anaemia to be 37.2% (10– 49%) Similarly, the latest prospective STAMINA-HFP (Study of Anaemia in a Heart Failure Population) Registry estimated a prevalence of 34% in a cohort study . The variability in the estimated prevalence of anaemia is partly attributable to use of different definitions of anaemia. Patients with CHF and anaemia tend to be older than their non-anaemic counterparts. Concerning the gender, in studies of CHF and anaemia enrolling a preponderance of men, the proportion of women steadily increases as Hb concentration falls to the point that women can predominate among patients with CHF and severe anaemia [86].

The major factors contributing to CHF-related anaemia involve chronic kidney disease(CKD), renin-angiotensin system, hematinic abnormalities, mainly iron deficiency, chronic inflammation and haemodilution[87]. Iron deficiency is common in patients with CHF especially when accompanied by CKD , whereas vitamin B12 and folic acid deficiencies or iron overload are not. It is of interest that the incidence of iron deficiency is increasing with the severity of heart failure . In half cases, iron deficiency is absolute with low transferrin saturation and serum ferritin, usually associated with decreased iron stores and reduced iron deposits in the bone marrow. In the other half cases iron deficiency is functional-relative, with low transferrin saturation and normal or elevated serum ferritin, usually associated with normal or elevated iron stores and iron deposits in the bone marrow [88].

Although the exact role of anaemia in promoting cardiovascular disorders (CVD) is currently not well understood, maintenance of adequate tissue oxygenation in the anaemic state is achieved by both non-hemodynamic and hemodynamic adaptations. Non-hemodynamic adaptations include increase in erythropoietin production and increase in intra-erythrocytic concentrations of 2,3-diphosphoglycerate . Hemodynamic changes include systemic arterial dilatation, which leads to a decreased systemic vascular resistance and reduced afterload, which in turn may increase stroke volume [89,90]. Anaemia also results in decreased blood viscosity, which increases venous return and thus, augments preload. Finally, the presence of anaemia activates the sympathetic nervous system, which results in

an increase in heart rate. Increased preload, heart rate and stroke volume as well as reduced after load, all of which act to raise cardiac output[90,91].

It is important to recognize that although studies may demonstrate an association between anaemia and CVD outcomes, this does not necessarily imply that anaemia is the cause and therefore, a treatable cause of CVD. That is, confounding from unmeasured factors, or residual confounding from measured factors may be playing a role. Two examples include (*1*) anaemia may be associated with an unmeasured risk factor such as inflammatory status, which in turn is the cause of both the anaemia and the causal risk factor associated with CVD; (*2*) anaemia may be a marker of the severity of underlying heart disease. For example, in patients with heart failure, anaemia may be due to hemodilution associated with the severity of heart failure[92.93].

There has been increasing appreciation of the significance of anaemia in the pathophysiology, treatment and prognosis of heart failure. Once considered a downstream complication of heart failure, anaemia is now emerging as a crucial and potentially modifiable factor in the overall treatment strategy for patients with chronic heart failure. Although the reports of prevalence of anaemia vary widely, it is unequivocal that anaemia is prevalent in patients with heart failure regardless of the clinical setting[94].

The prevalence of anaemia was reported to be 37.2% in a recent meta-analysis of a total of 153,180 patients with heart failure across 34 published studies over a seven-year period (2001–2007).This is consistent with the findings from the prospective STAMINA-HFP (Study of Anaemia in a Heart Failure Population) using the WHO definition of anemia[95].A recent Canadian study of a population-based cohort of 12,065 patients with new-onset heart failure from hospital discharges identified a prevalence of anaemia of 17%. In this study, more than half of the patients (58%) were classified as having anaemia of chronic disease based on International Classification of Diseases. As might be expected, in addition to variations in definitions, the different practice settings have different mechanisms and different prevalence rates of anaemia. For example, those patients in acute decompensated states likely experience more dilutional anaemia simply due to hypervolemia. Overall, the prevalence of anaemia varies widely, ranging from 14% to 56% in outpatient registries to 14% to 61% in hospitalized patients.[96,97].

The true frequency of anaemia in patients with heart failure is not only influenced by the definition used, but also differs according to the patient population and demographics in which anaemia is being assessed. Unfortunately, the precise cut-off to define anaemia in heart

failure has mostly been arbitrary and there is no consensus about definition of anaemia specific to patients with heart failure. Although a historical definition of anaemia was put forwarded by the World Health Organization (WHO). Such a definition has not been subjected to rigorous clinical validation, particularly in the setting of heart failure. Considerable variability exists in defining anaemia, particularly in the setting of chronic kidney disease (CKD), which often coexists with heart failure. Moreover, the threshold haemoglobin level at which anaemia treatment should be initiated is an even more complex and controversial clinical question[98,99].

Anaemia is a health problem mainly affecting developing countries. With economic growth and associated sociodemographic changes, the burden from under nutrition and infectious diseases has diminished. Concomitantly, changes in diet and other lifestyle factors have led to an increase in life expectancy but also to an increased prevalence of cardio metabolic diseases and other chronic diseases. The cardio metabolic disorders are characterised by a clustering of cardiovascular risk factors and being a powerful determinant of type 2 diabetes has been reported to be increasing in developing nations. An association between inflammation and the cardio metabolic risk has been reported in various populations. As inflammation is associated with anaemia, theoretically there could be a connection between anaemia and the cardio metabolic risk, if the anaemia is mainly caused by inflammation[100,101.]

2.7.CARDIO METABOLIC RISK, OXIDATIVE STRESS AND ANTIOXIDANTS:

Oxidative stress is a well-recognized mechanism playing an important role in many pathological conditions and several human diseases have been closely related to oxidative stress [102]. A number of cell functions appear to be regulated by free radical molecules, which may also act as intracellular and intercellular signals. Also, the protein redox state is implicated in the regulation of several cellular activities, including cell differentiation and activation of specific metabolic pathways Oxidative stress has been associated with all the individual components of various cardio metabolic risk complications in subjects .Three major cardio metabolic risk factors: impaired glucose tolerance, dyslipidemia and hypertension are caused by the same underlying mechanism—endothelial dysfunction primarily mediated by oxidative stress[103,104].

It is now apparent that visceral adipose tissue is an endocrine organ that secretes many bioactive molecules, known as adipocytokines. The production of adipocytokine is of particular interest, because their local secretion by perivascular adipose depots may provide a new mechanistic link between obesity and its associated cardiovascular complications. Increased oxidative stress to adipocytes causes dysregulated expression of inflammation-related adipocytokines. Increasing evidence supports the central role of adipose tissue in the development of systemic inflammatory state, which contributes to obesity-associated vasculopathy and cardiovascular risk [105,106]. These adipocytokines are generally divided into pro-inflammatory cytokines such as tumor necrosis factor-a, interleukin-6,monocyte chemo attractant protein-1, plasminogen activator inhibitor-1and anti-inflammatory cytokines such as adiponectin. Imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines is responsible for oxidative stress especially to endothelial cells and underlies the pathogenesis of the obesity associated insulin resistance, impaired glucose tolerance, type-2 diabetes mellitus , hypertension, dyslipidemia and vasculardisease[107,108].

In a recent study , the role of oxidative stress in the pathophysiologic interactions among the constituent factors of cardio metabolic risk in subjects has been evaluated[183]. Although some of the constituent characteristics of the metabolic syndrome are known to share common pathogenic mechanisms of damage, the impact of hereditary predisposition and the regulation of gene expression as well as the role of environment and dietary habit in determining inflammatory process triggered oxidation are still unclear . However, excessive free radical production and oxidative damage are found to be associated with cardio metabolic disorders in several experimental demonstrations and human observations[109,110].

High concentrations of H_2O_2 promote insulin signalling and induce typical metabolic actions of insulin. This result could be considered as the first documentation on the link between ROS and insulin · In particular, H_2O_2 uses the same pathway of insulin and causes downstream propagation of the signal producing typical metabolic actions of insulin. H_2O_2 induces an increase in glucose uptake by adipocytes and muscles and also stimulates GLUT4 translocation and lipid synthesis in adipocytes [111].Insulin receptor substrate (IRS) proteins are effectors for tyrosine kinase activity of the insulin receptor (IR) upon insulin binding. These proteins are involved in a critical step of insulin signalling. In normal physiological state, insulin signalling molecules are distributed between the cytosol and internal membrane pools. Whereas, after insulin stimulation, tyrosine residues on IR and IRS are phosphorylated through activated insulin receptor kinase. This leads to the enrolment of PI 3-kinase in the plasma membrane and in internal membrane pools. Subsequently, the activation of small GTPase Rac induces cytoskeletal reorganization that propagates the insulin signals. Finally, this induces to typical metabolic effects of insulin such as increased glucose uptake[112,113].

In conditions of increased oxidative stress, stress-responsive signalling cascades are activated, such as the MAP kinase cascades. This induces to increase Ser/Thr phosphorylation of IRS molecules. Modified IRS molecules are released from internal membrane pools and are subjected to increased protein degradation. In these conditions, insulin fails to gain normal metabolic effects. This happens because IRS molecules are decreased in content and cannot be normally tyrosine phosphorylated when hyper phosphorylated on certain Ser/Thr residues [114]. It is important to note that, receptor substrates are able to directly modify the expression of glucose/metabolic genes such as GLUT4 and adiponectin . In animal models of obese mice, an increased H_2O_2 generation by adipose tissue could be observed prior to diabetes onset . This event came with decreased mRNA levels of SOD, catalase and glutathione peroxidase. Developing diabetes in these mice increased these alterations, which remained unobservable in othertissues[115,116].

Obesity and related insulin resistance are frequently related with increased accumulation of lipids(triglycerides) in the liver[118,119.] Increased numbers of lipid peroxidation markers have been observed in the liver, in animal models of diabetes and obesity. Oxidation-induced disruption of cellular redistributed signalling molecules in response to insulin stimulation was associated with impaired insulin action. An animal model of oxidative stress provided support for this notion. In this in vivo model, oxidative stress was induced in rats using an inhibitor of glutathione biosynthesis enzyme. The reduction in tissue levels of glutathione, a cellular antioxidant, increased markers of oxidative stress and

impaired glucose homeostasis .Some studies in humans have evidenced the pivotal role of oxidative stress in insulin resistance states such as metabolic syndrome, obesity, and type 2 diabetes mellitus[120].

Oxidative stress with an imbalance between ROS and antioxidant defence mechanisms, contributes to the etiology of hypertension in animals and humans . ROS are generated by multiple cellular sources, including NADPH oxidase, mitochondria, xanthine oxidase, uncoupled endothelium-derived Nitric Oxide syntheses (eNOS), cyclo-oxygenase and lipo-oxygenase. Superoxide anion is produced by stimulation of angiotensin /angiotensin II type I receptor (AT1R) and NADPH oxidase by Angiotensin II. Superoxide anion is the predominant ROS species produced by these tissues, which neutralizes Nitric Oxide (NO) and leads to downstream production of other ROS such as Hydrogen Peroxide, hydroxyl radicals and peri-oxynitrite. Hypertensive patients have impaired endogenous and exogenous antioxidant defence mechanisms , increased plasma oxidative stress and an exaggerated oxidative stress response to various stimuli. Hypertensive patients also have lower plasma ferric-reducing ability of plasma (FRAP), lower vitamin C levels and increased plasma 8-isoprostanes, which correlate with both systolic and diastolic blood pressure (BP). Various single-nucleotide polymorphisms (SNPs) in genes that codify for antioxidant enzymes are directly related to hypertension including NADPH oxidase, xanthine oxidase, superoxide dismutase (SOD 3), catalase, glutathione peroxidase (GPx 1) and thioredoxin.

ROS directly damage endothelial cells and degrade NO . Various neurohormonal systems including the renin-angiotensin-aldosterone system and sympathetic nervous system also contribute to oxidative stress, inflammation and vascular immune dysfunctions. The increased oxidative stress, inflammation and autoimmune vascular dysfunction in human hypertension results from a combination of increased generation of ROS, an exacerbated response to ROS and decreased antioxidant reserve . There are also direct interactions of the central nervous system, inflammation and BP. Increased oxidative stress in the rostral ventrolateral medulla (RVLM) enhances glutamatergic excitatory inputs and attenuates GABAergic inhibitory inputs to the RVLM, which contributes to increased sympathetic nervous system (SNS) activity from the paraventricular nucleus . Activation of the AT1R in the RVLM increases NADPH oxidase and increases oxidative stress and superoxide anion. It will increase SNS outflow causing an imbalance of SNS/ Peripheral nervous system (PNS) activity with elevation of BP. There will be an increase heart rate, alterations in heart rate variability and heart rate recovery time, which can be blocked by AT1R blockers [121-23].

Atherogenic dyslipidemia is an important component of the cluster of cardio metabolic risk factors in subjects. There are three major components of dyslipidemia that are the part of cardio metabolic risk factors: an increase in triglyceride-rich lipoproteins (TRLs) both fasting and postprandial, a reduction in high-density lipoprotein (HDL) and elevated small, dense low density lipoproteins (LDL) particles. Because the metabolism of all lipoproteins is highly interrelated, it is believable that a common fundamental metabolic defect explicates all of the lipoprotein changes in the dyslipidemia related to insulin resistance. It is indeed rare that they are found separately in insulin resistant individuals [207]. During the postprandial state, dietary fatty acids are transferred from the intestine to peripheral tissues as chylomicron triglycerides. In blood of the peripheral tissues, chylomicron triglycerides are lipolyzed by lipoprotein lipase (LPL), conceding the delivery of nonesterified fatty acids to cells and resulting in production of smaller, cholesteryl ester-enriched chylomicron remnants. These particles are fast removed. Some studies have examined the relation between postprandial lipemia and insulin resistance, plasma glucose and insulin response to a dinner in healthy nondiabetic subjects [208]. Postprandial triglyceride levels, as an indirect measurement of chylomicron remnant particles were found to be significantly related to insulin action. A significant relation of triglyceride levels to post heparin plasma LPL activity was also demonstrated. Because LPL is an insulin-sensitive enzyme, which is suppressed in insulin resistant individuals, its deficiency might contribute to the abnormal levels of remnant particles in insulin resistance [124,124].

A correlation between elevated LDL and low HDL and oxidative stress in animal models is well established. LDL receptor-deficient mice fed a cholesterol-enriched diet developed elevated LDL levels and consequently oxidative stress . These observations extend to human studies. High plasma oxidative stress markers are positively correlated with elevated plasma triglycerides and are inversely correlated with low HDL in a group of metabolic syndrome patients with end-stage renal disease, after all other factors like presence of obesity, hypertension and/or type II diabetes mellitus were adjusted [125].

Lipid peroxidation, as an index of oxidative stress, is correlated with low HDL levels, irrespective of age, gender and presence of the other cardio metabolic risk components . It is also now accepted that the numerous positive effects of some statins in the cardiovascular system are independent of their lipid lowering effect and a consequence of a direct decrease in oxidative stress. For example, short-term pravastatin treatment reduced myocardial infarct (MI) size in hypercholesterolemic rabbits through reduction in peroxynitrate and nitrotyrosine formation. Similar results, with regards to the atherogenic index were achieved with

rosuvastatin, which lowered oxidative stress by elevating the expression of antioxidant enzymes (SOD, catalase, glutathione, glutathione peroxidase), LDL, triglycerides, and C-reactive protein (CRP) and elevated HDL [126].

In cross-sectional studies, obese subjects have higher levels of oxidative stress biomarkers compared with their leaner counterparts . Also, weight gain significantly increases the concentration of these biomarkers . There are multiple sources for oxidative stress in relation to obesity. Some of them are inherently related to increased adiposity and fat distribution, whereas others are the result of co-morbidities or behavioral changes associated with being obese. Increased adipose tissue and in particular, visceral adiposity are significantly correlated with systemic levels of oxidative stress biomarkers [127].

Adipose tissue-mediated systemic oxidative stress and systemic inflammation may be secondary to increased leptin-to-adiponectin ratio and increased levels of other adipokines, such as tumor necrosis factor and plasminogen activator inhibitor. Obesity is associated with several co-morbidities, including hypertension, insulin resistance, diabetes mellitus, and hyperlipidemia: each of these co-morbidities alone can increase the oxidative stress burden. Maintaining a healthy life style by eating a balanced diet rich in antioxidants and being physically active are associated with reduced oxidative stress. Unfortunately, this protection is less effective among obese subjects, who are more sedentary having reduced intake of dietary antioxidants and lower serum vitamin levels [128,129].

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CHAPTER 3:

STUDY PLAN AND PROCEDURE

3.1. Study Design:

i) The study was designed to obtain data on cardio metabolic risk profile and anaemia and their correlates in both pre and post menopausal Indian women comprising of subjects from two different ethnic and nonethnic communities.

ii) Ethical clearance for the study was obtained from Institutional Human Ethical Committee before onset of the study.

iii) Informed consent from all participating subjects were obtained before including them in the study.

3.2 Study population:

i) The study was conducted on apparently healthy subjects accompanying the patients attending the OPDs of Shri B.M. Patil Medical college, Vijaypur, Karnataka, and Tripura Medical College, Agartala, Tripura, representing both urban and rural subjects from a mixed Indian population. The subjects included women from two non ethnic and two ethnic groups of India in this Hospital based observational study. The groups are as follows :

a) Non ethnic subjects from Vijaypur, Karnataka.

b) Non ethnic subjects from Agartala, Tripura.

c) Ethnic Lambanis from Vijaypur, Karnataka, India.

d) Ethnic Riang tribe of Tripura, a North eastern state of India, and

ii) Age of the subjects varied from 25 to 65 years.

3.2.a. Sample size :

As the reported prevalence of cardio metabolic risk varies from 10% to 50% in Indian subjects from both sexes and all age groups, a prevalence of 30% was taken to calculate the sample size for a mixed Indian population with 95% interval and absolute precision of 5%. The formula for sample size calculation as mentioned in WHO Manual for Sample size calculation in Health studies was used to calculate the required sample size for the study[1].

The formula was : $4PQ/L^2$, where P= Prevalence (30%), Q= (1-P), L=Confidence interval (95%), Maximum allowable error=0.05. Thus the minimum sample size estimated was 323 (Table - Ia, page 25 of the Manual). The final sample size (335), however, was higher than this number.

3.2.b. Inclusion criteria :

Only healthy women who were non-smokers, non-alcoholics, nonusers of hormonal contraceptives and hormone replacement therapy were included for the study.

3. 2.c. Exclusion criteria :

i) Known diabetics.
ii) Known hypertensives.
iii) Subjects on lipid lowering drugs.
iv) Subjects with history of polycystic ovary or any other chronic diseases.
v) Subjects with other endocrine disorders (Thyroid, Adrenal).

3.2.d. Flow of the subject :

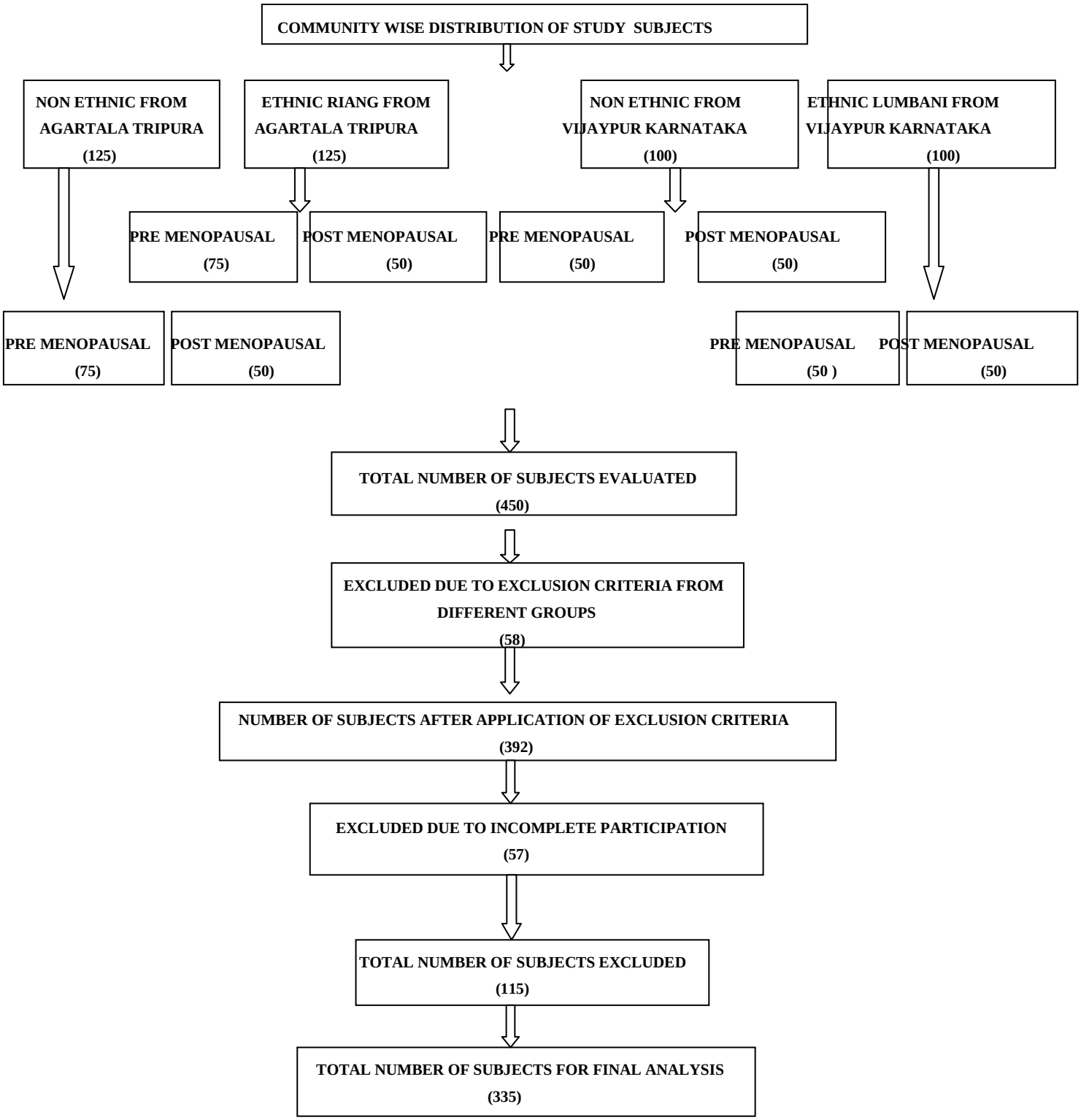

Figure I : Flow of study subjects.

3.2.e. Distribution of subjects from different Ethnic groups：

Groups	Premenopausal	Postmenopausal	Total
Non ethnic subjects from Agartala, Tripura.	61	36	97
Non ethnic subjects FromVijaypur, Karnataka	47	32	79
Ethnic Riang subjects From Tripura	59	33	92
Ethnic Lambani subjects FromVijaypur, Karnataka.	36	31	67
Total	203	132	335

Table II : Distribution of subjects from different ethnic groups.

3.3. STUDY PROTOCOL :

i) The subjects were first informed about the purpose of the study and the procedures involved. Once they volunteered for the study, the detailed procedure was explained to the subjects as outlined in the informed consent. Informed consent was taken from the volunteered subjects (ANNEXURE - I).

ii) At the onset of the study, a proforma was filled to evaluate general health status of the subject, history of past illness, information regarding menstrual and marital status, history of pregnancy along with personal and family history (ANNEXURE -II).

iii) Women who were still menstruating irrespective of regularities of their menses were considered as premenopausal women, while postmenopausal women were those women who had ceased menstruation for at least one year[2].

iv) In the second stage, various anthropometric and physiological parameters of the subjects were recorded.

v) In the third and final stage, venous blood was collected from each subject for analysis of various haematological and biochemical parameters.

vi) All the recordings were entered in a proforma (ANNEXURE - III)

3.3. a. Study period :

Study was conducted between November 2012 to October 2014.

3.3.b. RECORDING OF ANTHROPOMETIC PARAMETERS:

Anthropometric parameters recorded in the subjects were :

Height (cm), Body Weight (kg), Waist Circumference (cm) , Hip Circumference (cm), Body Mass Index (BMI) (kg/m^2), Waist -Hip Ratio (WHR), and Waist -Height Ratio (WHtR).

Height of the subject was recorded by using a stadiometer with subject standing erect.

Weight of the subject was recorded with subject standing erect on a human weighing machine in light clothing.

Waist circumference was measured at midpoint between the last rib and iliac crest by using a measuring tape.

Hip circumference was measured at the widest level over the greater trochanters to nearest centimetres by using a measuring tape.

BMI was calculated as Kg/m^2. WHR and WHtR were also calculated.

3.3.c. RECORDING OF BLOOD PRESSURE AND HEART RATE :

Heart Rate (HR) of the subject was recorded as beats/min in sitting posture.

Both systolic and diastolic blood pressures were recorded in each subject by using a sphygmomanometer in sitting posture. Two recordings were taken for each subject with a gap of five minutes. . Average of three measurements was used in the analysis. Pulse Pressure (PP) was calculated as difference of systolic and diastolic blood pressure.

3.3.d. COLLECTION OF BLOOD SAMPLES FOR HEMATOLOGICAL AND BIOCHEMICAL ANALYSIS :

Venous blood sample (10 ml) was drawn from each individual after an overnight fasting for haematological and biochemical analysis. A small drop of blood was collected in EDTA bulb for haematological analysis. The blood was centrifuged for plasma separation. 1.5 ml aliquots were pipetted in plastic Eppendrof tubes and were stored at -80^0C for future analysis.

3.3.e. RECORDING OF HAEMATOLOGICAL PARAMETERS:

Haematological parameters including Haemoglobin concentration were determined by an automated cell counter.

3.3.f. ESTINATION OF BLOOD GLUCOSE AND LIPID PROFILE :

All plasma samples were analysed in a semi-automatic biochemical analyser using commercial kits. ERBA kits supplied by Transia Biochemicals Ltd., Mumbai were used

3.3.f.i. Estimation of Blood Glucose Level :

The Fasting Blood Sugar (FBS) was analysed by glucose oxidase peroxidase (GPO-PAP) method,Trinder, 1969 [3].

Principle :

Glucose in the presence of atmospheric oxygen is completely oxidised by enzyme glucose –oxidase to gluconic acid and hydrogen peroxide. The hydrogen peroxide formed is broken down by peroxidase enzyme to water and oxygen. The latter oxidizes phenol which combines with amino -4- antipyrine to give a red coloured complex quinonimine The intensity of red colour is proportional to the concentration of glucose in the sample and the intensity of colour is measured colorimetrically at 520 nm using green filter.

Reaction :

$$Glucose + O_2 + H_2O_2 \xrightarrow{Glucose\ oxidase} Gluconic\ acid + H_2O_2$$

$$2H_2O_2 + phenol + 4\ Aminoantipyrine \xrightarrow{Peroxidase} Quinoneimine + 4H_2O$$

Reagents :

1. Enzyme reagent - Consisted of Amino 4 antipyrine, glucose, peroxidase and phosphate butter
2. Phenol (16 m mol /l)
3. Standard glucose (5.55 m mol /L)

Preparation of working enzyme reagent- The enzyme reagent was prepared in 500 ml distilled water to which 5 ml of phenol reagent was added. It was mixed gently and used after 3 hours.

Procedure :

Clean dry test tubes marked as T for test S for standard & B for blank were used.
To each test tube following solutions were added in ml in given order.
Sample / standard glucose / Distilled water - 0.2 ml
Working Reagent - 3.0 ml
All the contents of the test tubes were mixed well and were incubated for 15 minutes at 37^0C.
After incubation 2 ml distilled water was added, mixed well & O.D of test & standard were measured against blank using green filter (520nm)

3.3.f.ii. Estimation of Total Cholesterol level :

Total cholesterol was analysed by CHOD-PAP method[4].The method for this assay is based on that described by Trinder, (1969).

Principle :
Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free cholesterol produced as well as the preformed cholesterol is then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. However, the quinoneimine chromogen, which has its absorption maximum at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red colour produced is directly proportional to the total cholesterol concentration.

Reaction :

$$\textit{Cholesterol ester} + H_2O \xrightarrow{\textit{Cholesterol esterase}} \textit{Cholesterol} + \textit{Fatty acids}$$

$$\textit{Cholesterol} + O_2 \xrightarrow{\textit{Cholesterol oxidase}} \textit{Cholesterol -3-one} + H_2O_2$$

$$2H_2O_2 + \textit{4-Aminoantipyridine} + \textit{Phenol} \xrightarrow{\textit{Phenol peroxidase}} \textit{Quinoneimine} + 4\,H_2O$$

3.3.f.iii. Estimation of Triglyceride level:

Triglycerides was measured by GPO-PAP Trinder method[5].

Principle :

Triglycerides in the test samples are hydrolyzed by the enzyme, lipase to glycerol and fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate and adenosine-5-diphosphate (ADP) in a reaction catalysed by the enzyme, glycerol kinase. The glycerol-3-phosphate produced is then converted to dihydroxy acetone phosphate (DHAP) and hydrogen peroxide (H_2O_2) by glycerophosphate oxidase. The hydrogen peroxide then reacts with 4-aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene (Chlorophenol) in a reaction catalyzed by the enzyme, peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

Reaction :

$$\textit{Triglyceride} + H_2O \xrightarrow{\textit{Lipoprotein lipase}} \textit{Glycerol} + \textit{Fatty acids}$$

$$\textit{Glycerol} + \textit{ATP} \xrightarrow{\textit{Glycerol kinase}} \textit{Glycerol - 3- phosphatase} + \textit{ADP}$$

$$\textit{Glycero - 3- phosphatase} \xrightarrow{\textit{Glycerophosphateoxidase}} \textit{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \textit{4-Aminoantipyrine} + \textit{p-Clorophenol} \xrightarrow{\textit{Phenol peroxidase}} \textit{Quinoneimine} + \textit{HCL} + 4\,H_2O$$

3.3.f.iv. Estimationof HDL-C level :

HDL-C was measured by Phosphotungstic acid method[6]. Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of Mg2+ ions. The cholesterol concentration in the HDL is then determined by the method described by Trinder for the assay of cholesterol.

3.3.f.v. Estimation of LDL-C level :

LDL-C was calculated by subtracting VLDL-C and HDL-C from total cholesterol. The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald equation (Friedewald et al., 1972)[7].

$$\textit{LDL Cholesterol (mg/dl)} = \textit{(Total Cholesterol)} - \left(\frac{\textit{Triglyceride}}{5}\right) - \textit{(HDL Cholesterol)}$$

3.3.f.vi. Estimation of VLDL-C level :

VLDL-C was calculated by indirect method as VLDL-C is one fifth of triglyceride level.

3.3. g. EVALUATION OF LIPID PEROXIDATION AND ANTIOXIDANT STATUS OF THE SUBJECTS WITH CARDIO METABOLIC RISK :

3.3.g.i. Determination of Plasma Concentration of lipid peroxidation product Malondialdehyde (MDA) :

Plasma concentration of lipid peroxidation product Malondialdehyde (MDA) was estimated by the measurement of thiobarbaric acid reactive substance by the method of Yagi et.al.[8]

Principle:

Malondialdehyde (MDA) levels were determined by the MDA Thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution . MDA, a secondary lipid peroxidation product, reacts with thiobarbituric acid (TBA) to generate a red coloured product, which was detected spectrophotometrically at 535 nm.

Reagents:

1.Thiobarbituric acid (TBA): 0.67 % W/V.

2.Trichloroacetic acid (TCA): 0.5% W/V.

3.n-butanol

4.Standard malondialdehyde (1,1,3,3tetraethoxypropane**)** - 1 ml of standard contains 0.92 g MDA (M.W. 220).

Stock solution : 15µl of standard MDA was diluted to 10 ml with double distilled water.

Working solution : 1 ml of stock solution was diluted to 100 ml with double distilled water.

Procedure:

A volume of 0.5 ml of the serum sample was added to 2.5 ml of 20% trichloroacetic acid (TCA) in a centrifuge tube.

Then 1 ml of 0.67% thiobarbituric acid (TBA) was added to the mixture. The resulting mixture was then boiled in a water bath at $100^{o}C$ for 30 minutes.

The hot mixture was then allowed to cool using iced water bath.

After cooling, 4 ml of n-butanol was added to the tube and mixed using vortex mixture. The mixture was then centrifuged at 4000 rpm for 10 minutes.

The absorbance of supernatant was measured at 535 nm and the results were expressed as μmol per litre, using the extinction coefficient of 1.56 x 105 L mmol cm-1.

Standard curve of MDA was prepared by taking different amounts of standard working solution which were equivalent to 5, 10, 15, 25 and 30 nmoles of MDA per 0.5 ml and treated similarly as described in the experiment. The obtained ODs were plotted against the nmoles of MDA.

Calculation :

$Abs = C\varepsilon L$

Therefore, $C = Abs/\varepsilon L$

Abs = absorbance of the test sample

C = concentration of the test sample

ε = extinction coefficient

L = light path (1 cm).

3.3.g.ii. Determination of Erythrocyte Catalase (CAT) level :

Erythrocyte catalase (CAT) was assayed colorimetrically as micromoles of hydrogen peroxide consumed per minute per milligram of Haemoglobin as described by Sinha et.al.[9].

Principle :

In the ultraviolet range Hydrogen Peroxide (H_2O_2) shows a continuous increase in absorption with decrease in wavelength. The decomposition of Hydrogen Peroxide can be followed directly by decrease in extinction at 285 nm. The difference in extinction per unit time was a measure of the catalase activity.

Reagents:

1. Phosphate buffer [50mM; PH 7.0] - 681 mg of KH_2PO_4 was dissolved in 100 ml distilled water (Solution A) and 1.780gm of Na_2HPO_4 H_2O was dissolved in 200 ml-distilled water (Solution B). Solution A and B were mixed in the proportion 1:1.

2. Hydrogen Peroxide [30mM]- 0.34 ml of 30% Hydrogen Peroxide was diluted with phosphate buffer to 100ml.

Procedure:

1 ml of heparinized blood was used for assay. The sample was centrifuged at 3000 rpm for 10 min and plasma was removed. Then the erythrocyte sediment in centrifuge tube was washed with normal saline for 3 to 4 time and Hb was determined.

Stock haemolysate containing 5 gm % of Hb was prepared by adding 4 parts of distilled water by volume to erythrocyte sediment.

This concentrated haemolysate was diluted with phosphate buffer and Hb content was determined by using Drabkin method[10]. The Drabkin's reagent contains Potassium Ferrocyanide, Potassium Cyanide and Potassium Dihydrogen Phosphate. The ferrocyanide forms methaemoglobin which is converted to cyanmethaemoglobin by cyanide.

Then, the following additions were made in two Cuvettes marked as Reference Cuvette and Test Cuvette.

1.0 ml Phosphate buffer was added in the reference cuvettes and 1.0 ml H_2O_2 was added in sample cuvette.

To both the cuvettes 2.0 ml sample (hemolysate) was added.

The reaction was started by adding hydrogen peroxide and immediately the reading was taken at time t=0 against the reference cuvette at 285 nm.

Solution was mixed well and second reading was taken at time t=15 seconds at 285 nm.

Calculation:

Catalase activity unit/mg Hb = [2.3/15] [a/b] [logA1/A2]

A1 =Absorbance at t=0

A2 = Absorbance at t=15

a = dilution factor

b = Hb content of blood or erythrocyte sediment

3.3.g.iii. Determination of Erythrocyte Superoxide Dismutase (SOD) level :

Erythrocyte superoxide dismutase (SOD) was assayed in erythrocyte lysate by modified method of Das et.al,[11].

Principle:

Superoxide radicals generated by photo reduction of riboflavin. These radicals are allowed to react with hydroxylamine hydrochloride to produce nitrite. The nitrite in turn reacts with sulphanilic acid to produce diazonium compound, which subsequently reacts with napthylethylene diamine to form a red coloured azo- compound. The absorbance of coloured compound was measured at 543 nm.

Reagents:

1. Phosphate Buffer (pH 7.4): 1.375 gm Na2HPO4 and 1.0 gm KH2PO4 were dissolved in 500 ml of distilled water and pH was adjusted to 7.4. Finally, volume was made upto 1000 ml. with distilled water.

2. 20 mM L-Methionine: 149 mg of L-Methionine was dissolved in distilled water and diluted to 100 ml.

3. 10 mM Hydroxylamine Hydrochloride: 69 mg of Hydroxylamine Hydrochloride was dissolved in distilled water and diluted to 100 ml.

4. 50 μM Riboflavin: 1.88 mg of riboflavin was dissolved in distilled water and diluted to 100 ml.

5. Sulfanilamide: 2.5 gm. of Sulfanilamide was dissolved in 3M HCl and diluted to 250 ml.

6. N-napthylethylenediamine: 50 mg of N- napthylethylenediamine was dissolved in distilled water and diluted to 250 ml.

7. 1 % Triton X -100

8. 50 μM EDTA

Procedure:

Heparinized blood sample was centrifuged and plasma was removed. Hemolysate was prepared and treated with chloroform-ethanol mixture. The supernatant obtained was used as sample in the next step.

A set of test tubes was taken and labelled as test and control. In each test tube 1.110 ml of phosphate buffer was added.

To it, 0.075 ml L-Methionine, 0.0440 ml Triton- X 100 , 0.075 Hydroxylamine Hcl, 0.1 ml EDTA and 0.1 ml sample were added.

All the tubes were then incubated at 37^0C for 10 minutes.

After this, 0.05 ml Riboflavin was added to each test tube and the test tubes were exposed to light for 10 minutes , after which 0.75 ml Sulfanimide and 0.75 ml N-Napthylenediamine were added to each test tube, and the test tubes were kept at room temperature for 20 minutes.

After 20 minutes, absorbance was read at 543 nm.

Calculation:

Erythrocyte superoxide dismutase activity was estimated by following formula:

One SOD Unit = Amount of enzyme required to inhibit nitrite formation of control by 50% at 37°C for 10 min.

SOD unit = (OD of Control ÷ OD of Test) – 1.

3.3.g.iv. Determination of Erythrocyte Glutathione Peroxidase (GPx) level :

Erythrocyte Glutathione peroxidase (GPx) activity was assayed in erythrocyte lysate by the modified method of Paglia and Valentine[12].

Principle:

GPx activity was determined by a direct spectrophotometric procedure which depends on the reaction: $GSH + H_2O_2 = H_2O + GSSG$.

The activity was determined by measuring the decrease in the absorbance of the reaction mixture at 340 nm as NADPH,H+ was oxidized to NADP+. The decrease in the absorbance would reflect the amount of oxidized glutathione that had been formed and consequently the activity of GPx.

Reagents:

1- Phosphate buffer pH 7(0.05M KH_2PO_4/Na2HPO_4 and 0.005M EDTA): 0.2g potassium dihydrogen phosphate, 0.5g disodium hydrogen phosphate and 0.146g ethylene diamine tetra-acetic acid was diluted in 100ml distilled water.

2- Nicotinamide adenine dinucleotide phosphate reduced form- (0.0084 M-NADPH): 7mg/ml distilled water.

3- Glutathione reductase(GR): 2.3 mg/ml double water.

4- Glutathione(0.15 M GSH): 46mg/ml distilled water.

5- Sodium Azide(1.125 M NaN3): 73mg/ml distilled water.

6- Hydrogen Peroxide(0.0022 M H_2O_2): 0.025 ml of 30% H_2O_2/100 Ml distilled water.

Reaction medium:

Recombination of the buffer and other reagents into large fresh pools allowed more expeditious assays of multiple samples with no variance in the outcome. So that to 2.58 ml phosphate buffer pH 7, the following solutions were added in turn:

0.1 ml NADPH, 0.01 ml GR, 0.01 ml NaN_3 and finally 0.1 ml GSH.

Procedure:

25 µl of sample was mixed with 0.7 ml of the reaction mixture and the enzymatic reaction was initiated by the addition of 25 µl of H_2O_2. The conversion of NADPH to NADP was followed by continuous recording of the change in absorbance of the system at 340 nm at 0, 1, 2 and 3 minutes after the initiation of reaction.

A blank (to determine the contribution of the non enzymatic oxidation of NADPH) was similarly done but the tissue fraction was replaced by an equal volume of distilled water. The reaction rate of the blank was subtracted from the experiment to determine the true enzymatic activity.

Calculation:

A = [ΔE340 of sample - ΔE340 of blank] x V 6.22 x n

Where:

A = enzyme activity expressed as unit per gram , where one unit is the amount of enzyme which oxidize one µmol of NADPH,H+ per minute.

ΔE340 = the change in absorbance of NADPH,H+ per minute of sample

ΔE340 = the change in absorbance of NADPH.H+ per minute of blank resulting from non enzymatic oxidation of NADPH.H+.

V = total volume of the reagents used in the experiment and present in the cuvette.

6.22 = the molar absorptivity of the NADPH.H+ and it is the product of multiplication of the absorbance of 1 µmol of NADPH at 340 nm (8.34x 10-3) by the molecular weight of NADPH.H+ (744.5).

3.3.g.v.Determination of Blood Glutathione (GSH) level :

Glutathione (GSH) in whole blood was determined by method of Butler et.al[13].

Principle:

The method depends on the reaction of the free SH-group of the reduced glutathione molecule with 5,5'-dithiobis- (2-nitrobenzoic acid) [DTNB] yielding a yellow colour product (2 nitro-5- thiobenzoic acid) that can be measured colorimetrically.

Reagents:

1- Phosphate solution(0.3M Na2HPO4):42.6 mg disodium hydrogen phosphate / ml distilled water.

2- DTNB reagent:40 mg DTNB/100 ml of 1 % Sodium citrate.

3- Standard GSH: Stock standard was prepared by dissolving 7.5 mg GSH in 50ml 1% metaphosphoric acid. Different concentrations were prepared by serial dilution of the stock solution to get standard solutions of 0.92, 1.86, 3.7, 7.4 and 15 mg GSH per 100 ml.

Procedure:

0.5 ml of the sample was added to 2 ml of phosphate solution, followed by the addition of 0.25 ml of DTNB reagent.

The absorbance was measured at 412 nm within 5 minutes of the addition of DTNB reagent against blank (prepared using 0.5ml of 1% metaphosphoric acid.

Calculation:

GSH concentration =

Absorbance of sample X concentration of standard in 0.5 ml

3.3.g.vi. Determination of Vitamin - C level:

Plasma vitamin C was estimated by DNPH method[14].

Principle:

The ascorbic acid is oxidized to diketogluconic acid in presence of strong acid solution and the diketogluconic acid reacts with 2, 4 dinitro phenyl hydrazine to form dinitrophenylhydrazone which dissolves in strong sulphuric acid solution to produce red coloured complex which can be measured colorimetrically.

Reagents: -

1. 10%Trichloroacetic acid: 10 gm of Trichloroacetic acid (TCA) was dissolved in distilled water and diluted to 100ml.

2. 2, 4 dinitrophenylhydrazine (DNPH): 2 gm of crystalline DNPH was dissolved in 100 ml of 9N sulphuric acid. (75 ml distilled water + 25 ml concentrated sulphuric acid)

3. Thiourea solution: 10 gm of thiourea was dissolved in 100 ml of 50% ethanol and was stored in refrigerator.

4. Cupric sulphate (1.5%): 1.5 gm of $CuSO_4.5H_2O$ was dissolved in distilled water and the volume was adjusted upto 100ml.

5. Combined colour reagent: It was prepared freshly on the day of use by adding 5.0 ml of 2,4dinitrophenylhydrazine reagent + 0.1 ml of cupric sulphate solution + 0.1 ml of thiourea solution.

6. 85% Sulphuric acid: 10.0 ml of distilled water was added in 90 ml of concentrated sulphuric acid mixed and allowed to cool. It was stored in a glass stoppered bottle in the refrigerator or a portion was cooled in ice water bath before use.

7. Stock standard (1gm %): 1 gm of ascorbic acid was dissolved in distilled water and diluted to 100 ml.

8. Working standard (2mg %): 0.1 ml of stock was diluted up to 50 ml with distilled water just before use.

Procedure:

Deproteinization: 1.0 ml of plasma was taken in a clean and dry centrifuge tube. To this 1.0 ml 10% TCA and 0.5 ml of chloroform was added and mixed well by shaking vigorously for 10 to 15 minutes and centrifuged. The clear supernatant was collected as protein free filtrate (PFF).

Colour development : 500 µL of samples and standards were taken in separate test tubes to which 100 µL of colour reagent was added.

The test tubes were incubated at 37^0C for 3 hours and 750 µL 85% H_2SO_4 was added to each test tube.

The test tubes were then vortexed and kept for 30 minutes at room temperature.

The absorbance were read at 520 nm.

Calculation: -

$$\text{Plasma ascorbic acid} = \frac{\text{OD of Test}}{\text{OD of Standard}} \times 2 = \ldots\ldots. \text{ mg/dl.}$$

3.3.g.vii. Determination of Vitamin - E level :

Serum Vitamin E (VIT-E) was measured by the method described by Jargar et.al.[15].

Principle :

The method was based on previous Baker and Frank method and the method of Martinel by using 2.2'-bipyridal, ferric chloride and xylene. The complex of Ferrous ions generated in this reaction with 2-2'-bipyridal was determined by using plain enzyme linked immunoabsorbent assay microplate (non-antibody coated) at 492nm.

Reagents:

Stock standard solution of α-tocopherol(0.27% w/v) : 270 mg of α-tocopherol acetate diluted in 100 ml ethanol(aldehyde free) and mixed thoroughly.

2.2'—Bipyridyl (0.12% w/v): 120 mg 2.2'-Bipyridyl is dissolved and the volume is made upto 100 ml with n-propanol and is kept in a brown bottle.

Ferric chloride(0.12% w/v) : 120 mg $FeCl_3.6H_2O$ is dissolved in 100 ml ethanol and is also kept in a brown bottle.

All these solutions are stable at room temperature(24˚C ± 2˚C).

Working standard of α-tocopherol: 1 ml of stock standard solution was taken and the volume was made upto 100ml with ethanol (aldehyde free) to obtain concentration of 27μg/ml.

Procedure:

750 μl of ethanol (aldehyde free) and 750 μl serum were added in centrifuge tubes marked as sample.

The blank was prepared by adding 750 μl of distilled water and 750 μl of ethanol(aldehyde free).All tubes were covered tightly and shaken vigorously for 30 seconds.

Then 750 ml of xylene was added.All the tubes were again covered and shaken vigorously for another 30 seconds and then centrifuged for 10 minutes at 3000 rpm.

Xylene layer was transferred in small sized test tubes.

In each test tube, 50 µl of 2.2'-bipyridyle solution was added followed by 100 µl of ferric chloride solution and waited for 2 minutes.

The absorbance was measured at 492 nm.

The concentration of serum α-tocopherol of the sample was obtained by using standard curve prepared earlier.

4.4. EVALUATION OF CARDIO METABOLIC RISK PROFILE OF THE SUBJECTS:

Cardio metabolic risk profile of the subjects were evaluated according to consensus statement for diagnosis of general obesity, abdominal obesity and metabolic syndrome for Asian Indians[16]. Presence of three or more of the following risk factors in a single women was considered of having profound cardio metabolic risk :

Table III : Cut offs for different cardio metabolic risk factors according to harmonized criteria[16].

01. Increased waist circumference	≥ 80 cm.
02. Hypertriglyceridemia : (serum TG level)	≥ 150 mg /dl (1.7 mmol/L).
03. Low HDL-C	< 50 mg/dl (1.29mmol/L).
04. Elevated Blood Pressure	SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg.
05. Hyperglycemia : (fasting blood glucose level)	≥ 100 mg/dl (5.5 mmol/L) .

4.5. STATISTICAL ANALYSIS :

Statistical analysis was performed using SPSS 16.0 (statistical Program for the Social Sciences) for Windows. Continued variables (e.g- age) were reported as Mean ± Standard Deviation (SD). The mean for age, height, weight, BMI, WHR and WHtR were stratified by menopausal status and ethnicity. The t-test was used to test for the difference in measurements between various groups at the level of significance $P < 0.05$. Pearson's correlation coefficients between cardio metabolic risk factors and anthropometric parameters and atherogenic index were calculated. Linear regression analysis was performed to establish the relationship between anaemia, antioxidant status and cardio metabolic risk in subjects.

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CHAPTER 4:
FINDINGS AND INTERPRETATION OF DATA

4.1. Baseline characteristics of the study subjects:

The base line parameters evaluated in the subjects involved measurements of different anthropometric parameters, blood pressure, fasting blood sugar and lipid profile in each subject. Table IV represents anthropometric characteristics of the study subjects. The average age of the subjects was 41.88 ±10.04 years. The average BMI of the subjects was 22.24 ± 2.09 kg/m^2, which was within the normal range for Indian women. The average waist circumference of the subjects (86.61 ±5.91 cm) was higher than recommended waist circumference for Indian female. The cardio metabolic profile of the total subjects showed that average of all the cardio metabolic parameters were within the normal range (Table IV and V).

Table IV : The details of Anthropometric parameters evaluated in the subjects.
(Values are in Mean ± SD).

Parameters	Total subject (335)
Age (in years)	41.88 ±10.04
Height (cm)	150.59 ± 5.95
Weight (kg)	51.24 ± 5.47
BMI (kg/m^2)	22.24 ± 2.09
Waist circumference - WC (cm)	86.61 ±5.91
Hip circumferences HC (cm)	92.31 ± 5.31
Waist -Hip ratio (WHR)	0.94 ± 0.02

Table V : The details of Cardio metabolic parameters evaluated in the subjects (Values are in Mean±SD).

Parameters	Total subject (335)
Systolic blood pressure-SBP (mmHg)	122.50± 8.74
Diastolic blood pressure-DBP (mmHg)	79.30 ± 6.06
Pulse pressure -PP(mmHg)	43.29 ± 5.90
Fasting blood glucose-FBG (mg/dl)	100.08 ±9.48
Triglyceride-TG (mg/dl)	144. 11 ±10.66
Total cholesterol -TC(mg/dl)	238.28±12.67
HDL-C (mg/dl)	51.42± 4.79
LDL-C (mg/dl)	158.00 ± 14.62
VLDL-C (mg/dl)	29.49 ±11.19

HDL-High Density Lipoprotein, LDL- Low Density Lipoprotein, VLDL-C- Very Low Density Lipoprotein.

4.2. Clustering of cardio metabolic risk factors in pre and postmenopausal women:

Total number of premenopausal subjects were 203 whereas total number of postmenopausal subjects were 132. All the study parameters were evaluated in three hundred and thirty five (335) pre and postmenopausal women comprising of subjects from the communities described in the study protocol.

The cardio metabolic risk of each subject was evaluated as outlined in the consensus statement for Asian Indians. The difference in cardio metabolic risk profile between pre and postmenopausal women was assessed.

The average age of premenopausal subjects was 35. 21 ± 5.65 years and that of postmenopausal subjects was 52.64 ± 4.92 years. Height of the subjects did not vary significantly between pre and postmenopausal subjects. However, body weight, waist circumference and hip circumference were found to be significantly more in postmenopausal subjects compared to premenopausal subjects. BMI did not vary between premenopausal and postmenopausal subjects, but there was significant difference in WHR and WHtR between subjects from two groups (Table VI).

Cardio metabolic risk parameters including systolic pressure, diastolic pressure, pulse pressure, fasting blood glucose level and triglyceride level were found to be significantly high in postmenopausal subjects. Only HDL-C level was significantly less in postmenopausal women. Levels of total cholesterol, LDL-C and VLDL-C did not vary significantly between pre and postmenopausal women(Table VII) .

26.60% of premenopausal and 49.24% postmenopausal subjects were found to have profound cardio metabolic risk with three or more risk factors were found to be higher than the cut off limits outlined in consensus statement (Table VIII) . All the base line characteristics like age, WC,WHR, SBP, DBP, PP, TC, TG, LDL and VLDL were found to be significantly high in women having three or more cardio metabolic risks from both pre and postmenopausal subjects. HDL level was significantly less in subjects with cardio metabolic risk. BMI, however, did not vary significantly between women with and without profound cardio metabolic risk (Table IX).

Percentage of subjects with three or more cardio metabolic risk components in different age groups are presented in Figure II. The cardio metabolic risk in subjects increased with increasing age. Similar trend in increasing the cardio metabolic risk was seen for both premenopausal and postmenopausal subjects(Figures III & IV).

The single most prevalent cardio metabolic risk component identified in subjects was central obesity followed by elevated triglyceride, elevated blood pressure, reduced HDL-C and elevated fasting blood glucose level respectively (Table X). The trend was similar for both premenopausal and postmenopausal subjects with lesser prevalence of risk factors in premenopausal subjects in comparison to postmenopausal subjects (Figure V).

Table VI: The details of Anthropometric parameters evaluated in premenopausal and postmenopausal subjects. (Values are in Mean ± SD).

Parameters	Premenopausal subjects (203)	Postmenopausal subjects (132)
Age (in years)	35. 21 ± 5.65	52.64 ± 4.92***
Height (cm)	150.79 ± 6.07	150. 47 ± 5.89#
Weight (kg)	50.04 ± 4.78	53.18 ± 5.96*
Waist circumference - WC (cm)	85.64 ± 6.42	88.18 ± 4.57**
Hip circumference - HC (cm)	90.94 ±5.83	94.51 ± 4.73**
BMI ($kg/m^{2)}$	22.13 ± 2.03	22.42 ± 2.18#
WHR	0.93 ± 0. 03	0.95 ± 0.02*
WHtR	0.55 ± 0.07	0.59 ± 0.03*

Not significant, * $p<0.05$, ** $p<0.01$, ***$p< 0.001$

Table VII : The details of Cardio metabolic parameters evaluated in premenopausal and postmenopausal subjects.(Values are in Mean±SD).

Parameters	Premenopausal subject (203)	Postmenopausal subject (132)
Systolic Blood Pressure-SBP (mmHg)	120.26 ± 8.65	126.13 ± 7.63***
Diastolic Blood Pressure-DBP (mmHg)	77.72± 6.02	81.86 ± 5.20***
Pulse Pressure -PP(mmHg)	42.66 ± 6.39	44.31±4.87**
Fasting Blood Glucose-FBG (mg/dl)	97.98 ±8.93	103.48 ± 9.38**
Triglyceride-TG (mg/dl)	142.79 ± 10.33	146.24±10.87**
Total cholesterol - TC(mg/dl)	237.40 ± 14.77	239.70 ± 8.06#
HDL-C (mg/dl)	51.89 ± 4.91	50.66± 4.52*
LDL-C (mg/dl)	156.92 ± 9.24	156.92 ± 17.05#
VLDL-C (mg/dl)	29.66 ±14.15	29.22 ± 2.19

HDL - High Density Lipoprotein, LDL- Low Density Lipoprotein., VLDL-C- Very Low Density Lipoprotein.

Not significant, * $p<0.05$, ** $p<0.01$, ***$p< 0.001$

Table VIII : Percentage of premenopausal and postmenopausal subjects with three or more cardio metabolic risk factors according to modified consensus statement for Asian Indians (expressed in percentage of total subjects in particular group).

Age group of the subject	Consensus statements for Asian Indians
Premenopausal subjects 25-45 years (203)	54 (26.60%)
Postmenopausal subjects 46- 65 years (132)	65 (49.24%)
Total subjects 25-65 years (335)	119(35.52%)

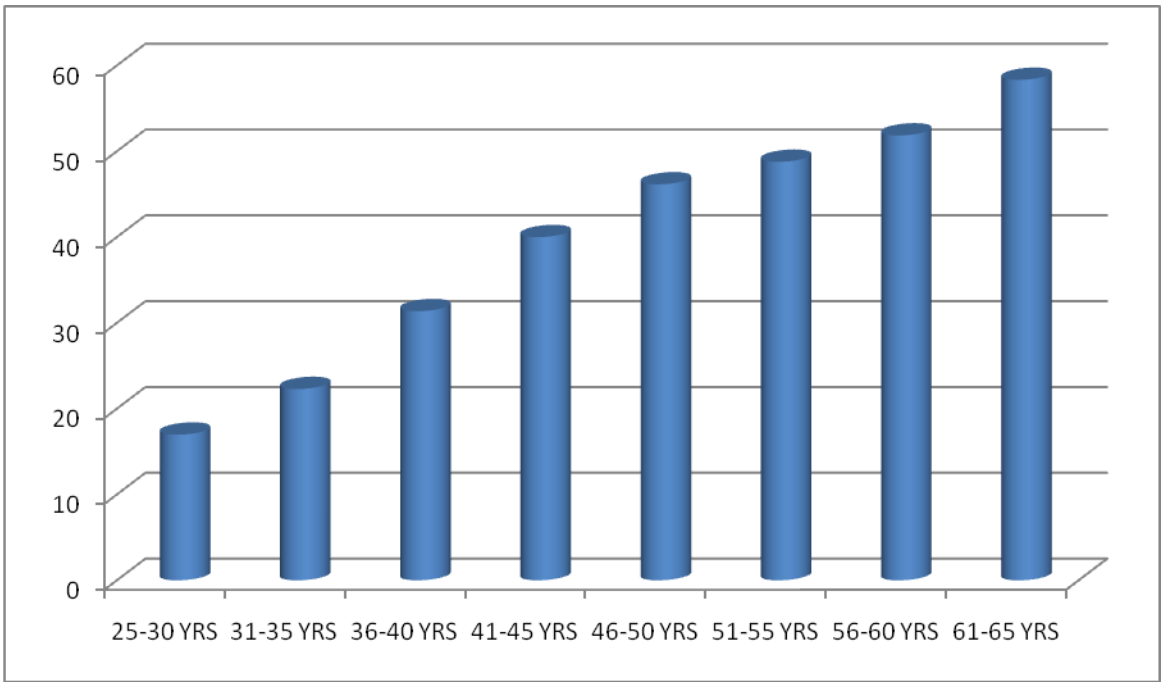
Table IX : Baseline characteristics of subjects (25-65 yrs) with and without cardio metabolic risk(Values are in Mean ±SD).

Parameters	Subjects without cardio metabolic risk (216)	Subjects with cardio metabolic risk (119)
Age(yrs)	35.67 ± 5.43	41.36 ± 4.35 ***
BMI(kg/m^2)	23.53 ± 1.57	23.86 ± 1.46#
WC(cm)	84.93± 10.89	92.85 ± 7.62***
WHR	0.87± 0.01	0.91 ± 0.01***
SBP(mmHg)	119. 17± 8.73	125.91± 8.80***
DBP(mmHg)	77.06 ± 6.54	86.81 ± 5.33***
PP(mmHg)	42.23 ± 5.76	46.38 ± 4.56***
TG(mg/dl)	137.24 ± 9.20	148.83 ± 10.91*
TC(mg/dl)	226.92 ± 13.70	247.64 ± 16.10*
HDL(mg/dl)	51.89 ± 2.62	46.35 ± 3.21**
LDL(mg/dl)	152.94 ± 11.16	157.90 ± 18.37**
VLDL (mg/dl)	28.36 ± 9.24	30.71 ± 4.92 *

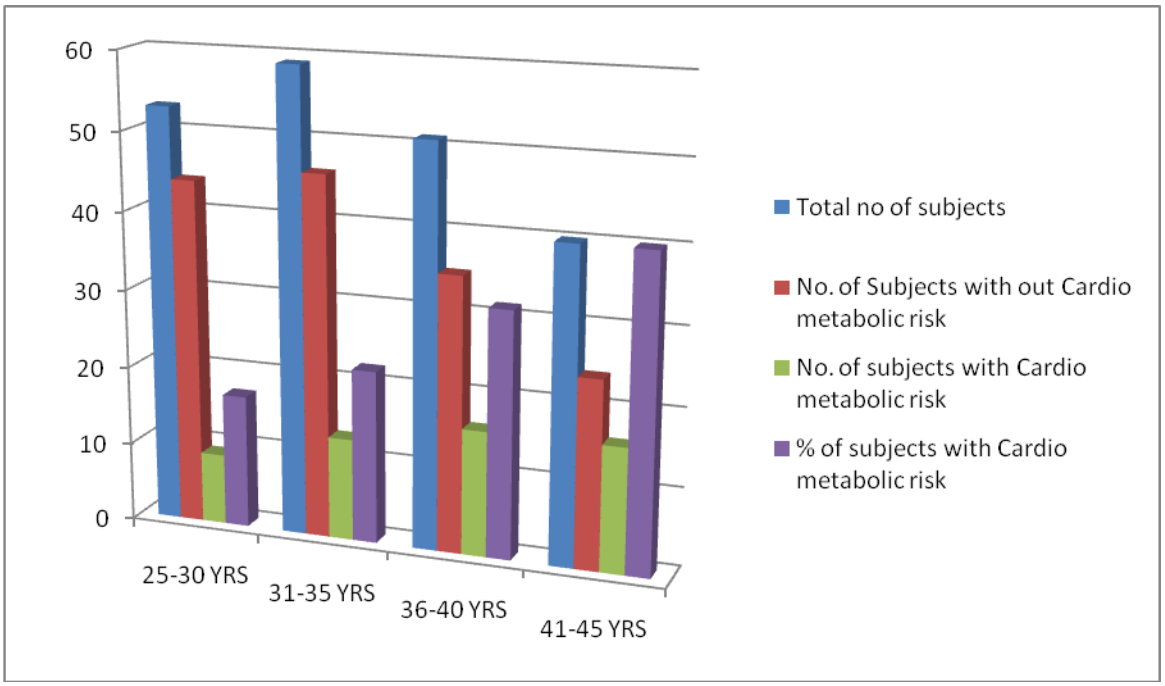
BMI – Body Mass Index, WC- Waist Circumference, WHR- Waist-Hip Ratio, SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, PP- Pulse Pressure, TC- Total Cholesterol, TG- Triglyceride, HDL - High Density Lipoprotein, LDL- Low Density Lipoprotein.

Not significant, * $p<0.05$, ** $p<0.01$, ***$p< 0.001$

FigureII : Age group wise prevalence of cardio metabolic risk in study subjects (values are in % of total subjects in a particular age group) .



FigureIII : Age group wise distribution of premenopausal subjects with and without cardio metabolic risk



FigureIV : Age group wise distribution of postmenopausal subjects with and without cardio metabolic risk.

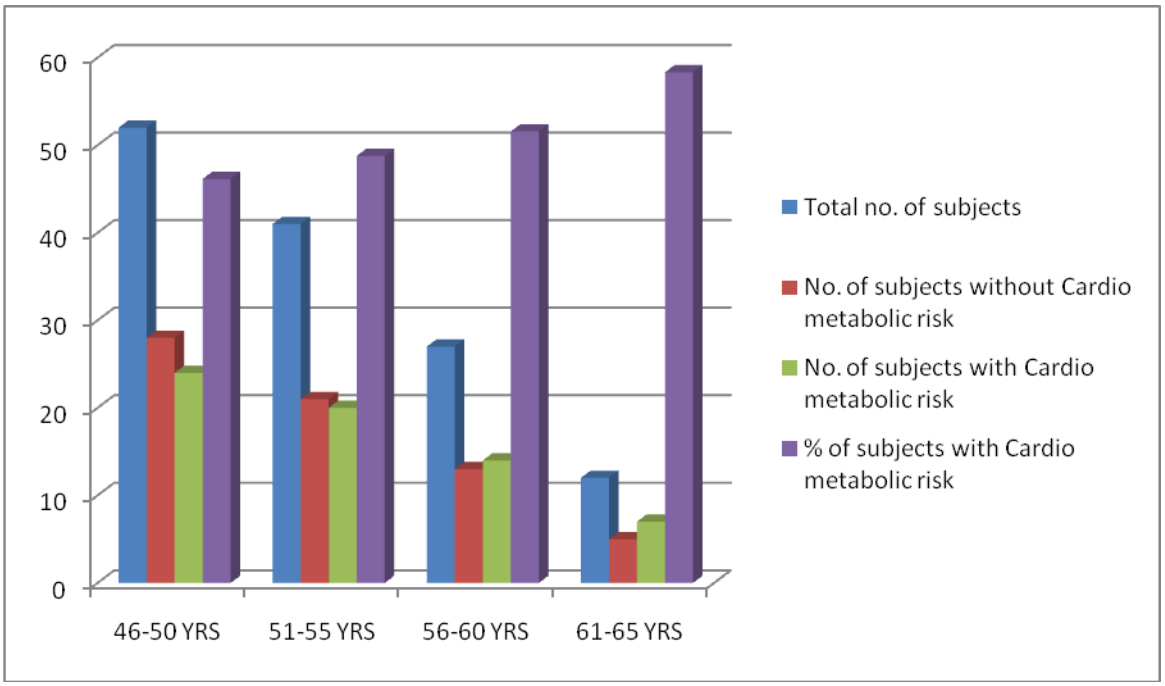
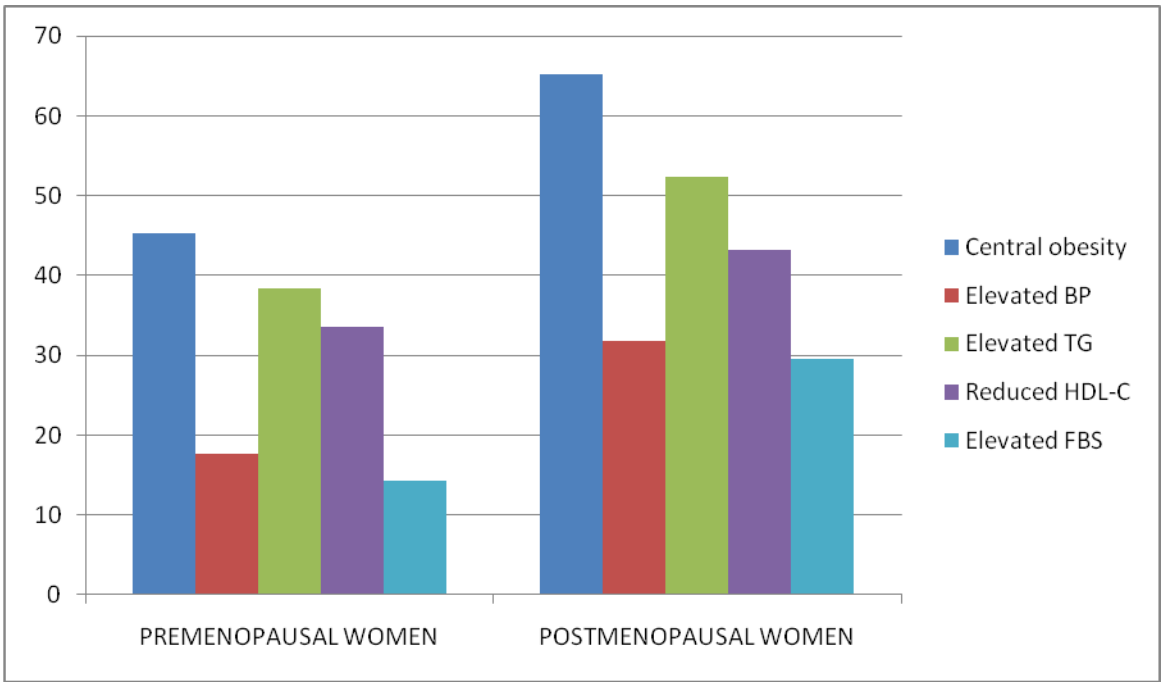


Table X : Prevalence of single cardio metabolic risk component among subjects.
(Number in the bracket indicates total number of subjects).

Cardio metabolic risk Component	**(%) Prevalence (335)**
Central obesity	51.44 (171)
Elevated Blood Pressure	28.95 (97)
Elevated Triglyceride	40.0 (134)
Reduced High Density Lipoprotein	23.58 (79)
Elevated Fasting Blood Glucose	14.92 (50)

Figure V: Prevalence of different cardio metabolic risk parameters in pre and postmenopausal subjects (values are in % of total subjects in the group).



4.3. Association among various obesity and atherogenicity markers and traditional cardio metabolic risk factors in women:

The obesity indices used for analysis included BMI, WC, WHR, WTR, HTR and WHtR. The atherogenicity markers included were HDL-C/TC ratio, HDL-C/LDL-C ratio, HDL-C/VLDL-C ratio and TG/HDL-C –ratio. The traditional risk factors included were systolic and diastolic blood pressures, fasting blood sugar, triglyceride and high density lipoprotein levels.

All the obesity indices and atherogenicity markers except BMI showed significant values in women with profound cardio metabolic risk in both premenopausal and postmenopausal women. The postmenopausal women showed higher values in comparison to their premenopausal counterparts (Table - XI) .

The influence of various obesity markers like BMI, WC, WTR, WHR and WHtR on the cut offs of SBP, DBP, FBS,TG and HDL-C according to consensus statement were analysed in both premenopausal and postmenopausal women separately.

The analysis revealed that premenopausal women with high BMI,WC.WHR,WTR and WHtR had raised SBP. Among premenopausal women, DBP was found to be high in women with higher BMI and WC. Premenopausal women with elevated WHR showed to have raised fasting blood glucose level . Raised WHR was associated with reduced HDL-C level (Table-XII).

In case of postmenopausal women, raised SBP and raised DBP were found to be associated with elevated BMI and WHR. Postmenopausal women with high WC,WHR, and WHtR had significantly raised FBS levels. Similarly, elevated WC, WHR, and less WTR, WHtR were associated with rise in the levels of triglyceride among postmenopausal women. Finally, like premenopausal women, only WHR had influenced on reduced HDL-C among post menopausal women (Table - XIII)

The influence of atherogenicity indices like TG/HDL-C, HDL-C/TC and HDL-C/LDL-C on cut off limits of traditional cardio metabolic risk factors revealed, premenopausal women with raised blood pressure had increased TG/HDL-C and HDL-C/LDL-C ratios. Among postmenopausal women, raised fasting blood glucose and triglyceride levels were common with higher TG/HDL-C and HDL-C/LDL-C ratios, but only with TG/HDL-C ratio in premenopausal group. Finally, reduced levels of HDL-C were noticeable in elevated TG/HDL-C ratio and reduced HDL-C/TC ratio in postmenopausal

group, whereas, it was apparent in higher TG/HDL-C ratio but in lower HDL-C/TC ratios among premenopausal women (Table - XIV).

The correlation between various obesity markers and traditional cardio metabolic risk indicators revealed a significant positive correlation between systolic blood pressure and WHR of the subjects. Diastolic blood pressure had a positive correlation with BMI and WHR. Triglyceride levels showed positive correlation with BMI, WHR and WHtR of the subjects. HDL-C showed a negative correlation with BMI, WC and WHtR. FBS correlated negatively with WHR and positively with WHtR of the subjects (Table- XV).

The correlation between various cardio metabolic risk indicators and atherogenicity markers revealed, TG: HDL-C ratio had a positive correlation with all cardio metabolic risk indicators except fasting blood glucose level (Table - XVI).

Table XI : Comparison of cardio metabolic risk indicators in pre and postmenopausal women with and without profound cardio metabolic risk.
(Values are in Mean±SD).

Parameters	Postmenopausal women with cardio metabolic risk (n= 65) GROUP - I	Postmenopausal women without cardio metabolic risk (n= 67) GROUP - II	Premenopausal women with cardio metabolic risk (n= 54) GROUP - III	Premenopausal women without cardio metabolic risk (n= 149) GROUP – IV
Age (yrs)	56.35 ± 1.13**	51.89 ± 1.45	41. 49 ± 1.81***	35. 83 ± 1.64
BMI (kg/m^2)	24.68 ± 1.36#	24.40 ± 1.07	23.19 ± 1.21#	22.83 ± 1.36
WHR	0.94 ± 0.01*	0.89± 0.01	0.89 ± 0.05**	0.84 ± 0.03
WTR	1.68± 0.05***	1.43 ± 0.06	1.48± 0.04**	1.24 ± 0.06
HTR	1.72 ± 0.06#	1.65± 0.02	1.63 ± 0.06#	1.61 ± 0.04
WHtR	0.63 ±0.01***	0.54 ±0.05	0.56 ±0.03**	0.47± 0.03
HDL-C/TC ratio	0.42 ±0.01*	0.37 ± 0.04	0.29 ±0.02**	0.34 ±0.01
HDL-C/LDL-C ratio	0.68 ±0.01***	0.59 ±0.05	0.82 ±0.09***	0.69 ±0.01
TG/HDL-C - ratio	0.84 ± 0.02***	1. 48 ±0.04	0.73 ±0.04***	1.37 ±0.05

BMI- Body Mass Index, WC-Waist Circumference, WHR- Waist- Hip Ratio, WTR- Waist-Thigh Ratio,WHtR- Waist-Height Ratio, HDL-C/TC- High Density Lipoprotein- Cholesterol-Total Cholesterol Ratio, HDL-C/LDL-C - High Density Lipoprotein - Cholesterol- Low Density Lipoprotein- Cholesterol Ratio, TG/HDL-C - Triglyceride- High Density Lipoprotein - Cholesterol Ratio.

Not significant, * $p<0.05$, ** $p<0.01$, *** $p< 0.001$

(Comparison between Groups I &II, and between Groups III& IV).

Table XII: Values of different obesity indices according to cut offs of different metabolic risk factors in premenopausal women using consensus criteria of cardio metabolic risk factors for Asian Indians. (Values are in Mean ± SD)

Cardio Metabolic Risk Factors	Obesity indices				
	BMI (kg/m^2)	WC (cm)	WHR	WTR	WHtR
SBP :					
< 130 mmHg	**22.37 ± 0.50**	**86.30 ± 1.20**	**0.83 ±0.06 0.88 ± 0.01*****	**1.38 ± 0.12 1.47 ± 0.16*****	**0.48 ± 0.01 0.54 ± 0.03*****
≥130 mmHg	**26.12 ± 0.70*****	**90.41 ± 1.90*****			
DBP :					
< 85 mmHg	**22.10 ± 0.50**	**83.97 ± 1.80**	0.84 ± 0.05 0.85 ± 0.03#	1.41 ± 0.11 1.43 ± 0.12#	0.47 ± 0.02 0.50 ± 0.01#
≥85 mmHg	**26.34 ± 0.70*****	**86.91 ± 1.10*****			
FBS :					
< 100 mg/dl	23.81 ± 0.90	84.79 ± 1.90	**0.83 ± 0.01 0.87 ± 0.03*****	1.42 ± 0.16 1.43 ± 0.13#	0.49 ±0.06 0.51 ± 0.03#
≥110 mg/dl	24.18 ± 0.60#	85.28 ± 1.70#			
TG :					
< 150 mg/dl	22.93 ± 0.60	83.68 ± 1.70	0.85 ± 0.03 0.86 ± 0.01#	1.47 ± 0.12 1.49 ± 0.13#	0.48 ± 0.03 0.51 ± 0.02#
≥150 mg/dl	23.16 ± 0.70#	84.87 ± 1.80#			
HDL-C:					
< 50 mg/dl	22.64 ± 0.60	84. 38 ± 1.60	**0.84 ± 0.03 0.87 ± 0.06*****	1.39 ± 0.18 1.46 ±0.11#	0.49 ± 0.05 0.52 ± 0.03#
≥ 50mg/dl	23.43 ± 0.60#	86.12 ± 1.90#			

BMI- Body Mass Index, WC-Waist Circumference, WHR- Waist- Hip Ratio, WTR- Waist- Thigh Ratio, WHtR - Waist-Height Ratio, SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, FBS- Fasting Blood Sugar, TG- Triglyceride, HDL-C- High Density Lipoprotein - Cholesterol.

Not significant, * $p<0.05$, ** $p<0.01$, *** $p< 0.001$

Table XIII: Values of different obesity indices according to cut offs of different metabolic risk factors in postmenopausal women using consensus criteria of cardio metabolic risk factors for Asian Indians.(Values are in Mean ± SD)

Cardio Metabolic Risk Factors	Obesity indices				
	BMI(kg/m^2)	WC(cm)	WHR	WTR	WHtR
SBP :					
< 130 mmHg	**21.19 ± 1.80**	92.10 ±1.70	**0.89 ±0.06 0.97**	1.57 ± 0.111.58	0.57 ± 0.020.56
≥130 mmHg	**24.86 ± 1.50*****	92.80 ± 1.90 #	**± 0.03****	± 0.13#	± 0.03#
DBP :					
< 85 mmHg	**22.16 ± 1.60**	93.10 ± 1.60	**0.89 ± 0.02 0.99**	1.52 ± 0.11 1.54	0.56 ± 0.03 0.57
≥85 mmHg	**25.76 ± 1.50****	94.30 ± 1.70 #	**± 0.02****	± 0.12#	± 0.01#
FBS :					
< 100 mg/dl	23.58 ± 1.30	**92.70 ± 1.60**	**0.86 ± 0.03 0.92**	**1.53 ± 0.12 1.56**	0.55 ±0.01 0.57
≥100 mg/dl	23.19 ± 1.60#	**96.30 ± 1.80*****	**± 0.03*****	**± 0.13*****	± 0.03#
TG :					
< 150 mg/dl	23.76 ± 1.60	**92.10± 1.70**	**0.87 ± 0.03 0.93**	**1.56 ± 0.18 1.14**	**0.59 ± 0.04 0.54**
≥150 mg/dl	23.35 ± 2.10#	**95.90 ± 1.70*****	**± 0.01*****	**± 0.11*****	**± 0.01*****
HDL-C:					
< 50 mg/dl	22.58 ± 1.50	92. 30 ± 1.60	**0.86 ± 0.01 0.92**	1.54 ± 0.11	0.58 ± 0.02 0.60
≥ 50mg/dl	23.96 ± 0.70#	93.10 ± 1.60#	**± 0.06*****	1.54±0.12#	± 0.02#

BMI- Body Mass Index, WC-Waist Circumference, WHR- Waist- Hip Ratio, WTR- Waist- Thigh Ratio, WHtR- Waist- Height Ratio, SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, FBS- Fasting Blood Sugar, TG- Triglyceride, HDL-C- High Density Lipoprotein - Cholesterol.

Not significant, * $p<0.05$, ** $p<0.01$, *** $p< 0.001$

Table XIV : Values of various atherogenicity markers according to cut offs of different metabolic risk factors in pre and postmenopausal women using consensus criteria of cardio metabolic risk factors for Asian Indians.(Values are in Mean ± SD)

Cardio Metabolic Risk Factors	Atherogenic Markers					
	Premenopausal women			Postmenopausal women		
	TG/HDL-C	HDL-C/ TC	HDL-C / LDL-C	TG/HDL-C	HDL-C/ TC	HDL-C/ LDL-C
SBP :						
< 130 mmHg	**2.78 ± 1.11**	0.37 ± 0.02	**0.56 ± 0.08**	3.90	0.31 ±0.06	0.76 ± 0.03
≥130 mmHg	**3.83 ± 1.58*****	0.39 ± 0.01 #	**0.86 ± 0.02*****	±2.133.48 ± 2.12 #	0.34 ± 0.01 #	0.79 ± 0.03 #
DBP :						
< 85 mmHg	**2.79 ± 1.30**	0.36 ± 0.01	**0.62 ± 0.04**	3.93 ± 2.21	0.29 ± 0.02	0.74 ± 0.01
≥85 mmHg	**3.82 ± 1.12*****	0.38 ± 0.02 #	**0.67 ± 0.05***	4.16± 1.90	0.26 ± 0.03	0.78 ± 0.03 #
FBS :						
< 100 mg/dl	**2.67 ± 1.19**	0.36 ± 0.03	0.63 ± 0.12	**3.23 ± 2.30**	**0.32 ± 0.01**	0.76 ± 0.06
≥100 mg	**3.93 ± 1.58*****	0.35 ± 0.02 #	0.65 ± 0.08 #	**4.97 ± 2.11*****	**0.39 ± 0.01*****	0.78 ± 0.02 #
TG :						
< 150 mg/dl	**2.72 ± 1.18**	0.37 ± 0.11	0.64 ± 0.14	**3.91 ±0.06**	**0.31 ± 0.01**	0.76 ± 0.01
≥150 mg/dl	**3.86 ±1.20*****	0.39 ± 0.02 #	0.65 ± 0.27 #	**4.18 ± 0.03*****	**0.37 ± 0.02*****	0.77 ± 0.02 #
HDL-C :						
< 50 mg/dl	**3.79 ± 1.31**	**0.32 ± 0.01**	0.66 ± 0.12	**4.96 ±0.06**	**0.31 ± 0.03**	0.74 ± 0.01
≥ 50mg/dl	**2.82 ± 1.47*****	**0.38 ± 0.01****	0.68 ± 0.14 #	**3.94 ± 0.01*****	**0.35 ± 0.03***	0.78 ± 0.03 #

TG/HDL-C - Triglyceride- High Density Lipoprotein - Cholesterol Ratio, HDL-C/TC- High Density Lipoprotein- Cholesterol- Total Cholesterol Ratio, HDL-C/LDL-C - High Density Lipoprotein - Cholesterol- Low Density Lipoprotein- Cholesterol Ratio, SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, FBS- Fasting Blood Sugar, TG- Triglyceride, HDL-C- High Density Lipoprotein - Cholesterol# Not significant, * $p<0.05$, ** $p<0.01$, *** $p< 0.001$

Table XV : Correlation between cardio metabolic risk factors and obesity markers.

Cardio metabolic risk factor	Obesity Markers							
	BMI		WC		WHR		WHtR	
	r	p	r	P	r	p	r	P
Systolic Blood pressure (mmHg)	0.152	0.077	0.121	0.160	**0.648****	0.000	-0.145	0.090
Diastolic Blood pressure (mmHg)	**0.173***	0.043	0.119	0.168	**0.677****	0.000	-0.112	0.193
Triglyceride	**0.352****	0.000	**0.197***	0.021	-0.100	0.247	**0.869****	0.000
High Density Lipoprotein	**-0.204***	0.017	**-0.198**	0.021	0.031	0.721	**-0.574****	0.000
Fasting Blood Glucose	0.051	0.557	0.080	0.354	**-0.233***	0.006	**0.214***	0.012

*** Correlation is significant at the 0.01 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed);* Correlation is significant at the 0.05 level (2-tailed).

Table XVI: Correlation between cardio metabolic risk factors and atherogenicity markers.

Cardio metabolic risk factor	Obesity/Atherogenicity Markers							
	TG : HDL-C		HLD-C :TC		HDL-C:LDL- C		HDL-C : VLDL-C	
	r	p	r	p	r	p	r	P
Systolic Blood pressure (mmHg)	**0.672*** **	0.000	0.213	0.262	-0.092	0.633	0.144	0.288
Diastolic Blood pressure (mmHg)	**0.783*** **	0.000	0.176	0.236	-0.116	0.432	0.157	0.282
Triglyceride	**0.546*** **	0.000	0.162	0.258	-0.229	0.187	**0.312***	0.018
High Density Lipoprotein	**0.351****	0.003	**0.242***	0.028	0.320**	0.006	**0.381****	0.000
Fasting Blood Glucose	0.131	0.357	0.136	0.376	0.163	0.218	-0.122	0.386

*** Correlation is significant at the 0.001 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

4.4 Screening of haematological parameters and evaluation of their association with cardio metabolic profile:

All the haematological parameters were recorded in a haematological analyser. Cardio metabolic risk parameters recorded in the subjects were utilized for analysis of association between Haemoglobin concentration as indicator of status of anaemia status and cardio metabolic risk of the subjects. A subject with Haemoglobin concentration less than 12gm% was considered as anaemic according to WHO criteria.

All haematological parameters studied were within normal limit for the age and sex of the subjects. The analysis of different haematological parameters between premenopausal and postmenopausal women revealed that there was a significant difference in total RBC count and Haemoglobin concentration between pre and postmenopausal women, which was less in postmenopausal women in comparison to premenopausal women in our study group.

Among the different ethnic groups, the Lambanis from Vijaypur were found to have largest number of subjects with Haemoglobin concentration less than 12 gm% in both premenopausal and postmenopausal groups.

The analysis of co-existence of both anaemia and cardio metabolic risk in subjects revealed that 42.42% of non-ethnic subjects from Tripura, 38.28% from Riang community of Tripura, 48.28% from non-ethnic subjects of Vijaypur and 51.58% Lambani subjects from Vijaypur with Haemoglobin concentration less than 12gm% were found to have co-existence with three or more cardio metabolic risk factors.

The analysis of difference in co-existence of anaemia and profound cardio metabolic risk in between premenopausal and postmenopausal subjects revealed, among premenopausal subjects, 26.6% had only anaemia without profound cardio metabolic risk, 22.65 % had only profound cardio metabolic risk without anaemia and 34.97% had coexistence of both anaemia and profound cardio metabolic risk. Among postmenopausal subjects, 31.69% had only anaemia, 29.48 % had only cardio metabolic risk and 39.66% had co-existence of both anaemia and profound cardio metabolic risk.

Table XVII: The details of Haematological parameters evaluated in the subjects.

(Values are in Mean ±SD)

Parameters	Total subject	Premenopausal	Postmenopausal
Total RBC count (millions/mm^3)	4.6 ± 0.80	4.9 ± 0.80	4.1 ± 0.63*
Total WBC count (thousands/mm^3)	5.3 ± 0.81	5.4 ± 0.80	5.2 ± 0.36#
Platelet count (lakhs/mm^3)	1.30 ± 0.32	1.38 ± 0.51	1.31 ± 0.62#
Neutrophils (%)	58.9 ± 3.9	58.7 ± 1.27	58.2 ± 2.56#
Eosinophils(%)	9.64 ± 0.31	9.71 ± 0.72	9.89 ± 0.75#
Basophils (%)	0.0	0.0	0.0#
Lymphocytes (%)	33.7 ± 1.58	36.3 ± 1.23	36.6 ± 1.38#
Monocytes (%)	7.1 ± 0.93	7.8 ± 1.3	7.3 ± 1.6#
Haemoglobin concentration (gm%)	12.11 ± 0.84	12.93 ± 0.56	12.18 ± 0.69*

Not significant, * $p<0.05$, ** $p<0.01$, ***$p< 0.001$

Figure VI: Percentage of subjects with Haemoglobin content less than 12gm% in different groups (values are in percentage of subjects).

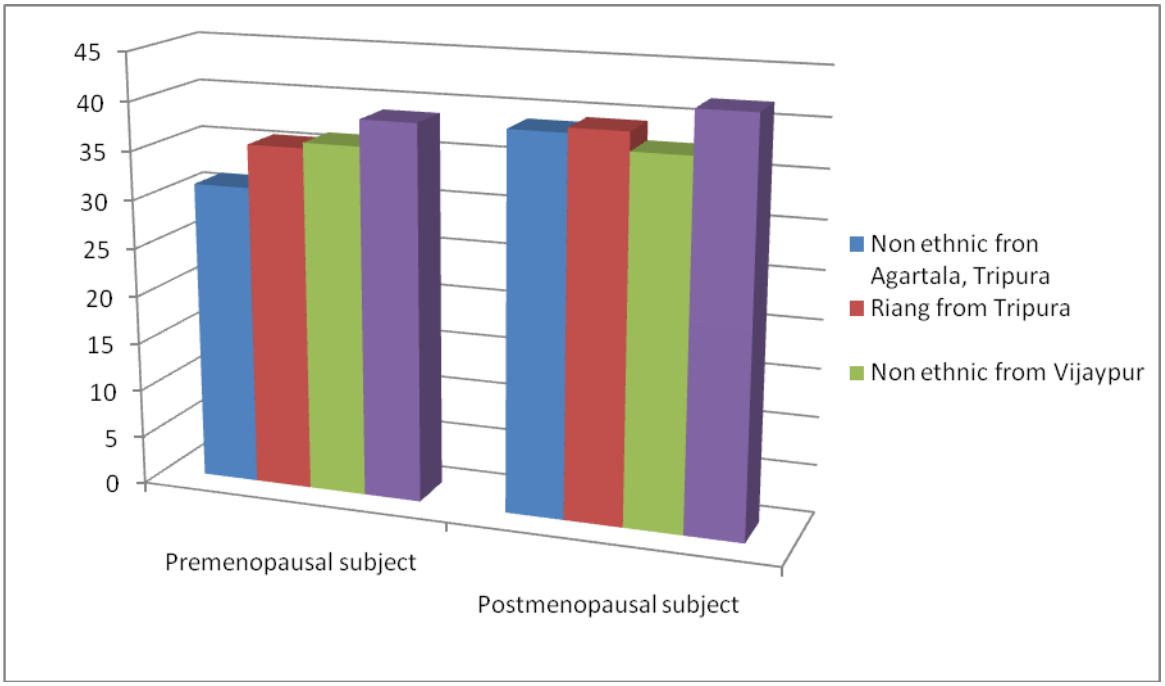
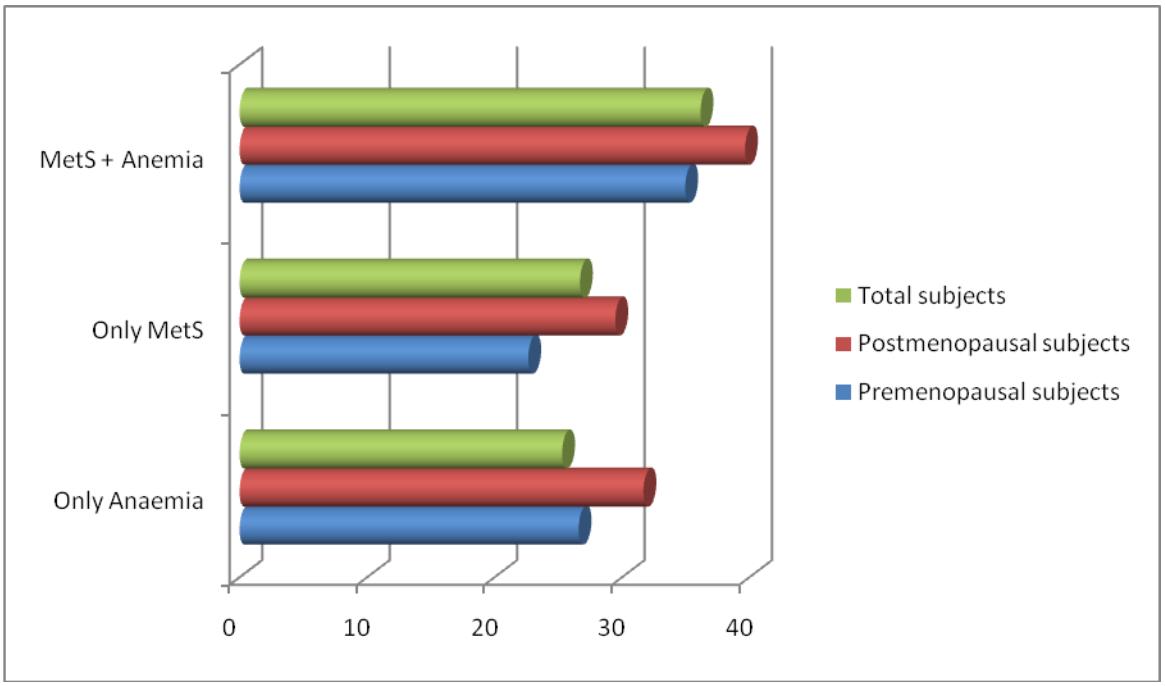


Table XVIII: Co-existence of cardio metabolic risk in subjects with anaemia (Hb concentration: ↓ 12.0 gm%).

Ethnicity of the subjects	Premenopausal subjects	Postmenopausal subjects	Total subjects
Non ethnic from Agartala, Tripura	7(19) - 36.84%	7(14)-50.0%	14(33)-42.42%
Riang from Tripura	6(21)-28.57%	7(13)-53.84%	13(34)-38.23%
Non ethnic from Vijaypur	6(17)-35.29%	8(12)-66.66%	14(29)-48.27%
Lambani from Vijaypur	6(14)-42.58%	8(13)-61.53%	14(27)-51.85%

Numbers within bracket indicate number of subjects with anaemia.

Figure VII: Association between anaemia and cardio metabolic risk in subjects (Values are in percentage of subjects in the category).



4.5. Role of nutritional status, sociodemographic characters and ethnicity on cardio metabolic risk of the subjects:

In this study, presence of various cardio metabolic risk factors in subjects from different ethnic backgrounds were analysed. The nutritional status was determined based on BMI of the subjects according to WHO criteria.

Analysis revealed that the percentage of subjects with three or more cardio metabolic risk factors observed from different ethnic groups varied within a narrow range. The maximum number of subjects with three or more cardio metabolic risk factors were observed among nonethnic subjects from Tripura (36.08%) and the minimum number was observed among Ethnic Lambani subjects from Vijaypur (34.32%). The nonethnic subjects from Vijaypur had an occurrence of 35.44%, and Riang subjects from Tripura had an occurrence of 35.86%. Both ethnic groups showed marginally lesser percentage of subjects with three or more cardio metabolic risk factors from their corresponding nonethnic counterparts (Table XIX).

The percentage of subjects with three or more cardio metabolic risk factors in premenopausal groups did not show much difference among different groups. In case of postmenopausal groups there was a marked difference in the percentage of subjects with three or more cardio metabolic risk factors which was found to be from 54.54 % in Riang women, 52.77% in nonethnic women from Tripura, 46.87 % in nonethnic women from Vijaypur and 41.93% in Lambani women (Table XIX, Figure VIII).

Analysis of cardio metabolic risk indicators in different groups of subjects revealed that central obesity marked by increased waist circumference was most prevalent risk factor. In Riang women from Tripura altered lipid profile marked by increased triglyceride level and reduced HDL-C level were more prominent than other groups. Other risk factors showed similar pattern in all the groups (Table XX, Figure IX).

Among the subjects with three or more cardio metabolic risk factors, 37.58% were urban subjects and 33.87 % were rural subjects. Among the urban subjects, 52.68% were having central obesity, 28.49% were having increased blood pressure, 44.08% were having increased TG, 19.35% were having reduced HDL-C and 14.51% were having increased FBS. Among rural women, 48.99% were having central obesity, 29.53% were having increased blood pressure, 34.89% were having increased TG, 28.85% were having reduced HDL-C and 15.93% of the subjects were having increased FBS (Figure X, Figure XI).

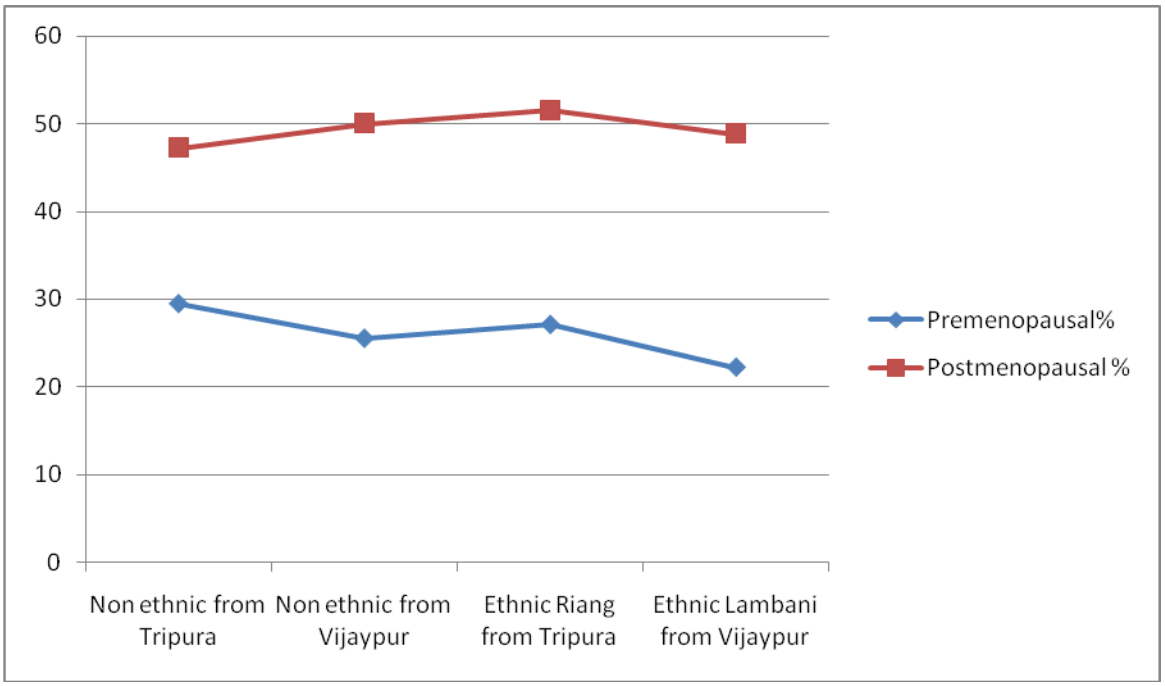
The percentage of subjects with three or more cardio metabolic risk factors was found to be highest in subjects having lesser educational level(less than 8th standard) and less in subjects with higher educational level(more than 12th standard, Figure XII)

The percentage of subjects with three or more cardio metabolic risk factors was found to be highest in subjects from higher family income groups with family income more than Rs. 30.000/= per month and lowest in subjects with lesser family income groups with income less than Rs. 10,000/= per month (Figure XIII). Non-vegetarian subjects were having more cardio metabolic risk than vegetarian subjects (Figure XIV).

Table XIX: Distribution of subjects with three or more cardio metabolic risk factors from different ethnic groups.
(Values are in percentage of subjects in particular group).

Groups	Premenopausal (%)	Postmenopausal (%)	Total (%)
Non ethnic subjects From Agartala, Tripura.	26.22	52.77	36.08
Non ethnic subjects From Vijaypur, Karnataka	27.65	46.87	35.44
Ethnic Riang subjects From Tripura	25.42	54.54	35.86
Ethnic Lambani subjects From Vijaypur, Karnataka.	27.77	41.93	34.32

Figure VIII: Cardio metabolic risk in premenopausal and postmenopausal subjects from different ethnic groups (% of total subjects in the group).



TableXX: Status of various cardio metabolic risk indicators in women from different ethnic groups.

Parameter	Non ethnic women from Agartala, Tripura.	Riang women from Agartala, Tripura.	Non ethnic women from Vijaypur	Lambani women from Vijaypur
↑WC (cm)	72 (74.22%)	67 (72.82%)	44 (55.69%)	41 (61.19%)
↑BP (mmHg)	40(41.23%)	37 (40.29%)	34 (43.03%)	28 (41.79%)
↑TG (mg%)	49 (50.51%)	55 (59.78%)	36 (45.56%)	26 (38.80%)
↓HDL-C (mg%)	41 (42.26%)	47 (51.08%)	31 (39.29%)	28 (41.79%)
↑FBS (mg%)	37 (38.14%)	33 (35.86%)	26 (32.98%)	25 (37.31%)

Figure IX: Comparison of prevalence of different cardio metabolic risk indicators in subjects from different ethnic groups.

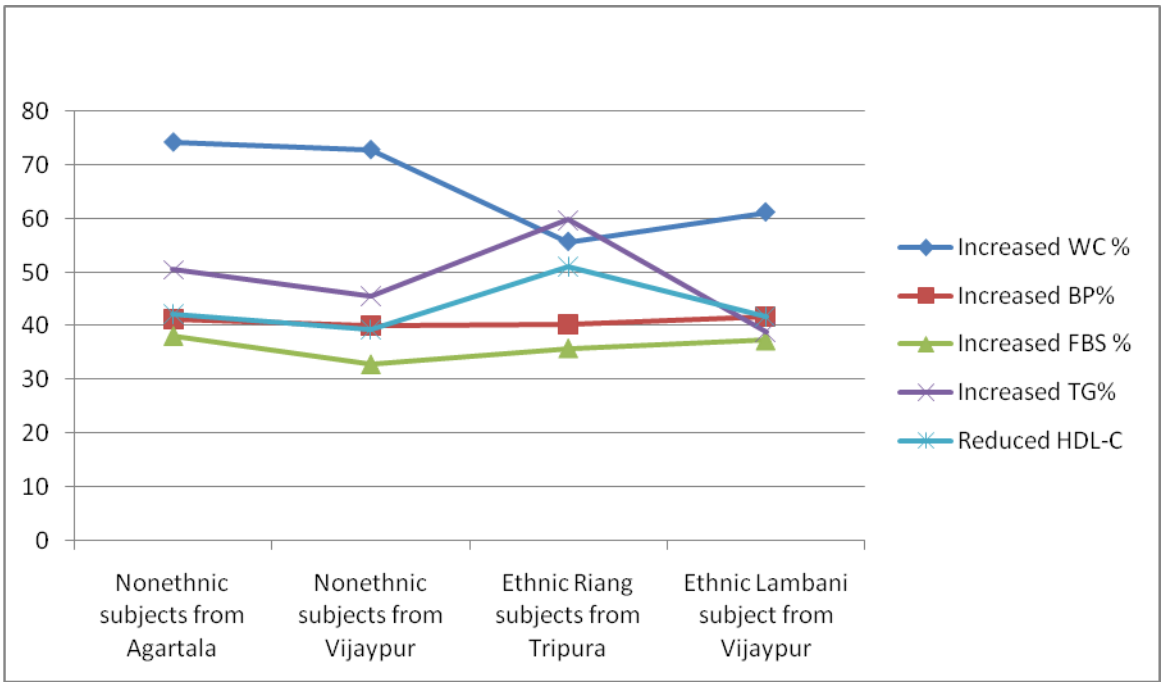


Figure X: Urban / Rural difference in profound cardio metabolic risk among subjects from different communities (Values are in percentage of total subjects).

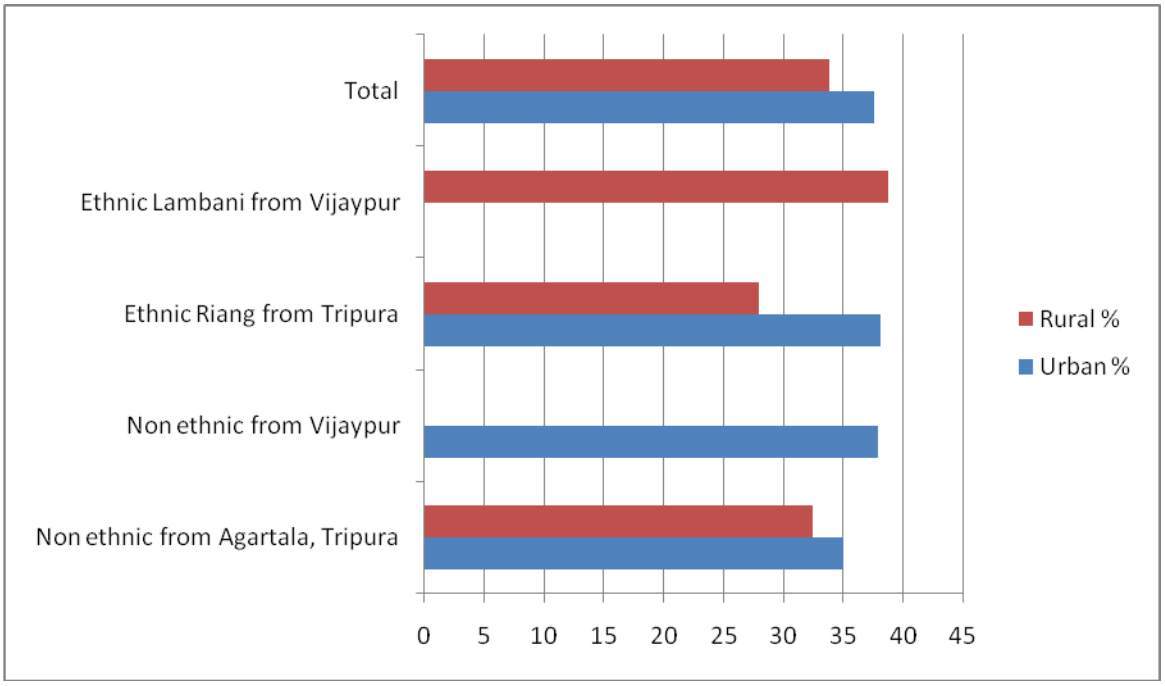


Figure XI: Urban / Rural difference in prevalence of individual cardio metabolic risk factor in subjects from different communities (Values are in percentage of subjects).

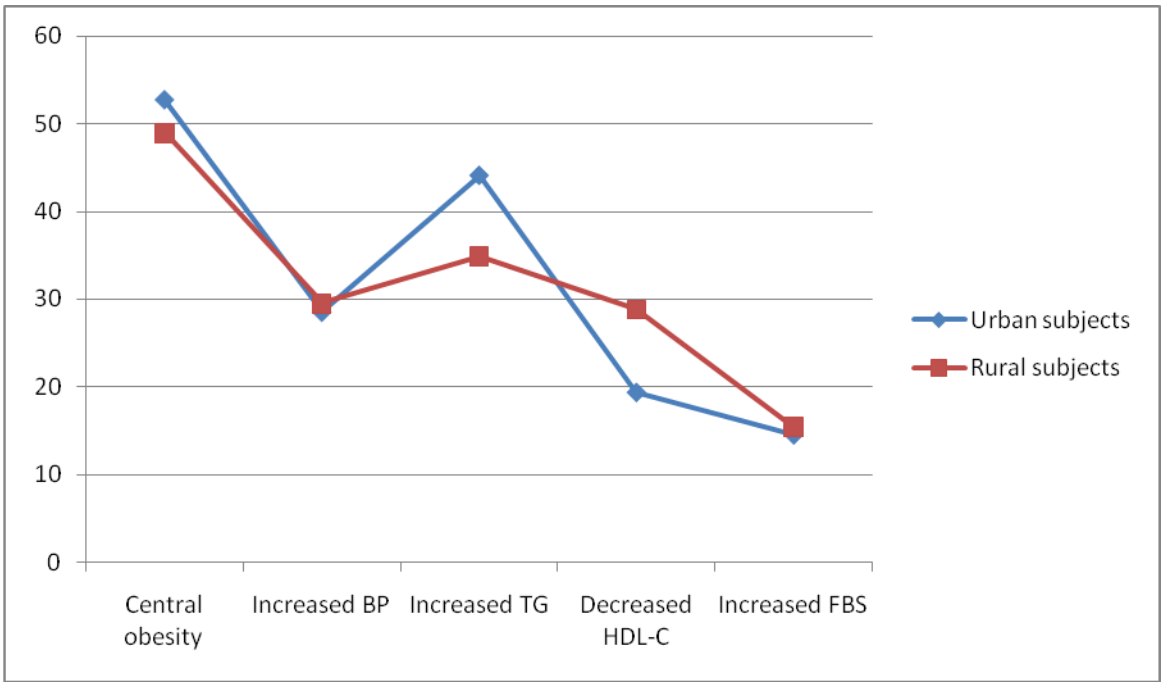


Figure XII : Role of level of education on cardio metabolic risk profile of the study population (Values are in percentage of total subjects of a category).

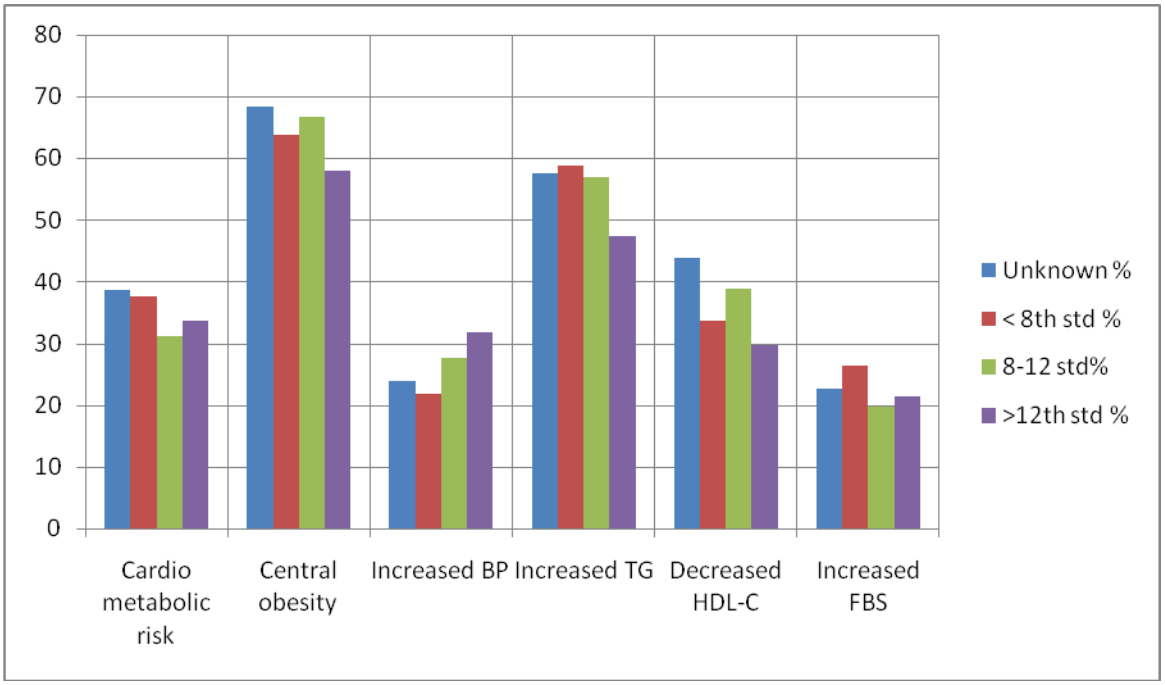


Figure XIII: Role of family income on cardio metabolic risk profile of the study population (Values are in percentage of total subjects in a category)

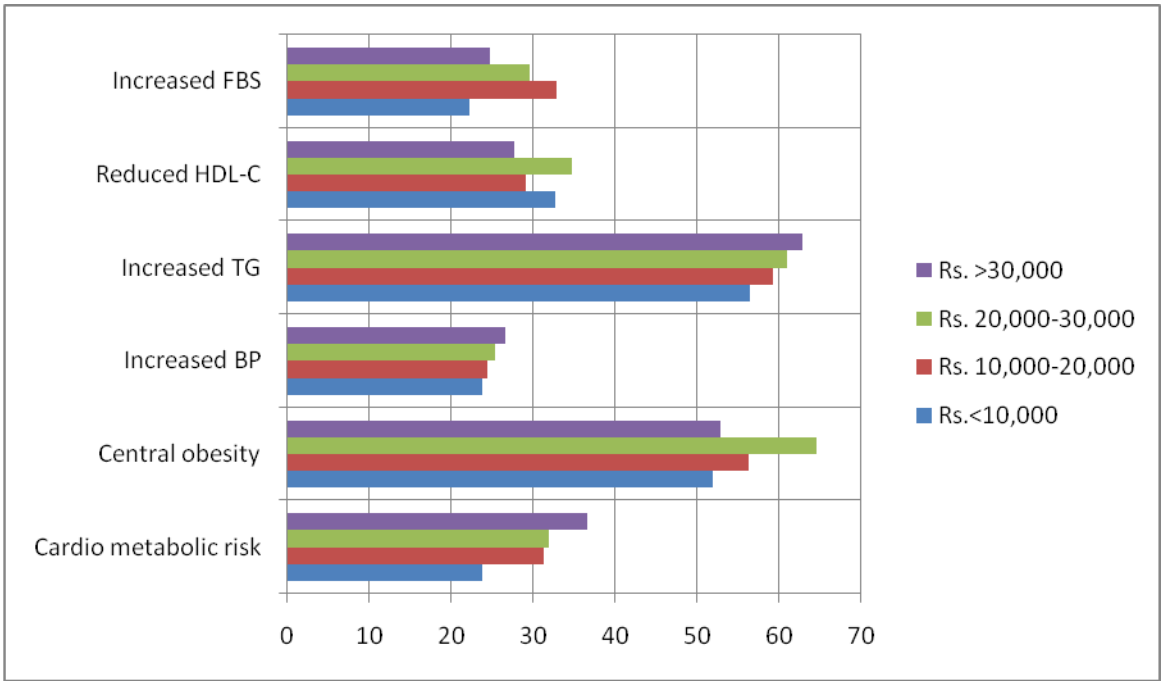
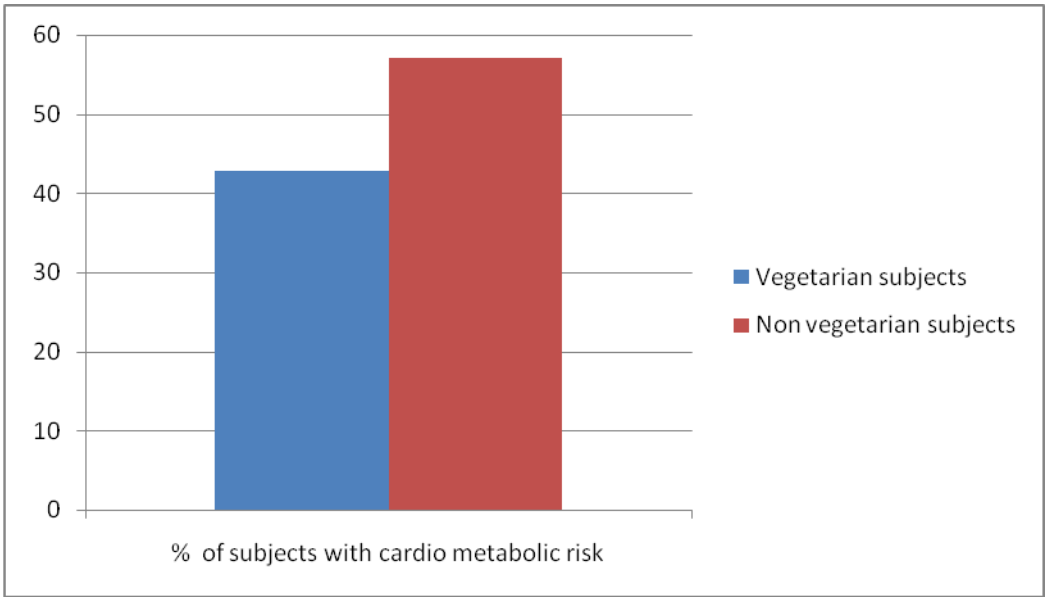


Figure XIV : Role of dietary habits on cardio metabolic risk profile of the study population (Values are in percentage of total subjects in a category)



4.6. Evaluation of association between antioxidant status and cardio metabolic risk of the subjects:

The association between cardio metabolic risk and antioxidant status was evaluated in subjects with three or more cardio metabolic risk factors and the subjects without cardio metabolic risk working as control group. Antioxidant status of the subjects was evaluated by measuring antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase activity (GPx). Plasma levels of two antioxidant vitamins, vitamin C and vitamin E were measured. Oxidative stress of the subjects was evaluated by measuring plasma concentration of lipid peroxidation product Malondialdehyde (MDA).

Analysis showed, there was a significant increase in MDA levels in subjects with cardio metabolic risk factors in comparison to control subjects. Antioxidant enzymes like CAT, SOD and GPx levels were reduced significantly in subjects with cardio metabolic syndrome in comparison to control subjects (Table XXI).

There was insignificant decrease in the levels of antioxidant vitamin C and vitamin E levels in subjects with three or more cardio metabolic risk factors in comparison to control subjects without cardio metabolic risk (Table XXI).

Table XXI: Comparison of oxidant and antioxidant levels in subjects with and without cardio metabolic risk.

Parameters	Subjects without cardio metabolic risk (control group)	Subjects with cardio metabolic risk
Plasma MDA (nmol /gm of Hb)	7.86 ± 0.24	8.29 ± 0.32 ***
Erythrocyte CAT (unit/mgHb)	42.32 ± 7.84	46.37 ± 6.12 *
Erythrocyte SOD (IU/gmHb)	789. 64 ± 169.39	892. 41 ± 162.93*
Erythrocyte GPx (IU/gmHb)	65.47 ± 2.58	68. 38 ± 1.86 ***
Plasma vitamin C (mg/dl)	1.91 ± 0.16	1.89 ± 0.24 #
Plasma vitamin E (µg/ml)	9.70 ± 2.18	9.46 ± 1.82 #

MDA- Malondialdehyde; CAT-Catalase; SOD- Superoxide dismutase; GPx - Glutathione peroxidase ;Hb- Haemoglobin;

#- Not Significant * $p< 0.05$;** $p< 0.01$; *** $p<0.001$;

CHAPTER 5 : DISCUSSION

Presence of several cardio metabolic risk factors especially central obesity and dyslipidemia, places women in high risk for development of insulin resistance and atherosclerosis. Physiological and hormonal changes during menopause acts as an additional risk factor for such diseases[1]. In the present study, 35.52% of women were found to have three or more cardio metabolic risk factors which had values higher than the cut off limits according to consensus statement on Asian Indians. The percentage of subjects with three or more cardio metabolic risk factors were significantly higher in postmenopausal women as compared to premenopausal women in the current study. Pandey et.al. in their study among pre and postmenopausal women from western India also observed a higher prevalence of metabolic syndrome in postmenopausal (56%) than premenopausal (44%) women[2]. Silvaraj et.al. observed 44.2% occurrence of metabolic syndrome among Indian women in the 41-50 years age group and 25.2% occurrence in women in the age group of 30-40 years [3]. Other studies in Austria, China, Germany, Iran and Canada showed a prevalence of 32.6%, 37.34%, 36.1%, 31% and 29.6% respectively which were in agreement with our findings. There were disagreement of findings between our study and some other studies done in Iran, Western India, Argentina and Ecuador with prevalence of 69%, 55%, 22% and 41.5% respectively[4,5]. These differences in prevalence of cardio metabolic risk factors in different studies can be due to different investigation criteria, socioeconomic and environmental differences, genetic factors and lifestyle of the subjects studied.

Sex difference in the occurrence of cardio metabolic risk and its individual components exists, suggesting sex-specific differences in the physiological mechanisms of occurrence of these risk factors[6]. Particularly in women, reproductive and hormone-related factors such as postmenopausal status, decreased parity and history of maternal gestational diabetes mellitus (GDM) have also been shown to increase the risk of condition. In case of menopause, for example, the biological plausibility of this relationship is explained by the fact that with menopause, women experience changes including increased abdominal adiposity, hyperglycaemia, hyperinsulinism, and dyslipidaemia[7,8]. Regarding menopausal status, NHANES and other studies had demonstrated strong association between menopause and cardio metabolic risk in women[9,10]. It has been suggested that the occurrence of cardio

metabolic risk after menopause is explained by oestrogen deficiency, because many of the risk factors are more prevalent in postmenopausal women. Also, oestrogen replacement improves insulin sensitivity and reduces the risk of diabetes mellitus[11,12]. Even though we

did not observe this association, our results are consistent with previous studies performed among postmenopausal women.

Different obesity and atherogenicity markers were found to be significantly higher among both post and premenopausal women with cardio metabolic risk in comparison to those without the risk. The most common cardio metabolic risk factor in our study, was found to be central obesity with a frequency of 79.48% in subjects with cardio metabolic risk in comparison to 38.77% in subjects without cardio metabolic risk. Majrjani et. al. also reported central obesity as the most important cardio metabolic risk factor in women in their study with an Iranian population[13]. Obesity increases cardio metabolic risk because it induces insulin resistance, increases blood pressure, triglyceride, low density lipoprotein cholesterol (LDL-C) and reduces high density lipoprotein cholesterol (HDL-C)[14,15]. However, in our study, BMI did not vary significantly between subjects with and without profound cardio metabolic risk. This might be due to the fact that people with normal weight might become metabolically obese due to abnormal distribution of body mass resulting into central obesity[16]. It is reported that substances released by intra-abdominal fat including inflammatory cytokines like tumour necrosis factor-alpha and interleukin-6 influence glucose metabolism as well as blood lipid profile producing insulin resistance. The second most prevalent cardio metabolic risk factor identified among pre and postmenopausal women, in our study was altered lipid profile. Various studies from different parts of the world have reported altered lipid profile as the main cardio metabolic risk factor in women[17-19].

Even though many features of cardio metabolic risk emerge with oestrogen deficiency characteristics of menopause, a considerable percentage of premenopausal women in our study as well as various previous studies were found to be predisposed for development of cardio metabolic risk. Central obesity observed among premenopausal women with cardio metabolic risk might have diluted the protective effect of premenopausal oestrogen level in the women[20]. Abdominal obesity is quite prevalent in South Asians with females outnumbering males[21]. Higher abdominal fat is known to be a risk factor for hypertension, hypertriglyceridaemia, hyperinsulinaemia and diabetes mellitus. The central obesity is also associated with changes in many biochemical variables especially with various adipokines like leptin and adiponectin which are found to be associated with insulin resistance and cardio metabolic risk components[22].

The second important component of cardio metabolic risk identified in our study was reduced HDL-C and elevated triglyceride. Atherogenic dyslipidaemia is common in South Asians who have lower HDL-C level and higher levels of small, dense and low density

lipoprotein (LDL-C) compared to Caucasians across all strata of the society[23]. Elevated serum triglyceride is more common in affluent Indians and migrant Indians[24]. These findings were in agreement with other findings that suggested a strong relationship between TG/HDL-C ratio with insulin resistance leading to cardio metabolic risk. Various studies reported a weak correlation between hypertension and insulin resistance. Consistent with findings of these studies, we also observed a weak correlation between hypertension and cardio metabolic risk in our study[25,26].

Studies on association between obesity markers and traditional cardio metabolic risk factors revealed that both waist circumference and waist hip ratio varied with almost all the traditional risk factors in pre and postmenopausal Indian women. Jouyandech et. al.[27] also observed similar relationship between waist circumference and traditional cardio metabolic risk components in women. The traditional cardio metabolic risk factors were also found to vary significantly with atherogenicity marker TG/HDL-C ratio. Similar observation was reported by Aurther et.al.[28] in their study with Ghanaian women. Our study showed that all the anthropometric and atherogenic risk factors showed a high risk value in subjects with increased cardio metabolic risk in comparison to subjects without the risk.

Reports suggest that ethnic difference in prevalence of cardio metabolic risk exists[29,30]. Consequently, we compared the cardio metabolic risk parameters in women from two different ethnic communities of India - Lambani from Vijaypur district of Karnataka state and Riang tribe from Tripura, a North Eastern state of India. For comparison, women from non ethnic population from both the study regions were also included in our study.

It is assumed that traditional societies and population residing in and around rural areas are expected to have lesser cardio metabolic risk as these are not exposed to modernization which is thought to be one of the principal causes of present day epidemic of cardio metabolic disorders[24]. The ICMR task force collaborative study reported general prevalence

of cardio metabolic risk to be 30% in urban areas of Delhi and 11% in rural Haryana[31].Mishra et. al. reported 30% prevalence among the urban slum population of Delhi[32]. Ramchandra et.al., reported 41% prevalence of cardio metabolic risk in urban area of Chennai among adults of 20-75 years with higher prevalence in women than men[33].Kamble et.al., in a study on adult population of Wardha, central India, observed that magnitude of cardio metabolic risk was low among rural adults as compared to urban areas[34]. Sarkar et. al., in their study with two tribal populations of India, namely Bhutia and Toto, have observed that Bhutia had a relatively more adverse cardio metabolic risk profile compared to Toto, as measured by

blood pressure, blood lipids and blood glucose. Bhutia had higher mean values in majority of the traits considered, except HDL-C, irrespective of age groups and gender. There was difference in cardio metabolic risk factors between urban and rural Toto and Bhutia population[24].

Our analysis revealed, overall cardio metabolic risk observed from different ethnic groups varied within a narrow range, where both the ethnic groups showed marginally lesser risk than their corresponding non ethnic counter parts. The risk in premenopausal groups did not show much difference, while in post menopausal groups there was marked difference in prominence of risk factors between the groups. Analysis of cardio metabolic risk indicators in different groups of subjects revealed that central obesity marked by increased waist circumference was most prevalent risk factor in all the groups. In Riang women from Tripura altered lipid profile marked by increased triglyceride level and reduced HDL-C level was more prominent than other groups. This might be connected to non-vegetarian nature of their diet which includes substantial quantity of red meat and other non-vegetarian products. Other risk factors showed similar pattern in all the groups. The cardio metabolic risk in urban subjects was found to be more in comparison to rural subjects, which might be associated with sedentary life style of most of the urban subjects evaluated. Subjects having higher educational level were found to have lesser risk of cardio metabolic disorders. The cardio metabolic risk was found to be highest in subjects from higher family income groups and lowest in subjects with lesser family income. Non-vegetarian subjects were having higher cardio metabolic risk than vegetarian subjects.

Similar findings in regard of influence of socio demographic status on cardio metabolic risk in women were reported from South Korean, Japanese and American studies[35-37]. Both higher income and educational levels were reported to be protective against different types of chronic diseases. In our study, we observed lesser percentage of subjects with cardio metabolic risk in higher educational but lesser family income groups. This might be explained by the fact that higher educational level increases health awareness, thereby helping in adopting life style modification to prevent cardio metabolic risk. The higher percentage of high income group may be explained by the fact that this group consumes more amount of high energy diet which predisposes such subjects to cardio metabolic risk. The same is true for non-vegetarian group also. Similar findings in regard to influence of family income, educational level and diet were reported by several other workers also[38,39].

Results of our study revealed that a good proportion of our subjects with predominant cardio metabolic risk also had lesser hemoglobin concentration. The co-existence of anaemia

and cardio metabolic risk being higher in postmenopausal subjects than premenopausal subjects. Similar observation of higher co-existence of anaemia and cardio metabolic risk in elderly women was reported by various workers[40]. The high rate of co-existence of anaemia and cardio metabolic risk in women might be explained by the fact that the prevalence rate of anaemia and altered lipid profile is consistently higher in women[41,42]. There were several studies that had reported positive correlation between anaemia and diabetes mellitus. However, reports on co-existence of anaemia and cardio metabolic risk were very few[43-45]. Shi et. al. had reported about co-existence of anaemia and cardio metabolic risk in adults for Jiangsu, China and they had suggested that the co-existence might be connected to inflammatory mechanism activated in both the conditions.

We have analysed oxidative stress and antioxidant status among subjects with three or more cardio metabolic risk factors and compared the data with the findings in control subjects without cardio metabolic risk. Our study revealed that lipid peroxidation product malondialdehyde (MAD) levels were higher in subjects with cardio metabolic risk in comparison to control subjects. Increase in MDA could be due to increased generation of reactive oxygen species due to oxidative damage generated in subjects with cardio metabolic risk. Similar study by Romero et.al. [46] had also reported a significant increase of MDA levels in subjects with cardio metabolic risk. In our study, we also observed a significant decrease in all the antioxidant enzyme levels in subjects with prominent cardio metabolic risk. Studies have shown that many cardio metabolic risk factors including hypertension, hypercholesterolemia, diabetes mellitus and decreased estrogen in postmenopausal women were associated with elevation of oxidative stress[47]. Oxidative stress phenomena occur, in particular, during the progressive steps of cardiovascular disorders[48]. Data from study conducted in patients with neurodegenerative disease showed that female patients presented with higher levels of oxidative stress compared to affected males, suggesting a higher susceptibility to oxidative stress in such female subjects[49.]However, there are very few reported studies on cardio metabolic risk profile of women that also included markers of oxidative stress[50.]

Our findings are in agreement with the findings of Shrestha et. al., who observed increase in oxidative markers in subjects with cardio metabolic risk from both the sexes[51,52]. Few earlier studies and a recent study by Vasselleet. al. had reported increase in oxidative stress biomarkers in women associated with cardiovascular events [53-55.]The levels of antioxidant vitamins E and C, in our study did not show significant difference between the groups. This might be due to the fact that our subjects were apparently healthy and were

taking adequate diet that helped them in maintaining the normal levels of these antioxidant vitamins[56]. A slight decrease in the levels of these vitamins in subjects with cardio metabolic risk may be explained by the fact that they were being utilized for preventing oxidative damage initiated in this group of subjects. However, the exact role played by the antioxidant vitamins in cardio metabolic risk still remains controversial[57,58].

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CHAPTER 6 :

SUMMARY AND CONCLUSION

6.1. SUMMARY & CONCLUSION :

The overall percentage of subjects with presence of three or more cardio metabolic risk factors higher than the recommended cut off limits among Indian women of 25-65 years of age was found to be 35.52%.

The cardio metabolic risk of the subjects in our study increased with increase in age.

Though the percentage of subjects with cardio metabolic risk was more in postmenopausal women (49.24%) , a substantial number of premenopausal (26.6%) subjects in our study were found to have three or more cardio metabolic risk factors higher than the recommended cut off limits.

The most common cardio metabolic risk factor identified in both premenopausal and postmenopausal women in our study was central obesity.

The second most important component of cardio metabolic risk factor identified in our study was reduced HDL-C and elevated triglyceride.

Both hypertension and hyperglycemia were found to be less common cardio metabolic risk factor in our subjects.

The most important obesity and atherogenic predictors of traditional cardio metabolic risk factors identified in both premenopausal and postmenopausal Indian women were waist circumference, waist to hip ratio and TG /HDL-C ratio.

There was high co-existence of combined anaemia and cardio metabolic risk in women from all age groups and ethnic backgrounds.

No significant difference in occurrence of cardio metabolic risk factor was observed among women from different ethnic groups.

However, there was a significant difference in occurrence of cardio metabolic risk between women from urban and rural locality.

The risk was high in women belonging to higher income group.

The risk was lesser among women with higher educational background.

There was an increase in oxidative stress parameter and decreased antioxidant defense mechanisms in subjects with profound cardio metabolic risk in women.

In conclusion, cardio metabolic risk in women is influenced by menopausal status, age, central obesity and altered lipid profile. There is high co-existence of anaemia and cardio metabolic risk in women. Ethnicity did not play important role in occurrence of cardio metabolic risk in women. There is a urban and rural difference and level of education and family income played an important role in determination of cardio metabolic risk. Finally, results of our studies on oxidative stress parameters supported the oxidative stress hypothesis in pathogenesis of cardio metabolic risk in subjects.

6.2. LIMITATIONS OF THE STUDY:

One of the limitations of our study is that, it is mainly a hospital based observational study which might have excluded certain section of the population from being included as subjects and the study did not evaluate prevalence of cardio metabolic risk separately in different groups of population included in the study as subjects.

The main reason for conducting hospital based study was feasibility to handle the samples with necessary care and performing biochemical assays and anthropometric measurements in a controlled set up.

Being an external Ph.D. scholar working with subjects from two locations, the scholar worked under controlled set ups of Sri B.M Patil Medical college and Tripura Medical College.

Since this study was carried out on women accompanying patients attending the OPDs of tertiary care medical hospitals in both the locations, the prevalence observed in our study may not be generalized for the entire population of both the study groups.

6.3. FUTURE PROSPECT OF THE STUDY :

The study had evaluated various combinations of cardio metabolic risk factors in a representative sample of Indian women that provides information regarding prevalence of cardio metabolic risk in regard to their menopausal status. The study also evaluated the relationship between various anthropometric and atherogenicity predictors of cardio metabolic risk , their relationship with hemoglobin concentration, oxidative stress parameters and antioxidant status of the subjects. There is a huge scope for further explaining the observed findings by conducting population based studies.

ANNEXURES

ANNEXURE - I

RESEARCH CONSENT FORM

TITLE OF THE RESEARCH PROJECT : STUDIES ON PREVALENCE OF CARDIO METABOLIC RISK FACTORS AND ANAEMIA IN RELATION TO ANTIOXIDANT STATUS OF POSTMENOPAUSAL WOMEN FROM DIFFERENT ETHNIC COMMUNITIES.

NAME OF THE RESEARCH SCHOLAR : MRS. SOMA CHAKRABORTI, M.Sc.
NAME OF THE GIUDE : DR. MANJUNATHA R. AITHALA, M.D.
NAME OF THE CO-GUIDE : DR. SANKAR ROY, M.D.

01. PURPOSE OF RESEARCH :

I have been informed that this study will evaluate the cardio metabolic risk factors present in women comprising of subjects from two different ethnic and non- ethnic communities of India and also evaluate the relationship among cardio metabolic risk profile of the subjects with their menopausal status , anaemia status and status of their antioxidant defence system. This will help to take measures to prevent future development of cardio metabolic disorders and also will be helpful to understand some of the causative factors behind development of such risk in subjects.

02. PROCEDURE :

I understand that the procedure of the study will involve recording of various anthropometric parameters, blood pressure, complete lipid profile, fasting blood glucose level, complete haematological parameters and ECG at rest. The procedure will require withdrawal of 10 ml venous blood from me and other non-invasive investigations. I understand none of the procedures will interfere with any of my physiological parameters.

03. RISK AND DISCOMFORTS :

I understand that the procedures involved in the study will not cause any discomfort to me and they do not involve any risk to my health.

04. BENEFITS :

I understand that my participation in the study may not have a direct benefit to me, but this may be a potential beneficial effect in the field of cardio metabolic risk in women.

05. CONFIDENTIALITY :

I understand that medical information produced by this study will become part of institutional record and will be subject to the confidentiality and privacy regulation of the said institute. Information of a sensitive personal nature will not be part of medical record, but will be stored in investigator's research file and identified only by a code number. The code key connecting name to numbers will be kept in a separate secured location.

If data are used for publication in the medical literature and for teaching purpose, no names will be used, and other identities such as photographs and audio or video tapes will be used only with my special written permission. I understand that I may see the photographs and video tapes and hear audio tapes before giving the permission.

06. REQUEST FOR MORE INFORMATION :

I understand that I may ask more questions about the study at any time. Concerned researcher is available to answer my question or concerns. I understand that I

shall be informed of any significant new findings discovered during the course of this study which might influence my continued participation.

07. REFUSAL OR WITHDRAWAL OF PARTCIPATION :

I understand that my participation is voluntary and I may refuse to participate or may withdraw my consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital.

I also understand that researcher may terminate my participation in this study at anytime after she has explained the reason for doing so and has helped to arrange for my continued care by my own physician.

08. INJURY STATEMENT :

I understand that in the unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided.

I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to …… the purpose of the research, the procedures required, and the possible risk and benefits to the best of my ability.

Signature of the Research Scholar Date

I confirm that

…… has explained to me the purpose of the research, the study procedure that I will undergo, possible risks and discomforts as well as benefits that I may experience. Alternatives to my participation in the study have also been discussed. I have read and understood this consent form. Therefore, I agree to give my consent to participate as a subject in this research project.

Signature of the participant Date

Signature of Witness Date

Modified from :Protney L.G., Watkins M.P. In. : Foundations of Clinical Research, second edn. Prentice Hall Health. New Jersey. 2000. (APPENDIX-E).

ANNEXURE - II

PROFORMA FOR RECORDING GENERAL AND SOCIODEMOGRAPHIC INFORMATION

SUBJECT IDENTIFICATION NUMBER :
NAME :
FATHER'S/ HUSBAND'S NAME :
COMPLETE RESIDENTIAL ADDRESS :
AREA OF RESIDENCE (URBAN/ RURAL) :
DATE OF BIRTH/ AGE :
RELIGION/CASTE :
ETHNICITY (SPECIFY) :
OCCUPATION (SPECIFY) :
LEVEL OF EDUCATION (Unknown/ 8th Standard/ 12th Standard/ Above) :
MONTHLY FAMILY INCOME (Rs. <10,000/, Rs. <20,000/, Rs. <30,000/, Rs. >30,000) :
PHYSICAL ACTIVITY (Sedentary / Mild/ Moderate/ Heavy) :
DIET (Veg/ Non-veg) :
HABITS (Alcohol/ Smoking/ Any other) :
MARITAL STATUS (Married/ Unmarried/ Widow/ Single) :
NUMBER OF CHILDREN :
HISTORY OF PREGNANCY (Miscarriage/ Normal/ Cesarean) :
HISTORY OF CONTRACEPTIVE USE :
HISTORY OF PAST ILLNESS :
HISTORY OF ANY ENDOCRINE DISORDER :
ANY SIGNIFICANT CHRONIC ILLNESS (DIABETES/ HYPERTENSION/ DYSLIPIDEMIA/ ANY SURGERY) :
HISTORY OF PRESENT DRUG USE:
MENSTRUAL STATUS / DATE OF LAST PERIOD :

ANNEXURE - III

PROFORMA FOR RECORDING OBSERVATIONS :

SUBJECT IDENTIFICATION NUMBER :

ANTHROPOMETRIC PARAMETERS :

01. Height (cm) :
02. Weight (kg) :
03. Waist circumference (cm) :
04. Hip circumference (cm) :
05. Thigh circumference (cm) :
06. BMI (kg/m^2):
07. WHR (Waist-Hip Ratio) :
08. WHtR (Waist-Height Ratio) :
09. WTR(Waist- Thigh Ratio) :
10 HTR (Height-Thigh Ratio) :

HAEMATOLOGICAL PARAMETERS :

01. Total RBC count (millions/mm^3)

02. Total WBC count (thousands/mm^3)
03.Platelate count (lakhs/mm^3)

04.Neutrophil(%)
05.Eosinophil(%)
06. Basophil(%)
07. Lymphocytes(%)
08. Monocytes(%)
09. Haemoglobin content (gm%)

PHYSIOLOGICAL PARAMETERS :

01. Heart Rate (beats/min) :
02 Blood Pressure (mmHg) :

BIOCHEMICAL PARAMETERS :

01. Fasting Blood Sugar (mg/dl) :
02. Triglyceride (mg/dl) :
03. Total cholesterol (mg/dl) :
04. HDL-C (mg/dl) :
04. LDL-C (mg/dl) :
06. VLDL-C (mg/dl) :
07. HDL-C/TC ratio :
08. TG/HDL-C ratio :
09. HDL-C/LDL-C ratio :
10. HDL-C/ VLDL-C ratio :

ANTIOXIDANTS :

01. Plasma Malondialdehyde (MDA) :
02. Erythrocyte Catalase (CAT) :
03. Erythrocyte Superoxide dismutase (SOD) :
04. Erythrocyte Glutathione peroxidase (GPx) :
06. Blood Glutathione (GSH) :
07. Plasma Vitamin C (VIT-C) :
08 Serum Vitamin E (VIT-E) :