Chapter 1

Introduction

For many decades, the term 'women's health' have been constrained to maternal and reproductive health in developing countries and many other dimensions of women's health have been overlooked. As a result of urbanization and nutrition transition, the burden of non-communicable diseases (NCDs) has shown a steep rise in both the genders. But the delusion of cardio vascular diseases (CVDs) being only men's health problem has led to underestimation of risk of these diseases for women. By the year 2030 the number of annual deaths caused by CVD in women is estimated to rise from 17.1 million to 23.6 million (NCD Alliance, 2011). Out of these women 80 percent may possibly be from low and middle income countries. The prevalence of breast and lung cancer is highest among all the cancers in women and around half of the cancer cases occurring among women are from developing countries (NCD Alliance, 2011). According to World Health Organization (WHO), NCDs are likely to account for 37% of the deaths in India. The estimated prevalence of four major NCD risk factors i.e. tobacco intake (18%), alcohol intake (4.3%), raised blood pressure (17%) and overweight/obesity (22.1%) is high among Indian women and is expected to rise in the next decade (WHO, 2014).

MENOPAUSE AND RISK OF NON- COMMUNICABLE DISEASES

As per definition by WHO (1996), the term menopause refers to 'the permanent cessation of menstruation and fertility resulting from the loss of ovarian follicular activity'. In this condition ovaries reduce their production of the female sex hormone-oestrogen, which is known to have protective effect against various chronic illnesses. Menopause can affect the body weight, composition and abdominal fat deposition and if obesity is prevalent among pre-menopausal women it can also alter the age of natural menopause *vice versa* (Davis et al, 2012). Obesity is characteristically more prevalent in females and reason behind is stated as fluctuations of sex hormones at different stages

of life, menopause being the predominant one. Due to the menopausal transition the gynoid type fat distribution among the females starts shifting to android type distribution. Obesity can also have an adverse effect on menopausal symptoms (Davis et al, 2012).

The Oestrogen hormone protects blood vessels by dilating the endothelium. The major effects of the decline in oestrogen levels on blood pressure are: relative increase in androgen levels, activation of renin-angiotensin system leading to high renin levels, increase in plasma endothelin levels, higher salt sensitivity, increase in insulin resistance, higher sympathetic activity and increase in weight (Mass and Franke, 2009).The two major hindering factors to reduce the incidence of cardiac events among females are inadequate recognition and poor control of hypertension (Coylewright et al, 2008).The risk of having heart diseases among females is 10 times higher than having breast cancer (WHO-NMH, 2002). Hormonal replacement therapy (HRT) has been used to prevent chronic diseases in postmenopausal women for many years but recent studies show controversial results of the impact of HRT, as it has more harms than benefits (Mass and Franke, 2009). As the prevalence of mortality due to NCDs and its risk factors is very high, developing countries like India need to focus on early prevention care as till now treatment regimen is followed only when disease has reached to its advanced stage. There is a need to educate people about the gender specific determinants of chronic diseases (WHO-NMH, 2002). The NCD alliance (2011) had recommended carrying out more women specific clinical research in the area of chronic diseases as majority of the studies till date have a focus on men.

VITAMIN B_{12} AND FOLIC ACID DEFICIENCY: EMERGING LINK WITH CARDIO VASCULAR DISEASES

Folate and vitamin B12 deficiency are the major factor responsible for increase in homocysteine levels which is a significant risk factor for cardiovascular disease (Klee, 2000). Homocystine produced during metabolism of methionine requires vitamin B12 and folate for remethylation into methionine. The Framingham study has indicated

folate, vitamin B_6 and vitamin B12 as key elements of plasma homocysteine concentration in the healthy population (Lindenbaum et al, 1994).

The mechanism proposed to link Homocystine to vascular damage, stroke and cardio vascular diseases include impairment of endothelial functions, endothelial desquamation, oxidation of LDL particles, increased monocyte adhesion to the vessel wall, impaired vascular response to nitric oxide and thrombotic tendency mediated by activation of coagulation factors and platelet dysfunction (Zhang et al, 2014; Murthy, 2005). A retrospective study (N=1743) performed at Germany evidenced significant association of high folate levels with favourable lipid profile irrespective of age and gender (Semmler, 2010).

The high prevalence of vitamin B12 and folate deficiency is seen worldwide without geographical differences indicating it as public health problem. As the deficiency of these vitamins have similar clinical and hematological features, at many times they cannot be differentiated easily from one another (Chandra, 2006). Population groups at risk for developing B12 deficiency are: Strict vegetarian, lactovegetarians, pregnant and lactating women, infants and young children with low B12 intake and elderly. According to Chandra (2006) "vitamin B12 deficiency has taken over as more common micronutrient deficiency as compared to folate and the shift has still not been explained". The effects of sub-clinical deficiency, absorption, bioavailability and metabolism of vitamin B12 are yet to be established (O'Leary and Samman, 2010).

Comprising a high percentage of vegetarian population, Vadodara's women are at high risk of developing B12 deficiency. A lot of efforts have been laid in to recognize the relation of homocystine as a risk factor for CVDs but there is dearth of data to assess the direct impact of underlying causes of high homosystine levels i.e. deficiency of B12 and folate. Homocystine is a good indicator of vitamin B12 and folate deficiency but assessing homocystine levels alone does not provide insight of effect of these deficiencies individually on development of NCDs. As, the emerging effect of B12 and

folate deficiency on cardiac health and the major factors lying behind these deficiencies have been understudied in India, these area need to be further explored.

INFLAMMATION AND NCD RISK AMONG FEMALES

Inflammation plays a major factor for development of atherosclerosis and Hs-CRP had been recognized as a novel indicator to assess inflammation in humans (Ridker, 2001). Addition of hs-CRP to the definition of the metabolic syndrome can improve the prediction of CVD. Elevated hs-CRP levels may also be predictive of development of the metabolic syndrome (Haffner, 2006). According to a study performed on 1035 subjects in Taiwan, sex difference exist between the association of metabolic syndrome and inflammation and concluded that inflammatory processes may be of particular importance in the pathogenesis of metabolic syndrome in women (Lai et al, 2010). Cook et al (2006) used Women Health study data to study the association of HsCRP and CVD risk among women. They added HsCRP into the Framingham prediction model and found that addition of HsCRP gave better prediction of actual CVD risk among women. In a study on south Indian females, HsCRP has been found to be early marker to predict the obesity related co-morbidities among obese females (Dev and Marcus, 2012). Therefore the HsCRP is a risk factor for CVDs with particular importance in females and should be studied extensively during various women centric studies.

INSULIN RESISTANCE AND NCD RISK AMONG FEMALES

According to International Diabetic Federation (2017) the prevalence of diabetes in India is around 8.8%. Around half of the people with diabetes in South East Asia are undiagnosed. The Asian Indian Phenotype characteristic (Joshi, 2003) and life style transition in the last few decades collectively poses higher risk for diabetes and other non-communicable diseases among Indian population. Cardio vascular disease is a major secondary complication of diabetes and presence of CVD risk factors can also lead to insulin resistance in early stages of life. According to Carey et al (1996) abdominal obesity can be a major factor in development of insulin resistance in obese as well as normal women. Insulin resistance has been closely interlinked with hyperlipidemia and

atherogenesis as it can increase the VLDL synthesis and also contributes to the elevated plasma triglyceride levels (Ginsberg, 2000). In the recent years the concept of inflammation induced insulin resistance is coming into focus and various mechanism have been proposed for such relationship (Shoelson et al, 2006). Screening of insulin resistance among female population can provide an insight into early irregularities in glucose homeostasis.

PREVENTION OF EARLY METABOLIC AND INFLAMMATORY ABERRATIONS

Various life style and dietary modification can be adopted to prevent the metabolic aberration and delay the onset of various non-communicable diseases. One such dietary modification approach is use of functional foods. There are various functional foods which have shown to exert beneficial effects in both prevention and control of metabolic derangements; like amla, wheat grass, flaxseeds, soyabean, oats, green tea, garlic etc.

FLAXSEED (*LINUM USITATISSIMUM*)

Flaxseed, also known as linseed is a functional food. It is a member of the genus Linum in the family Linaceae. Flaxseed contains around 19% of proteins, 36% of fats and 26% of dietary fiber (NIN, 2017). Flaxseed has a unique fatty acid profile, being fairly low in saturated fatty acid and rich in α-linolenic acid (ALA), the essential omega-3 fatty acid. ALA gets converted to DHA in vivo which is beneficial for heart health. In animal models, dietary enhancement of DHA was shown to promote neuronal membrane excitability, increase neurotransmitter levels, and reduce neuronal damage (Lim et al, 2009). Lignan is another major functional component of flaxseeds. Flaxseed lignans can exert beneficial impact on cardiovascular diseases and diabetes (Fukumitsu et al, 2010; Fukumitsu et al, 2008; Prasad, 2009).

A recent randomized control trial on effect of flaxseed supplementation on components of metabolic syndrome was performed by Yari et al (2016). Forty four subjects diagnosed with metabolic syndrome were supplemented with either a combination of

30g brown milled flaxseed and life style counselling or provided with life style counselling only (control). The results revealed that after 11 weeks supplementation the prevalence of metabolic syndrome decreased by 50% and 82% in the control and intervention group, respectively. Reduction in central obesity prevalence ($p<0.01$), insulin resistance ($p<0.001$), body weight and BMI ($p<0.05$) was significantly higher in flaxseed group. However the blood pressure levels were not significantly affected. Torkan et al (2015) studied the impact of 30g raw flaxseed powder on lipid profile of hyperlipidemic subjects and observed that after 40 days intervention, weight, BMI, TC, LDL-C and TG levels significantly reduced in the intervention group. In a study conducted on the effects of flaxseed- derived lignan supplement on glycemic control, lipid profile and insulin sensitivity in 73 NIDDM subjects, in which subjects were given 360mg lignan capsule every day for 12 weeks concluded that daily lignan supplementation resulted in modest, yet statistically significant improvements in glycemic control in NIDDM subjects without apparently affecting fasting blood glucose, lipid profile and insulin sensitivity (Pan et al, 2007). Six months supplementation of 30g flaxseeds significantly reduced different plasma oxylipin levels in supplementation group during a randomized, double-blinded, controlled clinical trial. These oxylipins were products of soluble epoxide hydrolase, which is a pharmacological target for antihypertensive treatment (Caligiuri et al, 2014). A departmental study on impact of flaxseed (20g) incorporated ladoos on cognitive impairment in institutionalized elderly of urban Vadodara did not show any detrimental effect of flaxseed supplementation on the health of participants (Chauhan and Kansara, 2012).

Literature review reveals the beneficial effects of flaxseed (*Linum. usitatissimum*) on the lipid profile, glycemic response and blood pressure, with supplementation of about 30g of flaxseed. 30g of flaxseed provides 6g ALA, whereas the recommendation for ALA is 1.1 g/d for women and 1.6g/day for men (US IOM, 2002). According to the American Heart Association (Kris-Etherton et al, 2002) 1.5-3g/d of ALA intake can have a beneficial effect on cardiac health. Therefore the doses of flaxseeds used for supplementation in various studies are quite high. Moreover, majority of the studies have been conducted

outside India. Very few studies are available on the effect of flaxseed supplementation in the Indian population. Flaxseeds are a cheap source of ALA in comparison to other supplements available in the market and small doses can be easily consumed in roasted form as "Mukhvas" (mouth freshner), commonly consumed in Gujarati society. Regular intake of moderate amount of flaxseed can exert beneficial effect even in apparently health but at risk population for developing NCDs. Most of the studies reported in literature are conducted on post-menopausal females with negligence towards pre-menopausal group. The increased prevalence of NCDs during early stages of life makes it indispensable to focus on preventive strategies for such population groups and use of functional foods like flaxseed is one of the rational approaches for the same. Based on the above introduction, various research questions aroused which are as follows:

- What are the dietary and life style habits of women residing in Urban Vadodara?
- What is the impact of menopause on lipemic, glycemic, biophysical, inflammatory and other physiological aberrations among women?
- Does age play any significant role in association between menopause and cardio vascular risk factors?
- What is the prevalence of vitamin B12 and folate deficiency among women?
- What are major causal factors of vitamin B12 and folate deficiency among women?
- What is the relationship of vitamin B12 and folate deficiency with cardio vascular risk factors?
- What are the predictor variables of inflammation and insulin resistance among women?
- What is the nutrient content of roasted flaxseeds?
- How effective would the low doses of flaxseeds be in improving the metabolic and inflammatory profile of the apparently healthy overweight/obese pre-menopausal women?

Subsequently, to address the above questions a three phase study was planned with the following objectives:

Objectives

1. To study the life style, behavioural and dietary differences, if any, among pre and post-menopausal women
2. To compare the physiological and metabolic aberrations in pre and post-menopausal females in terms of:
    - Body Composition
    - Blood Pressure
    - CHO metabolism
    - Lipid metabolism
    - Inflammatory markers
    - Nutritional anemia
    - Thyroid functions
    - Liver and kidney functions
3. To study the effect of age on association between menopause and metabolic aberrations
4. To explore the relationship of Vitamin B12 and Folic acid deficiency with risk factors of non-communicable diseases among women
5. To investigate the predictor variables of inflammation and insulin resistance among females
6. To assess the nutrient composition, fatty acid profile and antioxidant capacity of flaxseeds
7. To study the efficacy of two different doses of whole roasted flaxseeds on lipid profile and inflammatory markers of pre-menopausal overweight or obese female subjects