Abstract

Menopause is emerging as a distinctive risk factor for non-communicable diseases in women. Estrogen hormone plays as a protective element against various diseases and its deficiency can negative impact the body composition and metabolic regulations in post-menopausal women. Still there is an ongoing debate whether menopause can independently impact the development of non-communicable disease irrespective of age factor. Concurrently there are other upcoming metabolic risk factors i.e. vitamin B12 and folic acid deficiency, inflammation and insulin resistance which needs to be explored to outline their prevalence and association with other risk factors of non-communicable diseases in women population. Vitamin B12 and folic acid deficiency can lead to high homocysteine levels, which is an independent risk factor for NCDs. As vitamin B12 is mostly present in non-vegetarian foods, predominantly vegetarian population of Guajarati women have high risk of developing B12 deficiency. HsCRP which is a novel marker of inflammation is emerging as a strong predictor of cardiovascular diseases among women. Similarly detection of insulin resistance at the early stages of aberrations in glucose metabolism can lead to prevention of morbidities associated with it. Therefore it is necessary to study prevalence of women specific risk factors of NCDs among pre and post-menopausal women and explore their inter-relationship. For prevention of various metabolic and inflammatory anomalies, diet and lifestyle modifications needs to be focussed from the early stages of women's life cycle. Flaxseed is an important functional food rich in α linolenic acid, lignans, different polyphenols and fiber. Due to the presence of these bioactive compounds, different forms of flaxseeds like whole flaxseeds, ground flaxseeds, flax oil, flaxseed incorporated recipes have been studied in managing metabolic aberrations by various researchers. However, the use of high doses/amount of flaxseeds in different studies makes it difficult to adapt its intake in the regular diet. Hence there is a need to explore the effect of low doses of flaxseeds, which can be feasibly incorporated in the regular diet

without any added effort. 'Mukhwas' is an integral part of Gujarati diet and whole roasted flaxseeds can be used in the form of 'Mukhwas' for this purpose.

Thus the present study was planned with the following major objectives:

1. To compare the life style, behavioural, dietary differences, if any, along with physiological and metabolic aberrations in pre and post-menopausal women
2. To explore the relationship of Vitamin B12, Folic acid deficiency, inflammation and insulin resistance with risk factors of non-communicable diseases among women
3. To assess the nutrient composition of flaxseeds and 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

The study was divided into three phases. First phase consisted of a cross sectional study in which detailed non-invasive risk analysis for NCDs was performed on 131 women of urban Vadodara which included data regarding medical history, life style pattern, dietary habits, physical activity, food frequency, 24 hour dietary recall, anthropometric measurements, blood pressure and body fat percent. Further biochemical estimations were performed on 90 women in terms of lipid and glycemic profile, nutritional anemia, HsCRP, thyroid, liver and kidney function tests.

The results of first phase revealed negligible number of women consumed tobacco (0.8%) or alcohol (0%). 70% of the subjects were moderately physically active. Mean consumption of oil (45.2±18.6g), sugar (32.5±17.9g) and salt (9.8±4.5g) was higher than the recommendations with post-menopausal women consuming significantly higher amounts than the pre-menopausal ones. Mean energy (1504±415 Kcal) and protein intake (42.8g) was below whereas fat intake was higher (34% of total energy intake) than the recommended intake. No significant difference between nutrient intake of pre and post-menopausal women was observed. The prevalence of overweight/obesity (74.8%) and hypertension (42%) was high among the subjects. The prevalence of high TC, high LDL-C levels, low HDL-C levels and hypertriglyceridemia was 45.6%, 72.2%, 28.9% and 12.2% respectively. Around 12% of the subjects were found to be diabetic. In univariate analyses the major variables associated with menopause were high body fat

percent, hypertension, diabetes, high HbA1C levels and high alkaline phosphatase levels. Through logistic regression analysis menopause was found to be independently associated with body fat percent irrespective of age and obesity status of women. The prevalence of vitamin B12 deficiency was quite high (71%) in the women, with a low prevalence of folic acid deficiency (5.5%). Two key causal factors identified through multivariate analysis for vitamin B12 deficiency were: Very low dietary intake of vitamin B12 (<0.3µg/day) and no regular health check-up. Vitamin B12 deficiency emerged as independent predictor for development of hypertension among women after adjustment for age and BMI using logistic regression. The prevalence of high Hs-CRP levels was around 64%. Metabolic Syndrome was found to be the key variable associated with Hs-CRP. About 21.5% of the non-diabetic women were suffering from insulin resistance. The major determinants of insulin resistance predicted through logistic regression model were low HDL-C, high BMI and high energy intake in women.

To conclude, a trend of higher prevalence of anthropometric and metabolic aberrations was seen in both pre and post-menopausal women. Therefore a healthy life-style should be focused from the early stages of life with extra caution during and post menopause. Menopause can indirectly affect risk of cardio vascular disease through these fat distribution changes. However, still larger longitudinal studies are required to establish menopause as a primary risk factor for non-communicable diseases, as various other factors like age, decreased physical activity etc. can act as confounders. The prevalence of vitamin B12 deficiency was found quite high in the present study and was found to be strongly associated with hypertension. The area needs to be further explored considering the very high prevalence of Vitamin B12 deficiency in the population. HsCRP can be used as suggestive tool while assessing metabolic syndrome as well as diabetes and CVD risk in women.

In phase two, PKV-NL 260 variety of the flaxseed was selected to use for the supplementation. Carbohydrate, fats, proteins, crude fiber, sodium, potassium, calcium, iron, fatty acid profile, total phenols, flavonoids and antioxidant capacity of the flaxseeds (PKV-NL 260) were determined using standard methods. The fatty acid profile showed the highest

percent (78.04%) of n-3 fatty acid in the PKV NL-260 variety of flaxseeds. Flaxseeds contained 365.8±18.2 mgGAE/100g of total polyphenols and 148.6±4.0 mgRE/100g of flavonoids. The antioxidant capacity of the flaxseeds using DPPH RSA and FRAP methods was 1776.6±80.1 mmolTE/100g and 643.7±2.6 mmolTE/100g respectively.

Phase three of the study was an open labelled randomized control trial. It included an initial screening of the women using inclusion and exclusion criteria. Pre-menopausal overweight/obese female subjects (20-50y) were selected (N=151) for the study and the subjects with history of chronic illnesses, allergy to flaxseeds diabetes, smoking or tobacco chewing, pregnancy, vigorous physical activity, peri and post menopause, hysterectomy, currently (for last 4 weeks) taking n-3 rich foods or supplements, rapid or dramatic weight gain or loss in last year were excluded. 90 subjects out of those who consented to participate were randomly selected and divided into three groups. Biochemical estimations, anthropometric and biophysical assessment were performed pre and post supplementation. Biochemical estimation included lipid profile, fasting blood glucose, insulin, HsCRP, haematological profile, liver and kidney function tests. Experimental group I was asked to consume 5g of roasted flaxseeds (in from of one dose) and group II was asked to consume 10g of roasted flaxseeds (in form of two doses) for a period of 8 weeks. The control group was advised not to consume flaxseeds during the study period. 5g of flaxseeds contained 1.17g of ALA whereas 10g of flaxseed contained 2.34g of ALA.

The results of phase three indicated that no significant difference in the mean values of lipid profile was observed in the two supplementation groups (5g and 10g) post supplementation. In the control group a significant rise of TC ($p<0.001$), LDL-C ($p<0.001$), TC/HDL-C ratio ($p<0.001$) and LDL-C/HDL-C ratio ($p<0.001$) was found. Both 5g and 10g flaxseed supplementation brought better impact on the lipid profile of the subjects with initially high levels of LDL-C or AIP, though not statistically significant. HOMA IR and insulin values indicated a non-significant decrease after supplementations of both 5g and 10g flaxseeds. % insulin sensitivity fairly increased after 5g flaxseed supplementation. Insulin, HOAM IR and % insulin sensitivity improved greater in

subjects having initially high HOMA IR levels (>1.2) post 5g flaxseed supplementation. Insulin ($p<0.05$) and HOMA IR ($p<0.05$) levels significantly decreased and % insulin sensitivity ($p<0.05$) significantly increased in subject with high HOMA IR levels (>1.2) post 10g flaxseed supplementation. Mean body fat percent ($p<0.01$) and SBP ($p<0.01$) levels significantly reduced in 5g flaxseed group post supplementation. However 10g flaxseed supplementation showed only a non-significant decrease in these parameters. Prevalence of pre/stage I hypertension significantly reduced in both 5g ($p<0.05$) and 10g ($p<0.05$) supplementation group.

Thus, flaxseeds exerted beneficial impact on blood pressure, insulin resistance (in subjects with HOMA IR >1.2) and body fat percent (5g flaxseed group), maintained lipid profile, anthropometric indices and failed to alter inflammation in the healthy overweight/obese subjects. Therefore hassle free strategies like inclusion of 5-10g of roasted flaxseeds in the form of “mukhwas” in the daily diet can be adapted to reduce the risk of metabolic aberrations in population.