



A novel non-hormonal therapeutic study to alleviate perimenopausal symptoms in women

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ABSTRACT

Perimenopause is associated with unpleasant symptoms which affect quality of life. As hormone replacement therapy (HRT) is associated with certain adverse events including life threatening effects there is a need for alternate forms of treatment for relieving menopausal symptoms. As a pilot project we prospectively studied 200 perimenopausal women aged between 40 to 55 years at the Government Maternity Hospital, Tirupati in South India. They were allocated to receive the following interventions: HRT; omega-3- fatty acids and vitamin E; isoflavones; diet and exercise; and placebo. The effect of these therapies in relieving menopausal symptoms was analyzed. Women who received omega-3- fatty acids, isoflavones, diet and exercise and HRT had beneficial effect on all groups of menopausal symptoms and (placebo had beneficial effect) on urogenital and somatic symptoms. Weight was influenced by HRT and calcium levels by omega-3-fatty acids. No significant change was noted in serum levels of hormones with HRT for six months. Reassurance and diet and exercise can be advised initially to prevent or treat perimenopausal symptoms. Omega-3-fatty acids and isoflavones appear to be potentially beneficial alternate therapies if there is no relief. HRT is to be reserved as a final short term therapy to relieve perimenopausal symptoms.

INTRODUCTION

Perimenopause is a phase of adjustment between the active and inactive ovarian functions and it covers the period of 2 to 3 years immediately prior to and 12 months after the last menstrual period (menopause) [1]. It is a period of physiological transition associated with temporary, unpleasant symptoms which affect quality of life. Where the endocrinological and biological changes result in physical, sexual and psychological adjustments [2]. The average age of menopause in India is reported to be 47 years [2]. The morbidity due to menopause ranges from perimenopausal symptoms to more serious problems, like osteoporosis and cardiovascular manifestations which in turn affect their physical and mental health.

Menopausal symptoms may be grouped into vasomotor, psychological, genito-urinary and somatic disturbances [3], where in loss of ovarian function may be the primary cause of these perturbations, the role of general ageing and socio-economic stresses peculiar to midlife cannot be underemphasized.

Although hormone replacement therapy (HRT) could alleviate majority of these symptoms in most of the women, undesirable side effects like (venous thromboembolism, gall bladder disease, irregular vaginal bleeding, carcinoma of breast and endometrium, etc were reported.[3] The exorbitant cost of HRT particularly for women in developing countries necessitated the research on non-hormone therapy (NHRT) to relieve menopause symptoms. While several NHRTs including vitamin E[4], isoflavones [5] and life style changes such as diet and exercise [6] were reported to alleviate menopause symptoms, the relative efficacy of these agents vis-à-vis HRT was not probed. The present study has investigated the efficacy of omega-3 fatty acids with vitamin E, isoflavones, diet and exercise relative to a placebo or standard HRT, in relieving different menopause symptoms in peri-menopause women.

Alternate therapies for treatment of menopausal symptoms are popular in USA where women can spend more than US 600 million dollars per year for purchasing them [7]. In India, no such published data are available, but, the average cost for HRT ranges between 100 to 1000 rupees (1 US dollar = rupees 50) per month.

Keeping in view the unwanted adverse drug reactions, exorbitant cost of HRT and other treatment regimens; and unknown effectiveness of some of the alternate therapies, the present study was conducted.

MATERIALS AND METHODS

Study design

A pilot clinical study with placebo and standard treatment (HRT) as control groups was conducted from March 2005 to September 2006 to evaluate the efficacy of alternate therapies on menopause symptoms. For this placebo controlled study, two hundred symptomatic women aged between 40-55 years who attended gynecology out-patient service at the Government Maternity Hospital, Tirupati in South India were screened and prospectively studied.

Study population

Tirupati is a famous pilgrim centre in Andhra Pradesh, India, with a large floating population. And people from surrounding four districts and from borders of adjacent states, come to Government maternity hospital for outpatient and inpatient care representing the population of South India.

Details about all the 200 women in perimenopausal age: the various demographic, socio economic details were recorded in a structured proforma by a thorough detailed gynecological history, dietary habits, daily physical activity, attitude towards menopause, common stress inducing factors in middle age, systematic physical and clinical examination, any treatment taken for relief of menopausal symptoms in 200 women.

Sampling method

A sample of 200 women manifesting perimenopausal symptoms with uterus intact were selected for follow-up with alternate non-hormonal therapy. None of them had any history of a medical or surgical illness and was taking treatment for any chronic illnesses. Written informed consent was obtained from all the two hundred subjects who participated in the clinical trial. The study was approved by the Institutional Ethical Committee. A detailed dietary history was recorded. Investigations such as a cervical pap smear examination, electro cardiography, trans-abdominal ultrasonography by real time 'B' mode ultra sound ['ESOATE' model 'MYLAB-50', Italy] using a 3.5 MHz curvilinear probe to obtain global view of the pelvis were done. Haemoglobin estimation, urine analysis, fasting blood glucose, blood urea, serum calcium, total cholesterol, triglycerides, high- and low-density lipoproteins were also estimated using semi auto-analyzer [BTS 320 Biosystems, Spain]. Bone mineral density studies were carried out by digital X-ray radiogrammetry (DXR) [Pronosco and Posure system V2 in 2000](8). The digital image was subjected to a combination of computerized radiogrammetric and textural analysis of the 2nd, 3rd and 4th metacarpal bones of a non-dominant hand. The average age of bone loss is 2% per year after menopause and rate of bone loss in metacarpals was 1.2 to 1.5 per year. The HRT group has lower annual decrease in BMD. The combination of the low precision error of the Pronosco X posure system V2 and the high yearly bone change at metacarpals results in a superior minimum monitoring time interval of 6 months to compare the bone mineral density of an individual with the young adult mean BMD(T Score) [9].

The serum concentrations of FSH, 17 β -oestradiol and serum progesterone were estimated by radio-immuno assay (RIA) kit (RADIM, Italy) following the procedure specified by the

manufacturer [10,11] only in the placebo and HRT groups both at the beginning and conclusion of the study.

Of the 200 patients who were subjected to detailed investigations, 8 patients were excluded from the study as they were (i) diagnosed to have medical (two patients with diabetes mellitus) or gynecological disorders (three patients with fibroid uterus, one with endometrial hyperplasia) (n=6) (ii) drug allergy or intolerance (n=2). Also excluded were 32 women who were unwilling to continue the suggested treatment intervention and those who were not willing for further follow up (n=17); women who were unwilling to change their dietary habits (n=7), and women who could not carry out the exercise fitness tests (n=8) were excluded from the study.

The remaining 160 women were assigned by open label to receive (i) oral placebo (60mg/day of starch) (n=30); (ii) oral omega-3 fatty acids as a capsule containing 90 mg eicosa pentanoic acid, 60 mg of docosa hexanoic acid and 100mg of alpha tocopherol (Cadvin; Merck India) once-daily (n=35); (iii) isoflavones 60 mg/d containing genistin 39 mg (65%); daidzin 18 mg (30%); and glycitin 1.8 mg (3%) (Isoflav; Raptakos, India) (n=35); (iv) diet (*vide infra*) and exercise (defined as half-hour of brisk walking (2-2.5 Kms) per day) (n=35); and (v) HRT administered as a tablets containing 0.625mg of conjugated equine estrogens (Wyeth) + 2.5 mg of medroxy progesterone acetate (Serum Institute), once-daily (n=25). To know the exercise tolerance, Canadian home fitness test (12) and 12-minute walking test [13] were done on subjects who follow the prescribed diet and exercise.

At the baseline, every woman was given a form with menopausal symptoms, graded according to Greene climacteric scale (Appendix 1) [14] and was asked to mark the presence or absence of the symptoms, graded as '0' (nil); '1' (mild); '2' moderate; and '3' (severe). Each subject was followed-up for 6 months on a monthly basis. The patients were asked to maintain a diary and note down whether they were following the intervention therapy regularly or not. After 6 months, all the subjects were again subjected to Greene climacteric scale [14] to indicate the perimenopausal symptoms and to know the effectiveness of the intervention method. The blood investigations, serum calcium, lipid profile and bone mineral density were repeated after 6 months.

Women in the diet and exercise group were advised to consume five meals (Appendix 2) between 8.00AM and 10.00PM to maintain blood glucose levels within a narrow range. These women were advised to obtain their calories mostly from cereals, vegetables, and fruits and avoid sweets and fats in diet and coffee, tea, alcohol and other similar stimulants. They were also asked to drink 2-3 liters of water a day. Further, women in this group were prescribed half an hour of brisk walking each day (2.0 - 2.5 Km). All the women were asked to maintain a record of compliance with the prescribed treatment. At the end of the study 22, 32, 34, 21 and 14 women reported perfect compliance in placebo, omega-3-fatty acids, isoflavones, diet and exercise and HRT groups respectively. Thus the data pertaining to 123 women in different treatment groups were analysed.

STATISTICAL ANALYSIS

The severity of menopausal symptoms was compared before and after the treatment by cross tabulation and analysis done by Wilcoxon-rank test. The 'Z' value and significance was also shown in the Table 1. A p-value less than 0.05 was considered as

Table 1: Effect of treatments on different groups of menopause symptoms in women

| Treatment | Severity of symptoms | Vasomotor symptoms | | Anxiety symptoms | | Depression | | Urogenital symptoms | | Somatic symptoms | |
|--|----------------------|-----------------------|-------|-----------------------|-------|-----------------------|-------|-----------------------|-------|-----------------------|-------|
| | | Before | After | Before | After | Before | After | Before | After | Before | After |
| Placebo (n=22) | Mild | 7 | 13 | 10 | 14 | 9 | 15 | 9 | 13 | 7 | 15 |
| | Moderate | 9 | 5 | 9 | 5 | 9 | 4 | 9 | 9 | 11 | 7 |
| | Severe | 6 | 4 | 3 | 3 | 4 | 03 | 4 | 0 | 4 | 0 |
| | Total | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| | Wilcoxon Test | Z = -1.814; P = 0.07 | | Z = -1.265; P = 0.206 | | Z = -1.645; P = 0.1 | | Z = -2.138; P = 0.033 | | Z = -2.652; P = 0.008 | |
| Omega-3 Fatty Acids with Vitamin E (n=32) | Mild | 6 | 25 | 9 | 28 | 7 | 27 | 8 | 19 | 8 | 25 |
| | Moderate | 17 | 7 | 9 | 0 | 15 | 3 | 14 | 11 | 10 | 3 |
| | Severe | 9 | 0 | 14 | 4 | 10 | 2 | 10 | 2 | 14 | 4 |
| | Total | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| | Wilcoxon Test | Z = -4.008; P = 0.001 | | Z = -3.540; P = 0.001 | | Z = -3.716; P = 0.001 | | Z = -2.906; P = 0.004 | | Z = -3.629; P = 0.001 | |
| Isoflavones (n=34) | Mild | 10 | 29 | 16 | 31 | 13 | 32 | 9 | 21 | 13 | 30 |
| | Moderate | 18 | 5 | 9 | 2 | 12 | 2 | 18 | 12 | 13 | 4 |
| | Severe | 6 | 0 | 9 | 1 | 9 | 0 | 7 | 1 | 8 | 0 |
| | Total | 34 | 34 | 34 | 34 | 34 | 34 | 34 | 34 | 34 | 34 |
| | Wilcoxon Test | Z = -4.134; P < 0.001 | | Z = -3.302; P = 0.001 | | Z = -4.179; P = 0.001 | | Z = -3.819; P = 0.001 | | Z = -3.917; P = 0.001 | |
| Diet and Exercise (n=21) | Mild | 5 | 15 | 10 | 16 | 4 | 13 | 4 | 11 | 7 | 17 |
| | Moderate | 12 | 5 | 7 | 3 | 12 | 7 | 12 | 7 | 10 | 4 |
| | Severe | 4 | 1 | 4 | 2 | 5 | 1 | 5 | 3 | 4 | 0 |
| | Total | 121 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| | Wilcoxon Test | Z = -3.127; P = 0.002 | | Z = -2.138; P = 0.033 | | Z = -3.153; P = 0.002 | | Z = -2.179; P = 0.029 | | Z = -2.841; P = 0.005 | |
| HRT (n=14) | Mild | 3 | 10 | 4 | 12 | 2 | 12 | 4 | 9 | 5 | 12 |
| | Moderate | 4 | 4 | 6 | 2 | 9 | 2 | 5 | 5 | 8 | 2 |
| | Severe | 7 | 0 | 4 | 0 | 3 | 0 | 5 | 0 | 1 | 0 |
| | Total | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 |
| | Wilcoxon Test | Z = -2.581; P = 0.001 | | Z = -2.762; P = 0.006 | | Z = -3.127; P = 0.002 | | Z = -2.271; P = 0.023 | | Z = -2.530; P = 0.011 | |

Table 2: Influence of different treatments for the alleviation of menopause symptoms on serum biochemical profiles of women

| Parameter | Before/After Treatment | Placebo | Omega-3 Fatty acids and Vitamin E | Isoflavones | Diet and exercise | HRT |
|---|------------------------|--------------|-----------------------------------|-------------|-------------------|--------------|
| | | Mean±S.E. | | | | |
| Serum calcium (mg/dL) | Before | 8.49±0.43 | 8.45±0.17* | 8.94±0.18 | 9.19±0.20 | 8.75±0.35 |
| | After | 8.51±0.17 | 9.41±0.20* | 9.39±0.20 | 9.33±0.17 | 8.85±0.37 |
| Total cholesterol (mg/dL) | Before | 170.05±4.74 | 199.28±5.82 | 191.03±5.40 | 185.76±4.32 | 187.93±8.90 |
| | After | 176.89±9.41 | 195.33±3.77 | 187.06±4.77 | 190.24±5.78 | 188.70±8.29 |
| High density lipoproteins (mg/dL) | Before | 42.86±0.55 | 41.81±0.63 | 40.47±0.75 | 42.57±0.54 | 41.57±1.14 |
| | After | 43.78±0.64 | 43.72±0.64 | 43.47±0.63 | 43.19±0.43 | 43.40±0.64 |
| Low density lipoproteins (mg/dL) | Before | 105.14±4.78 | 130.66±5.60 | 125.29±5.87 | 120.90±4.66 | 125.07±8.02 |
| | After | 108.78±8.20 | 122.97±3.87 | 118.49±4.73 | 117.14±7.41 | 122.00±8.20 |
| Very low density lipoproteins (mg/dL) | Before | 21.82±2.32 | 24.84±1.78 | 23.59±1.47 | 22.00±1.34 | 21.14±2.24 |
| | After | 22.94±1.77 | 28.03±1.93 | 24.94±1.55 | 23.48±1.44 | 23.20±2.36 |
| Serum triglycerides (mg/dL) | Before | 109.73±11.61 | 123.78±8.94 | 118.12±7.36 | 110.62±6.72 | 105.64±11.20 |
| | After | 114.78±8.96 | 138.50±9.75 | 125.09±7.70 | 118.14±7.43 | 116.90±11.80 |
| Bone mineral density (g/cm ²) | Before | 0.53±0.01 | 0.55±0.008 | 0.54±0.008 | 0.53±0.01 | 0.54±0.01 |
| | After | 0.54±0.01 | 0.55±0.008 | 0.54±0.008 | 0.53±0.01 | 0.51±0.01 |
| Body weight (Kg) | Before | 63.32±2.15 | 65.00±2.03 | 62.68±1.57 | 63.33±1.97 | 57.36±2.99* |
| | After | 63.68±2.00 | 63.81±2.06 | 63.00±1.65 | 62.24±1.74 | 59.07±3.21* |

statistically significant.

The effect of treatment on different parameters was tested for its significance by Kruskal-Wallis test with family size adjusted level of significance. A 'p-value' less than 0.05 was considered as statistically significant. Duncan's multiple regression test was done for the parameter which had significant change by different treatments. Student's 't' test was done for comparing serum hormone level with treatment between placebo and HRT groups (Table 2). Statistical analyses were undertaken using SPSS 13.0 software (SPSS Inc, Chicago, IL, USA).

RESULTS

Out of the 160 patients assigned to various treatment groups, 123 women reported perfect compliance at the end. The effect of treatment on the severity of symptoms was shown in Tables 1. Placebo had no significant effect on vasomotor, anxiety and depressive symptoms. However there was beneficial effect on urogenital and somatic symptoms. Omega-3 fatty acids and vitamin E, isoflavones, diet and exercise and HRT had beneficial effect on all groups of menopausal symptoms.

Certain physicochemical parameters like weight, serum calcium, total cholesterol high density lipoproteins, low density lipoproteins, very low density lipoproteins, triglycerides and bone mineral density were measured before and after intervention therapy to know the influence on them due to the administered therapy. Kruskal-Wallis test showed that significant differences were found in the moderate change in weight and serum calcium levels; in all other parameters, treatment did not result in statistically significant difference (Table 2). The moderate increase in weight was 2 kg after HRT treatment. Though a moderate change in serum calcium of 1mg/dl was observed in the omega group, this difference was not clinically significant because the serum calcium values before and after treatment were within the physiologically normal range. The effect of hormone replacement therapy on serum hormone levels was not

significant, which was observed between placebo and HRT group (Table 3).

DISCUSSION

Placebo was found to be effective in relieving urogenital and somatic symptoms but not vasomotor, anxiety or depressive symptoms (Table 1). Similar effect was reported in another study [15] with reduction of perimenopausal symptoms by 30% to 40% within a period of 12 weeks. In a long term randomized controlled trial, i.e. for 2 years, there was relief of menopausal symptoms more in placebo group than in women given isoflavones as this is a physiological transition [16]. Except for sleeplessness and dyspareunia, most of the perimenopausal symptoms responded to placebo treatment indicating that they are not estrogen dependent. Thus, the results of the present study indicated that placebo and reassurance might have started early to relieve menopausal symptoms.

Although omega-3 fatty acids were suggested to alleviate menopausal symptoms by reducing the production of eicosanoid pro-inflammatory molecules derived from omega-6 fatty acids [17], no systematic studies were reported on the use of these agents for the therapy. Further it was suggested that therapy with omega-3 fatty acids must always be accompanied by administration of an antioxidant to avoid induction of lipid per oxidation [18]. Vitamin E was earlier reported to decrease the frequency of vasomotor, somatic and anxiety symptoms in perimenopausal women [19,20]. For these reasons a combination of omega-3 fatty acids with vitamin E, rather than omega-3 fatty acids alone, was used in the present investigation. Present results indicated that a therapeutic synergy between the omega-3 fatty acids and vitamin E as the women in that group experienced significant relief from all the five classes of menopausal symptoms. Based on the present observations omega-3 -fatty acids and vitamin E may be used to treat menopause symptoms. Use of this particular combination may also benefit the

Table 3: Hormone profiles of women treated with a placebo or HRT for the relief of menopause symptoms

| Hormone | Treatment | Before/After Treatment | Concentration (mean \pm SE) | t - value |
|---------------------------------|-----------|------------------------|-------------------------------|-----------|
| FSH (mIU/mL) | Placebo | Before | 41.79 \pm 3.34 | t = 1.638 |
| | | After | 53.96 \pm 14.44 | |
| | HRT | Before | 44.89 \pm 17.15 | p= 0.111 |
| | | After | 53.71 \pm 17.28 | |
| Oestradiol - 17 β (pg/mL) | Placebo | Before | 74.90 \pm 12.93 | t= 0.9566 |
| | | After | 73.57 \pm 17.11 | |
| | HRT | Before | 47.79 \pm 10.41 | p= 0.346 |
| | | After | 59.64 \pm 11.66 | |
| Progesterone (ng/mL) | Placebo | Before | 4.38 \pm 1.39 | t= 0.2627 |
| | | After | 4.13 \pm 1.12 | |
| | HRT | before | 4.09 \pm 1.72 | p= 0.795 |
| | | After | 4.31 \pm 1.71 | |

cardiovascular system and lipid metabolism especially in obese women [20,21]. Omega-3 fatty acids may be effective in the treatment of depressive symptoms and hot flushes in women during the menopausal transition [22]

Isoflavones were reported to decrease the frequency of hot flushes and reduce menopausal symptoms within 6-12 weeks of continuous use [23]. 60 mg/day of isoflavones was found to be ideal for the therapeutic effect [24]. Amelioration of all the classes of menopause symptoms by 60mg/day of isoflavones in the present study corroborates the earlier results [24]. Isoflavones are diphenol compounds with structural and functional resemblance to human 17- β -estradiol [25]. If the circulatory estrogen level is low as during perimenopause, isoflavones bind to oestrogen receptors and mimic the oestrogen effects on menopause symptoms. However, they were not observed to influence the vaginal cytology, serum FSH (Follicle stimulating hormone), LH (Leutinising hormone), oestradiol, high density lipoproteins, cholesterol, triglycerides, urinary hydroxyl proline and calcium excretion [25]. In the present study also isoflavones did not affect the serum biochemical profiles of the women. Thus isoflavones may be prescribed to relieve menopausal symptoms.

Women on prescribed diet and regular exercise experienced relief from all the classes of menopausal symptoms (Table 1). The effect might be due to avoidance of fats in food and the absence of stimulatory influence of coffee, tea and others on the central nervous system. High intake of calcium, maintenance of consistent blood sugar levels and maintenance of normal body mass index with regular exercise were reported to be important for the alleviation of menopausal symptoms [26]. Vasomotor symptoms, negative moods, decreased sexual desire during perimenopause were reportedly relieved by regular exercise [27, 28]. Regular exercise, creates a sense of wellbeing supposedly triggered by increased noradrenaline secretion that reduces muscle tension and anxiety symptoms [29]. The improvement or preservation of muscle function with high protein, high dairy and low carbohydrate diet and weight loss with aerobic and resistance exercise could have important implications in elderly population [30].

In the present study HRT also ameliorated all classes of menopausal symptoms (Tables 1). Although there was a significant increase in weight in this group (Table 2), serum biochemical and hormone profiles (Table 3) were not affected. Estrone levels was not measured in this study. Also no other adverse influences of HRT could be noted in the present study. However, six months is relatively a short period for any adverse effects to be observed. In this connection it must be noted that women often experience menopausal symptoms for 4-5 years and sometimes for longer periods. Therefore continued long-term use of HRT may lead to adverse side effects.

None of the alternate therapies used in the present study influenced the serum biochemical profiles. One exception, however, was the increase in serum calcium levels observed in the omega-3 fatty acids group (Table 2). However, this increase was within the normal physiological level and therefore considered to be clinically not significant. As mentioned earlier six months might be relatively a short period to observe significant changes on these parameters. Therefore it would be necessary to undertake studies for longer periods of time with more sample size. From the present results, it may be concluded that reassurance, diet and exercise are the first line of therapy for menopausal symptoms as they have known benefits on the cardiovascular system and lipid

metabolism [29]. This may be followed by administration of isoflavones or omega-3 fatty acids with vitamin E in that order. If the symptoms were not relieved by any means, short term HRT may be advised.

In the present study due to small sample size and short duration of follow up i.e. for 6 months, suggests a promising direction in which large research efforts are needed to develop more safe and universal therapy protocols that replace HRT for menopause related symptoms and problems.

Further studies are needed to develop safest alternative therapeutic protocols replacing HRT for menopause related symptoms and problems.

Values for each parameter within a treatment were compared before and after treatment and assigned different superscripts if the difference was statistically significant ($p \leq 0.05$).

In the HRT group, compared with the base-line values, a statistically significant weight gain was observed at the end of treatment ($p=0.005$)

Though a median change in serum calcium of 1mg/dl was observed in the omega group, this difference was not clinically significant because the serum calcium values before and after treatment were within the physiologically normal range.

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