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Original Research Article

To Analyze Postmenopausal Women's Micronutrient Status and Metabolic Syndrome Biomarkers

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Abstract

Background: A "clustering" of metabolic abnormalities, such as raised blood sugar, abnormal lipid profile, high blood pressure, and abdominal obesity, that increase a person's risk of cardiovascular disease and type 2 diabetes is known as metabolic syndrome (MetS). India and other emerging nations are seeing an increase in the prevalence of metabolic syndrome. In middle age, the prevalence of obesity is higher in women than in men because urban lifestyle factors such as poor dietary choices, sedentary behaviors, physical inactivity, and menopause make women more susceptible to obesity. It is believed that homocysteine poses a separate risk for the onset of cardiovascular illnesses.

Material and Method: The institutional ethics committee gave its approval before the study could begin. 350 women, ages 35–64, make up the sample. They were split equally into three groups based on inclusion and exclusion criteria: premenopausal, perimenopausal, and postmenopausal. Biochemical measures included serum insulin, FBG, TGs, HDL, homocysteine, and plasma levels of vitamin B12, folic acid, vitamin D3, and LCPUFA.

Results: When comparing premenopausal and postmenopausal women, the mean levels and percentages of all components of the metabolic syndrome—aside from HDL—were greater in perimenopausal women. All three groups had similar and lower-than-normal HDL cholesterol levels. In addition, perimenopausal and postmenopausal women had considerably greater levels of homocysteine and HOMA IR than premenopausal women did. Compared to premenopausal women, the concentrations of vitamin B12, folic acid, and vitamin D were significantly reduced in perimenopausal and postmenopausal women. When compared to the premenopausal group, the perimenopausal and postmenopausal groups exhibited notably lower omega-3 fatty acid and greater omega-6: omega-3 ratio.

Conclusion: The current study did not assess parathyroid hormone. Future research should use PTH as a functional indicator of vitamin D levels. Future research should consider including vitamin B6, which has a significant role in the breakdown of homocysteine, in addition to vitamin B12, folic acid, and vitamin D. The current study could not identify the minimal amount of micronutrients required to prevent MetS. There is potential to expand this study to other urban areas and other demographic groups because the majority of the women are housewives from the lower middle class who are also less educated.

Keywords: PTH, vitamin D, HOMA IR, HDL, Vitamin B12 and Omega-6: omega-3.

Introduction

A "clustering" of metabolic abnormalities, such as raised blood sugar, abnormal lipid profile, high blood pressure, and abdominal obesity, that increase a person's risk of cardiovascular illnesses is known as metabolic syndrome. [1] In developing nations, the prevalence of metabolic syndrome is rising, particularly in South Asian nations. [2,3] According to recent data, between one-fourth and one-third of India's urban population suffers from metabolic syndrome. [4] Additionally, women's prevalence at middle age 2 is 1.5–2 times higher than men's due to their greater propensity for

obesity, impaired fasting glucose, low high density lipoprotein, and elevated triglycerides. Insulin Resistance Syndrome is another term for metabolic syndrome. [2,5,6]

Due to substantial changes in food and physical activity patterns brought about by socioeconomic transition, obesity is more common in middle-aged women living in metropolitan areas than in men. Furthermore, postmenopausal women who experience central obesity due to an estrogen deficit are included in the middle-aged female population. [7] Central obesity leads to abdominal adiposity, dyslipidemia and insulin resistance in postmenopausal women. [8] It is known that people with metabolic syndrome have more morbidity and mortality for coronary artery disease or stroke. In addition, people with metabolic syndrome have a fivefold greater risk of developing type2 diabetes [9] which further raises susceptibility to cardiovascular diseases. [10-11] Numerous studies show that elevated homocysteine levels are caused by the aforementioned conditions, including obesity, physical inactivity, poor dietary habits, metabolic syndrome, and estrogen shortage in postmenopausal women. [12] Vitamin B12 and folic acid are two micronutrients that are crucial for the metabolism of homocysteine. Vitamin B12 functions as a cofactor and folic acid as a methyl donor in the metabolism of homocysteine.

Indians are more likely to have hyperhomocysteinemia because they often have folic acid and vitamin B12 deficits, possibly as a result of improper cooking techniques and insufficient intake of foods derived from animals. [13,14,15] When these vitamins are lacking, homocysteine does not get converted to methionine, which causes homocysteine levels to rise. In addition to raising insulin resistance, a vitamin D deficit also raises homocysteine levels. [16,17] It also affects the renin-angiotensin system, which raises the risk of CVDs. Moreover, high body fat and a lack of sun exposure are thought to be the main reasons for vitamin D insufficiency in Indians. [16] Long chain polyunsaturated fatty acid (LCPUFA) deficiency causes methyl groups to be directed toward DNA, which changes the expression of important genes involved in a number of metabolic processes and eventually results in insulin resistance and metabolic syndrome. [14] Interventional trials [17,18] with these nutrients found inconsistent results. This could be the result of relationships that are not causative or interventions made too late in the course of the illness.In this regard, very few research have been conducted in India.

Limited studies reported lower levels of either vitamin B12 [19] or vitamin D among the middle aged women with metabolic syndrome. A negative association of vitamin B12 levels with body mass index [18] and adverse lipid profile has been reported. [20] It has also been observed that there is an inverse link between vitamin D and the risk of metabolic syndrome. [20] To the best of our knowledge, no research has been done to compare the basal levels of any of the aforementioned nutrients with the different metabolic syndrome components. India currently has a pandemic of middle-aged urban women who are more susceptible to non-communicable diseases like cardiovascular disease (CVDs). [21] Understanding the relationship between elements of the metabolic syndrome and micronutrients including LCPUFA, vitamin B12, folic acid, and vitamin D is therefore crucial, especially for postmenopausal women. This would support the idea that micronutrients can prevent cardiovascular diseases (CVDs) in middle-aged urban women for a reasonable price.

Material and Methods

This study was an observational cross-sectional study. The study was conducted in Department of Obstetrics and Gynaecology.

Sample Size: 350 women volunteers between 35 - 64 yr were included in this period of the study.

Inclusion Criteria: All non-pregnant women volunteers between age group of 35 to 64 yrs were included in the study.

Exclusion Criteria:

- Consists of subjects with morbid conditions like diabetes, hypertension, ischemic heart disease, cancer, thyroid disease, or any other acute or chronic liver or kidney disease or subject who underwent hysterectomy surgery or any current infectious condition
- Those taking treatment of anemia or taking hormonal supplementation or phytoestrogens were also excluded from the study.

Biochemical Analysis:

- Plasma levels of vitamin B12, folic acid, vitamin D3 and homocysteine were assessed by Chemiluminescence method. [23]
- Fasting plasma glucose was assessed by GOD -POD (mg/dl) [24]
- Plasma insulin was analyzed using the Mercodia insulin ELISA kit. [25]
- Plasma levels omega 3 fatty acids were assessed by Gas chromatography. [25]
- The estimation of plasma HDL and TG was carried out using enzymatic kit method. [26]

Specimen Collection and Storage:

All women were asked to come to the hospital for blood sample collection 10-ml of fasting venous blood sample was taken in the morning (7:00–8:00 A.M.) after 12 to 14 hrs of overnight fast. Blood was collected by venipuncture of median cubital

vein in the antecubital fossa. The area around intended puncture site was cleaned with prepackaged alcohol swab. The skin was allowed to dry as alcohol may cause hemolysis of collected blood. The plasma & serum were separated and frozen at - 80°C for later analysis.

Statistical Analysis: Data is represented as mean (standard deviation). SPSS version 17.0 for Windows (SPSS Inc, Chicago) was used for the statistical analysis. Variables with skewed distribution were log transformed to satisfy the 56 assumptions of normality. In such cases, the data has been represented as median (inter quartile range, IQR). ANOVA (Analysis of Variance) and chi square test were used for comparison between three groups.

Result

350 women volunteers were equally divided into pre, peri and postmenopausal groups according to their menstrual history.

Table 1: Comparison the Components of FBG, HDL, TG, homocysteine and HOMA IR in the three groups.

	Group I Premenopausal N=116	Group II Perimenopausal N=116	Group III Postmenopausal N=118
Fasting glucose (mg/dL)	90.3±80.4	102.9±91.43	113.7±97.81
HDL cholesterol (mg/dL)	44.5±3.7	47.1±4.2	46.1±6.1
Triglycerides (mg/dL)	105.1±35.8	100.7±42.3	115.5±44.1
Homocysteine (mM/L)	18.9±9.1	21.9±9.9	23.91±11.8
HOMA IR	5.76±1.78	6.23±2.91	5.78±3.4

Table 1 shows that mean levels of all metabolic syndrome components except HDL, are higher in peri & highest in postmenopausal women as compared to premenopausal women. HDL cholesterol levels are similar & lower than normal in all three groups. Homocysteine and HOMA IR levels are also significantly higher in peri and postmenopausal women as compared to premenopausal women

Table 2: Comparison the Levels of micronutrients and fatty acids in the three groups.

	Group I Premenopausal N=116	Group II Perimenopausal N=116	Group III Postmenopausal N=118
Vitamin B_{12}(pg/mL)	348.4±14.5	280.6±14.7	290.7±111.9
Folic acid(ng/mL)	17.8±6.9	13.12±7.2	12.9±6.8
Vitamin D (ng/mL)	19.7±6.6	18.2±7.4	17.5±7.8
SFA (gm/dL)	37.4±5.7	34.9±5.8	33.9±6.6
MUFA	19.8±5.3	19.6±6.9	20.10±6.3
w-3	2.17±0.53	1.24±0.44	0.98±0.41
w-6	46.9±9.4	48.10±9.8	48.6±6.4
w-6:w-3	44.13±22.4	65.9±43.5	61.7±33.4

Table 2 shows that vitamin B_{12}, Folic acid & vitamin D concentrations are significantly lower in peri and post-menopausal women as compared to premenopausal women. Peri and postmenopausal group show significantly lower omega-3 fatty acid and higher omega-6/omega-3 ratio as compared to premenopausal group.

Table 3: Comparison the Levels of micronutrients and fatty acids in women with and without metabolic syndrome.

	Metabolic syndrome N=200	Normal N=150
Vitamin B_{12}(pg/mL)	290.5±15.1	333.5±26.7
Folic acid(ng/mL)	14.8±6.8	16.3±6.4
Vitamin D (ng/mL)	15.2±7.4	19.5±7.2
SFA (gm/dL)	35.3±6.9	33.4±6.5
MUFA	20.4±7.4	19.21±7.9
w-3	1.03±0.62	1.12±0.52
w-6	47.8±8.6	49.17±9.8
w-6:w-3	61.7±43.9	53.9±32.4
Homocysteine (mM/L)	25.6±10.9	18.9±12.4
HOMA IR	7.53±4.28	5.56±2.9

There is a significant difference between levels of vitamin D, SAFA, homocysteine and HOMA IR in subjects with & without metabolic syndrome after adjustment for age. There is no significant differ-

ence between vitamin B 12, folic acid, w-3 fatty acids and w-6/w-3 ratio. The proportion of women with lowest micronutrients levels was highest in postmenopausal women.

Discussion

The goal of the current study was to investigate the relationship between middle-aged urban women's metabolic syndrome components and micronutrients such as folic acid, vitamin B12, vitamin D, and LCPUFA.

In the current investigation, peri- and postmenopausal women had significantly higher mean fasting blood glucose levels and a greater proportion of women with impaired fasting glucose than premenopausal women did. In postmenopausal women, elevated fasting glucose was detected in 82% of cases. Whereas studies by Sapana Goyal et al(2013) [27] and Haidari et al(2010) [28] which did not observe higher FBG in postmenopausal women as compared to premenopausal women suggesting that, high fasting blood glucose levels itself is not sufficient to diagnose metabolic syndrome. Rather, fasting glucose levels may be within normal range in presence of insulin resistance.[5]

Triglycerides (TG) levels were highest in postmenopausal group and there was a significant difference between peri and postmenopausal group. Similar findings were reported by Ainy E et al(2007) [29], Arthur FK et al(2013) [30], Maharlouei N et al(2013) [31] did not find high TG, in postmenopausal women after age adjustment. Plasma TG and HDL-cholesterol are known to be inversely correlated as observed by various epidemiological studies. [32,33] The enzyme cholesteryl ester transfer protein (CETP) balances the levels of TG and HDL-cholesterol. Cholesteryl ester transfer protein mediates the exchange of cholesteryl ester for triglycerides between HDL, VLDL and LDL. Manisha Chandalia et al (2001), [34] Smith J et al found low HDL-cholesterol values in South Asians. All premenopausal, perimenopausal, and postmenopausal groups had similar and below-normal HDL cholesterol levels. The levels of homocysteine and HOMA IR varied significantly between pre-menopausal and post-menopausal women, with the latter showing the highest levels. Even after controlling for body mass index, plasma homocysteine levels were still linked to menopause, suggesting that menopause had an impact on plasma homocysteine levels. [35]

Ross AC et al(2011) [36] in human tissues after injection of radioactive cholecalciferol. However no correlation of waist circumference with vitamin B12, folic acid and SAFA, MUFA, omega 6 fatty acids and w-6:w-3 ratio were observed with any of the group in the present study. Negative correlation of waist circumference with omega 3 fatty acids was also reported by Poudyal H et al. (2011) [37] This may be due to omega 3 fatty acids suppressing fat synthesis but increasing metabolism in adipose tissue via multiple mechanisms involving altered expression of nuclear transcription factors.

Conclusion

The current study did not test parathyroid hormone (PTH). Future research should use PTH as a functional indicator of vitamin D levels. Future research should consider including vitamin B6, which has a significant role in the breakdown of homocysteine, in addition to vitamin B12, folic acid, and vitamin D. The current study could not identify the minimal amount of micronutrients required to prevent MetS. There is potential to expand this study to other urban areas and other demographic groups because the majority of the women are housewives from the lower middle class who are also less educated.

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