RESEARCH ARTICLE

Association of homocysteine with metabolic syndrome risk factors in postmenopausal women

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ABSTRACT

Background: The prevalence of cardio-metabolic disorders is highest among the elderly population and homocysteine (Hcy) is considered as a major risk factor. Vascular aging in women is exacerbated by reproductive aging in response to hormonal changes during the menopause. The present study assessed the levels of Hcy, Vitamin B<sub>12</sub>, folate, and examined their association with metabolic syndrome risk factors in menopausal women. Aim and Objectives: The objectives of the study were to examine the levels of Hcy, Vitamin B12, and folate and their association with various components of metabolic syndrome in menopausal women. Materials and Methods: We recruited a total of 300 women between 35 and 64 years old from Bharati Hospital, Pune. They were classified into pre-, peri-, and post-menopausal groups according to their menstrual history. Results: Lower Vitamin B<sub>12</sub> and folic acid and higher Hcy concentrations were found in peri- and post-menopausal women as compared to premenopausal women. Women with metabolic syndrome showed lower micronutrients and higher Hcy compared to those without metabolic syndrome. An inverse association of Vitamin B<sub>12</sub> with waist circumference (WC), diastolic blood pressure, triglycerides and HOMA-IR and inverse association of folate with fasting glucose and HOMA-IR were observed. A positive association of Hcy with WC, fasting glucose, systolic and diastolic blood pressure, triglycerides, and HOMA-IR was also seen. In a logistic regression model, metabolic syndrome was found to be independently associated with age and Hcy. Conclusion: This study reports elevated Hcy and low micronutrient levels in postmenopausal women. Hcy was found to be independently associated with metabolic syndrome risk in these women. Monitoring plasma Hcy concentrations with adequate B vitamin stores could be an effective strategy to minimize metabolic syndrome risk in middle-aged women.

KEY WORDS: Homocysteine; Metabolic Syndrome; Postmenopausal Women

INTRODUCTION

Metabolic syndrome represents a cluster of metabolic abnormalities that directly aggravate the risk of type 2 diabetes mellitus and cardiovascular diseases (CVD). The component features of metabolic syndrome include hyperglycemia, obesity (particularly abdominal obesity), high blood pressure, increased triglycerides, and decreased high-density lipoprotein cholesterol. The incidence and prevalence of metabolic syndrome are highest among the elderly population because of adverse effects of advancing age. Emerging data suggest that gender differences also exist between men and women who develop metabolic syndrome. Hyperglycemia and diabetes pose a greater risk of CVD mortality in women than in men. Moreover, higher prevalence of the metabolic syndrome has been observed in female diabetic population. Age-standardized prevalence of metabolic syndrome is reported to be 24.9% in males.
and 42.3% in females in Asian Indians and prevalence was much higher in the postmenopausal as compared to the premenopausal women. It is reported that the risk increases slowly in the early stage in women and then rapidly rises in their middle-age after 50s. This is mainly because of the changes in hormonal secretion caused due to a decrease in ovarian function, which results from the menopause. The menopause (a physiological aging process) is known to change body fat distribution leading to central adiposity which increases the risk for developing metabolic syndrome. Moreover, ageing also contributes to clustering of cardio-metabolic risk factors. Thus, understanding the factors associated with adverse metabolic outcomes and their aggressive control in middle-aged women is of importance.

Elevated homocysteine (Hcy) is considered as a marker of systemic or endothelial oxidant stress and acts as a major risk factor for atherothrombotic or cardiovascular disorders. Studies have shown that low concentrations of folate and Vitamin B12 are associated with insulin resistance diabetes and higher incidence of acute coronary events. In Hcy metabolism, micronutrients such as folic acid and Vitamin B12 act as methyl donors for remethylation of Hcy to methionine. Various studies have also shown a negative association of folic acid and Vitamin B12 levels with Hcy. Very few studies have reported Vitamin B12 levels in postmenopausal group. However, the association between micronutrients and Hcy with metabolic syndrome risk factors in postmenopausal women is relatively less explored.

In the previous research, we found that postmenopausal women have higher waist circumference (WC), blood pressure and triglyceride levels, and important risk factors for metabolic syndrome. We have also reported an inverse association of serum 25 (OH) D with elevated WC, blood pressure (BP), and triglycerides. This study has been conducted to examine the levels of Hcy, Vitamin B12, and folic acid and their association with various components of metabolic syndrome in menopausal women. This would help to understand the influence of Hcy and micronutrients on metabolic syndrome risk in elderly population.

MATERIALS AND METHODS

The present cross-sectional study was performed at Bharati Vidyapeeth University Medical College and Hospital, Pune. Ethical approval was obtained by institutional human ethics committee (BVDU/MC/42). All non-pregnant women volunteers between age group of 35 and 64 years were included in the study. Women who were attending free camps organized by Department of Obstetrics and gynecology, Bharati Hospital, Pune for the measurement of bone mineral density and detection of anemia were included from 2012 to 2014. It also includes women who were attending Women Wellness Clinic conducted by the Department. A written informed consent was taken from each participant. Detailed history was taken regarding age, menopausal status, socioeconomic status, dietary pattern, and physical exercise.

A total of 300 participants were divided into three groups (pre, peri, and post-menopausal) according to their menstrual history. They were categorized as: (i) premenopausal if they had regular menstrual periods; (ii) perimenopausal if they had irregular interval menstrual periods, that is, more than 2–3 months; and (iii) postmenopausal in case of the absence of menstrual periods for 12 consecutive months and thereafter.

Subjects with morbid conditions such as 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 having any current infectious condition were excluded from the study. Those taking treatment of anemia or taking hormonal supplementation or phytoestrogens were also excluded.

A venous blood sample was collected from all the participants after an overnight fast. The plasma was separated and frozen at −80°C for analysis. Plasma folate and Vitamin B12 concentrations analyzed by radioimmunoassay and expressed as pg/mL. Hcy was estimated using the chemiluminescent microparticle immunoassay technology and expressed as mM/L. Fasting plasma glucose was assessed by GOD-POD and expressed as mg/dL. The plasma lipid estimation was carried out using enzymatic kit method and expressed as mg/dL. Women were considered for clinical identification of the metabolic syndrome if they had any three or more of the following, as per the joint interim statement is as follows: abdominal obesity by WC ≥80 cm; triglyceride ≥150 mg/dL; high-density lipoprotein cholesterol <50 mg/dL; blood pressure ≥130/85 mm Hg; and fasting glucose ≥100 mg/dL.

Statistical Analysis

Data are represented as mean and standard deviation. Variables with skewed distribution were log transformed to satisfy the assumptions of normality. In such cases, the data have been represented as median inter quartile range. Analysis of variance and Chi-square test was used for comparison between three groups. Bonferroni correction for multiple comparisons was used to identify difference between the groups. Pearson correlation coefficient was used to measure the linear correlation between two variables and “r” and “P” value were calculated. Odds ratios were calculated to measure the association between an exposure and an outcome, that is, metabolic syndrome. Multiple logistic regression analysis was used to find out determinants of metabolic syndrome.
All the results were age adjusted. $P < 0.05$ was considered as statistically significant. Statistical Package for the Social Sciences version 17.0 for Windows (SPSS Inc., Chicago) was used for the statistical analysis.

RESULTS

Homocysteine and Micronutrients Concentrations

Higher Hcy levels were found in 13.3% ($n = 40, >30 \mu\text{mol/L}$) among the whole population of women. The percentage of women deficient in Vitamin $\text{B}_12$ was 19% ($n = 57, <203 \text{pg/ml}$) and folate was 7.7% ($n = 23, \leq 5.38 \text{ng/ml}$).

Table 1 shows concentrations of micronutrients in different groups. Peri and postmenopausal group showed higher Hcy concentrations as compared to premenopausal group. Vitamin $\text{B}_12$ and folic acid concentrations were significantly lower in peri and post-menopausal women as compared to premenopausal women.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All women ($n=300$)</th>
<th>Group I premenopausal women ($n=100$)</th>
<th>Group II perimenopausal women ($n=100$)</th>
<th>Group III postmenopausal women ($n=100$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (mM/L)</td>
<td>20.1 (8.2)</td>
<td>17.3 (7.7)</td>
<td>20.1 (7.9)*</td>
<td>22.9 (8.1)**</td>
</tr>
<tr>
<td>Vitamin $\text{B}_12$ (pg/mL)</td>
<td>302.2 (113.8)</td>
<td>347 (111)</td>
<td>277 (111)**</td>
<td>282 (106)</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>12.3 (5.2)</td>
<td>14.2 (5.1)</td>
<td>11.9 (5.3)**</td>
<td>11.0 (4.7)**</td>
</tr>
</tbody>
</table>

Values represented as mean (SD): *Different from group I $P<0.05$, **Different from group II $P<0.05$. All the values are age adjusted.

Levels of Hcy and Micronutrients in Women with and without Metabolic Syndrome

We observed higher Hcy and lower Vitamin $\text{B}_12$ and folate concentrations in women with metabolic syndrome compared to those without metabolic syndrome [Figure 1].

Associations of Vitamin $\text{B}_12$, Folate, Hcy with HOMA-IR

There was a direct association of Hcy with HOMA-IR ($r = 0.22, P < 0.01$). On the other hand, there was an inverse association of Vitamin $\text{B}_12$ ($r = -0.013, P < 0.05$) and folate ($r = -0.012, P < 0.05$) with HOMA-IR.

![Figure 1: Levels of micronutrients in women with and without metabolic syndrome. Values represented as mean (SD): *Different from women with metabolic syndrome $P < 0.05$](image-url)
Associations of Vitamin B_{12}, Folate, and Hcy with Metabolic Syndrome Components

Hcy was directly associated with all the metabolic syndrome components except for HDL cholesterol [Table 2]. Vitamin B_{12} was inversely associated with WC, diastolic blood pressure, and triglyceride concentrations.

Folic acid was inversely associated only with fasting glucose. There was no correlation of folate with other metabolic syndrome components.

Regression Analysis

In a logistic regression model including age, Vitamin B_{12}, folate, Hcy concentrations, and metabolic syndrome was independently associated with age and Hcy concentrations [Table 3].

Women in 2nd and 4th quartile of age were more likely to have metabolic syndrome compared to those in the lowest quartile of age. Women in top two quartiles of Hcy concentrations were 3.16 times and 3.33 times likely to have metabolic syndrome when compared to those in the lowest quartile.

DISCUSSION

In women’s life, menopause is one of the crucial stages which lead to various physiological and psychological changes. These changes occurring due to hormonal transition can influence the long term risk for developing cardio-metabolic disorders where genetic, environmental and dietary factors also play an important role. In the current study, we estimated the levels of micronutrients, Hcy and their association with metabolic syndrome risk factors in menopausal women.

We found higher concentrations of Hcy in peri and post-menopausal women as compared to premenopausal women. Our findings are consistent with other studies which show lower Vitamin B_{12} to Vitamin B_{6} require B vitamins as cofactors and its levels are responsive to Vitamin B_{12} and folic acid status. In this study, we found lower Vitamin B_{12} and folic acid in peri and post-menopausal women as compared to premenopausal women. A study reported lower dietary intake and levels of Vitamins B_{12}, B_{6} folate in postmenopausal women and significantly of the menopause transition lead to elevations in Hcy that contribute to endothelial dysfunction in postmenopausal women. We found a positive association of Hcy with WC, fasting glucose, systolic and diastolic BP, triglycerides, and insulin resistance index. Moreover, women with metabolic syndrome also showed the higher Hcy compared to those without metabolic syndrome. Several studies in human and rodents have shown that hyper Hcy mia is associated with obesity, higher cholesterol and triglyceride synthesis and diabetes. Elevated Hcy is also considered as a marker of systemic or endothelial oxidant stress, result in endothelial dysfunction, and also acts as a major risk factor for atherothrombotic or cardiovascular disorders reviewed by Wierzbicki, Bhagwat et al., Namekata et al. In women, vascular aging appears to be exacerbated by reproductive aging, particularly due to changes in gonadal hormones during the menopausal transition (i.e., perimenopause). The mechanisms underlying the association between hyperhomocysteinemia and endothelial dysfunction are unclear but could be related to factors involved in Hcy metabolism.

Hcy metabolism is catalyzed by a number of enzymes that require B vitamins as cofactors and its levels are responsive to Vitamin B_{12} and folic acid status. In this study, we found lower Vitamin B_{12} and folic acid in peri and post-menopausal women as compared to premenopausal women. A study reported lower dietary intake and levels of Vitamins B_{12}, B_{6} folate in postmenopausal women and significantly lower Vitamin B_{12} and folic acid in peri and post-menopausal women compared to premenopausal women. Evidence suggests that declines in estradiol across stages

Table 2: Associations of Vitamin B_{12}, folate, homocysteine with metabolic syndrome components; Pearson correlations (n=300)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Homocysteine</th>
<th>Vitamin B_{12}</th>
<th>Folate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference</td>
<td>0.33***</td>
<td>0.03***</td>
<td>0.03***</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.15**</td>
<td>0.01**</td>
<td>0.01**</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>0.30***</td>
<td>0.13**</td>
<td>0.13**</td>
</tr>
<tr>
<td>Systolic</td>
<td>0.055</td>
<td>0.055</td>
<td>0.055</td>
</tr>
<tr>
<td>Diastolic</td>
<td>0.28***</td>
<td>0.28***</td>
<td>0.28***</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001

Table 3: Multivariate associations of metabolic syndrome

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Odds ratios</th>
<th>95% Confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td>1.04, 4.31</td>
<td>0.04</td>
</tr>
<tr>
<td>Q1</td>
<td>2.11</td>
<td>0.68, 2.85</td>
<td>0.37</td>
</tr>
<tr>
<td>Q2</td>
<td>2.37</td>
<td>1.11, 5.04</td>
<td>0.025</td>
</tr>
<tr>
<td>Q3</td>
<td>1.39</td>
<td>0.47, 2.03</td>
<td>0.96</td>
</tr>
<tr>
<td>Q4</td>
<td>0.60</td>
<td>0.28, 1.31</td>
<td>0.20</td>
</tr>
<tr>
<td>Vitamin B_{12}</td>
<td>1</td>
<td>0.39, 1.80</td>
<td>0.64</td>
</tr>
<tr>
<td>Q1</td>
<td>0.67</td>
<td>0.32, 1.41</td>
<td>0.29</td>
</tr>
<tr>
<td>Q2</td>
<td>0.77</td>
<td>0.36, 1.64</td>
<td>0.50</td>
</tr>
<tr>
<td>Q3</td>
<td>0.84</td>
<td>0.39, 1.80</td>
<td>0.64</td>
</tr>
<tr>
<td>Q4</td>
<td>1.69</td>
<td>0.82, 3.50</td>
<td>0.16</td>
</tr>
<tr>
<td>Q5</td>
<td>0.77</td>
<td>1.52, 7.27</td>
<td>0.002</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>1</td>
<td>0.39, 1.80</td>
<td>0.64</td>
</tr>
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Odd ratios with 95% confidence interval are shown, Q1 to Q4 represent quartiles of distribution for the whole group.
negative correlation of serum Vitamin B₁₂ and blood folate concentrations with total plasma Hcy.[31] Further, in patients with coronary artery disease, low concentrations of serum Vitamin B₁₂ and hyperhomocysteinemia have been shown to be related with cardiovascular risk factors.[31]

We observed an inverse association of Vitamin B₁₂ with metabolic syndrome risk factors like WC, diastolic BP, triglycerides and HOMA-IR index and an inverse association of folic acid with fasting glucose and HOMA-IR index in women of our study. In addition, women with metabolic syndrome also showed lower levels of these vitamins compared to those without metabolic syndrome. Low Vitamin B₁₂ levels are shown to be associated with adverse lipid profile, obesity[31] and higher risk of coronary artery diseases.[32] It is reported that deficiency of dietary methyl donors can cause adverse metabolic changes in the cell which may be indicated by higher Hcy levels.[33] It is reported that, in postmenopausal women, in addition to the loss of the protective effects of estrogen on their cardiovascular physiology and lipid metabolism, they are exposed to higher plasma Hcy concentrations and have deleterious cardiovascular effects. It has been suggested that B vitamins inhibit atherogenesis by decreasing plasma Hcy levels through their antioxidant properties and thus can be used to prevent CVD.[34] Low dose supplementation of folic acid, Vitamin B₁₂ and B₉ can ameliorate cardiovascular disease risk in healthy Chinese elderly subjects.[35]

CONCLUSION

Our data show lower Vitamin B₁₂ and folic acid and higher Hcy concentrations in postmenopausal women and have a higher risk for metabolic syndrome. Hcy was found to be independently associated with metabolic syndrome risk factors in postmenopausal women suggesting their vulnerability to develop cardiovascular disease in future. Monitoring plasma Hcy by improving micronutrient status could be a preventive measure against metabolic syndrome and related disorders.

REFERENCES


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