Evaluation of Risk of Atherosclerosis in Indian Adults

Deepa Pandit1, Shashi Chiplonkar2, Anuradha Khadilkar3, Arun Kinare2, Vaman Khadilkar2, Uma Divate2

Abstract

Objective: To investigate interrelationship of arterial measurements with metabolic syndrome (MS) components and zinc status in apparently healthy Indian adults.

Methods: Anthropometry and biochemical data were recorded in 110 men and 139 women (25-50 yr). Carotid Intima media thickness (CIMT), stiffness (β), pulse wave velocity (PWV), elasticity modulus (Ep), and arterial compliance (AC) of the right carotid artery were evaluated ultrasonically. According to definition of MS, subjects were categorized as MS-I, MS-2, MS-3. Further, normal and MS subjects were divided as zinc sufficient and deficient.

Results: In all, 12.1% subjects had 3 risk factors for MS. Mean CIMT, β, Ep and PWV were significantly higher by 6%, 11.6%, 29.5% and 12.4% in subjects with MS than normal (p<0.05). AC showed significant decline in MS subjects by only 3% than normal (p<0.05). Serum zinc was inversely correlated with β, Ep and PWV in both the genders in subjects with MS (p<0.05). A synergistic effect of serum zinc deficiency with MS further envisages the elevated risk of arterial stiffness.

Conclusion: Risk of atherosclerosis is marked by increase in stiffness parameters even in presence of a single MS risk and zinc deficiency may further aggravate the risk indicating need for early diagnosis.

Introduction

Metabolic syndrome (MS) is a cluster of conditions including glucose intolerance, central adiposity, hypertension, insulin resistance and lipoprotein abnormalities.1 Almost all of these conditions are considered as risk factors for cardiovascular disease (CVD). In addition, increased carotid intima-media thickness (CIMT) is reported to be an important risk factor for CVD.2 Increased arterial stiffness also affects vascular walls leading to worsening of haemodynamics, which enhance the risk of stroke and heart failure.3 Moreover, it has been observed that there is a higher risk of CVD and stroke mortality in patients with MS relative to those without the syndrome.4 Prevalence of metabolic syndrome is high in urban Indian adults (8% in men and 46% in women)5 exposing them to a greater risk of cardiovascular disease.

Some noninvasive tools have been shown to be clinically useful to obtain measures of arterial function and morphology, including measurement of CIMT, stiffness, pulse wave velocity and arterial compliance.6 As one of the better CVD markers, intima-media thickness can be measured noninvasively throughout life, yielding a risk assessment for future events.

The relationship between MS and subclinical atherosclerosis has been reported in young and middle-aged adults.7 Reports have also indicated that adults with metabolic syndrome may have increased CIMT, PWV and decreased AC.8,9 Although hypertension and other risk factors are major contributors, ethnicity is an important independent risk factor.10 However, there are very limited studies reporting carotid CIMT and stiffness measurements in Indians who differ from the Western population in cardiovascular risk profile, morbidity, and mortality.11

1Agharkar Research Institute, Agarakar Road, Pune 411 004, India;
2Hirabai Cawasji Jehangir Medical Research Institute, 32, Sassoon Road, Pune 411 001, India.
Received: 05.03.2012; Accepted: 26.07.2012

Considering the long latent phase of atherosclerotic progression before symptoms are manifest ability to evaluate arterial function and morphology before the development of atherosclerotic plaques is an important aspect of early detection and risk classification. Therefore, linkage of metabolic syndrome components with stiffness parameters in Indian adults needs to be explored in order to make an early diagnosis of atherosclerosis in young adults.

Zinc deficiency could be another important risk factor for atherosclerosis. Studies have shown protective role of zinc on vascular endothelium. Zinc is also involved in maintenance of cell integrity. Zn deficiency can enhance oxidative-stress-related signaling processes in endothelial cells which could be a risk factor for CVD.12 However to the best of our knowledge, association of zinc with atherosclerosis in humans has not been reported in India. It is therefore, worth investigating zinc profile of Indian adults and examining its association with arterial stiffness.

Thus, the aim of our study was i) to assess early markers for functional and structural changes in the carotid artery in presence of metabolic risk factors and ii) to examine association of arterial stiffness with zinc status in young Indian adults.

Methods

Study population

In a cross-sectional study a random sample of 110 men and 139 women (25-50 yr) was selected, who were apparently healthy from the routine health checks at Jehangir Hospital in Pune city, India on voluntary basis during 2008-2009. All adults were inhabitants of Pune city or surrounding suburbs. A written informed consent was obtained from participants prior to actual commencement of the study. The research protocol was approved by the Ethics Committee of Hirabai Cawasji Jehangir Medical Research Institute (HCJMRI), Pune, India. A clinical examination of all the adults was performed by a physician to...
assess their health status. None of the participants were suffering from any established cardiovascular disease. Adults who were on anti-hypertensive and lipid lowering medications and having frank Diabetes Mellitus (DM type I and II) were excluded from the study. With the sample size as mentioned above and the margin of error of 10% for the difference between means, power of the study was found to be 0.84.

**Anthropometric measures**

Height was measured to the nearest 0.1 cm (Leicester height meter, Child growth foundation, UK, range 60-207 cm). Weight was measured on an electronic digital scale to the nearest 0.1 kg. Waist circumference was measured with an inelastic tape to the narrowest point between the lower borders of the rib cage and the iliac crest. Hip circumference was measured as maximal circumference at the level of the trochanters. Body mass index [BMI] was computed using the following formula: BMI = Weight (kg)/Height (m)^2.

**Biochemical observations**

A venous blood sample (8 ml) was collected at 9:00 am from each adult after an overnight fast of not more than 12 hours using (BD Franklin lakes NJ USA) vacutainers. Samples were immediately brought to the laboratory in ice bags and serum was separated after centrifugation at 2500 rpm for 15 minutes at room temperature within two hours of collection. Lipid profile of each serum sample was estimated on a Siemens analyzer Siemens Health Care Diagnostics, Deerfield, IL, USA (Dade Dimension RXL Max) with enzymatic procedures for measurement of cholesterol, triglycerides and HDL. The LDL cholesterol level was calculated by using the formula [LDL = TC – (HDL + TG /5)] Friedewald equation. All biochemical analyses were performed at the Biochemistry Unit of the hospital. Hemoglobin (Hb) was estimated using Automated Cell counter (Beckman Coulter, Coulter Corporation Maima USA). Glucose concentrations were analyzed enzymatically and serum insulin and apolipoprotein-B (Apo-B) were measured by micro-particle enzyme immunoassay kit. Serum samples were digested and analyzed for zinc content and readings were recorded using atomic absorption spectrophotometry (Perkin Elmer, Model 3110, Norwalk, CT, USA).

**Percent body fat by DXA (Dual energy X-ray absorptiometry)**

DXA measurements were performed using Lunar DPX-PRO total body pencil beam Densitometer (GE Healthcare, Wisconsin, USA) using a medium mode scan (software encore 2005 version 9.30.044). We have standardized our measurements by running daily quality assurance scans. All scans and scan analysis were performed by the same operator.

**Carotid arterial measurements**

Arterial measurements were carried out at right carotid artery using a Prosound a 10 equipment (Model SSD- α 10; No: M00542; 2007; Aloka Co. Ltd, Japan). Using a high-frequency linear array probe (range 6.67 to 13 MHz) change in vessel diameter was measured by a radiologist with e-tracking. Three readings for each participant were taken by a single observer and the best wave forms were considered. The four physiological parameters; stiffness (β), elastic modulus (Ep), arterial compliance (AC) and pulse wave velocity (PWV) along with intima media thickness (CIMT) were recorded. Blood pressure was measured in the right arm with the adult lying down quietly. The average of the 3 measurements was used as the final measurement.

Reliability of stiffness parameters was assessed using two repeat measurements of CIMT, Ep, PWV and AC in 12 adults (36.7±8.9 yr). Cronb’s alpha was obtained as 0.93 for CIMT, Ep, PWV and AC. Reproducibility of stiffness parameters was derived from the root mean square standard deviation (RMSD) of two repeat measurements for Ep, PWV, AC, and CIMT in 12 adults. The RMSD was 7.8 units for Ep (11.7% cv), 0.3 for PWV (6.11% cv), 0.008 for CIMT (1.66% cv) and 0.08 for AC (8.83% cv).

**Identification of adults with metabolic syndrome**

Participants were classified as per the definition of the MS for Asian adults. They were considered to have the metabolic syndrome if they had any three of the following abnormalities:

1. Abdominal obesity (waist circumference >90 cm in men and > 80 cm in women)
2. Hyper-triglyceridemia ≥ 150 mg/dl
3. Low high density lipoprotein (HDL) cholesterol ≤ 40 mg/dl
4. High blood pressure ≥ 130/85 mm Hg
5. Fasting blood glucose ≥ 110 mg/dl

Adults were categorized according to the number of components of MS into one of four groups (normal with no risk components, 1-risk with any one component of MS as MS-1, 2-risk with any two components of MS as MS-2 and MS as with three or more components of MS as MS-3).

**Statistical analyses**

All the statistical analyses were performed using SPSS version 11.0 for windows. Prior to the statistical analyses, all the study parameters were tested for normality. The gender differences of various parameters were tested using Student’s t-test. Anthropometric, dietary, blood, CIMT and stiffness parameters were compared across categories of MS amongst MS-3 over normal using student’s t-test. Pearson’s correlation was used to examine association of serum zinc with stiffness parameters and CIMT. Differences in the stiffness parameters were tested between normal and MS with zinc sufficiency and deficiency using student’s t-test. Univariate analysis was performed to calculate adjusted means for stiffness parameters.

**Results**

**General characteristics of the study population**

Age of the study participants was between 30 to 50 years in both men and women respectively. Weight, height and waist were significantly higher in men than women, while hip circumference and percent body fat was higher in women than men (p<0.05) (Table 1). Mean BMI was higher than the Asian cutoff of 23 in 80% men and 81.3% women indicating high percentage of overweight and obesity. They were considered to have the metabolic syndrome if they had any three of the following abnormalities: abdominal obesity (waist circumference >90 cm in men and > 80 cm in women), hyper-triglyceridemia ≥ 150 mg/dl, low high density lipoprotein (HDL) cholesterol ≤ 40 mg/dl, high blood pressure ≥ 130/85 mm Hg and fasting blood glucose ≥ 110 mg/dl.

In biochemical parameters, hemoglobin was found to be normal in all the study adults (>12mg/dl) in females and (>14mg/dl) in males. Fasting sugar was normal in 88.8% men and 95.5% women (<110mg/dl). Sixty percent men and 66.9% women had total cholesterol within the normal range (<200mg/dl). Similarly, 64.3% men and 79.9% women had triglycerides within the normal range.
Table 1: General characteristics of the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Men (n=110)</th>
<th>Women (n=139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>39.5 ± 0.5</td>
<td>38.5 ± 0.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.4 ± 1.1</td>
<td>66.0 ± 1.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.2 ± 0.7</td>
<td>155.8 ± 0.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.1 ± 0.4</td>
<td>27.2 ± 0.4</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>94.5 ± 1.4</td>
<td>87.5 ± 1.1</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>102.1 ± 0.9</td>
<td>106.9 ± 1.1</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>34.6 ± 0.6</td>
<td>46.6 ± 0.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125.2 ± 1.0</td>
<td>122.8 ± 1.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82.6 ± 0.6</td>
<td>79.9 ± 0.8</td>
</tr>
</tbody>
</table>

(All the values are expressed in mean ± SE; Significance level was set at 0.05; *indicates p<0.05; **indicates p<0.01)

Table 2: Biochemical and metabolic parameters of the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Men (n=110)</th>
<th>Women (n=139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting serum glucose (mg/dl)</td>
<td>96.7 ± 1.9</td>
<td>88.6 ± 1.9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>191.3 ± 4.2</td>
<td>188.2 ± 3.5</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>121.5 ± 3.5</td>
<td>117.7 ± 2.7</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>43.4 ± 1.2</td>
<td>48.6 ± 1.0</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>142.5 ± 8.2</td>
<td>111.3 ± 5.4</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>14.7 ± 1.3</td>
<td>11.8 ± 0.9</td>
</tr>
<tr>
<td>Apo-lipoprotein B (mg/dl)</td>
<td>83.6 ± 2.3</td>
<td>83.1 ± 2.2</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>14.7 ± 0.1</td>
<td>12.6 ± 0.3</td>
</tr>
<tr>
<td>Serum zinc (mg/L)</td>
<td>0.79 ± 0.3</td>
<td>0.80 ± 0.3</td>
</tr>
<tr>
<td>Intima media thickness (mm)</td>
<td>0.45 ± 0.008*</td>
<td>0.41 ± 0.006*</td>
</tr>
<tr>
<td>β (stiffness index)</td>
<td>6.8 ± 0.2</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>Elasticity modulus (Kpa)</td>
<td>91.3 ± 3.2</td>
<td>86.1 ± 3.1</td>
</tr>
<tr>
<td>Arterial compliance (mm²/Kpa)</td>
<td>0.77 ± 0.02*</td>
<td>0.71 ± 0.02*</td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td>5.9 ± 0.09</td>
<td>5.6 ± 0.09</td>
</tr>
</tbody>
</table>

(All the values are expressed in mean ± SE; Significance level was set at 0.05; *indicates p<0.05; **indicates p<0.01; *indicates p<0.01; when MS-3 is compared with normal (no-risk) BMI; Body mass index; LDL: Low density lipoprotein; HDL: High density lipoprotein; LDL: Low density lipoprotein; Apo-B: Apo-lipoprotein B)

Overall, zinc deficiency (serum zinc < 0.7 mg/L) was found to be 44.9% in men and 38.5% in women respectively. When percent zinc deficiency was estimated separately for normal subjects with MS, more subjects from MS group (48%) were found to be zinc deficient than normal group (38.1%).

Mean CIMT and compliance were significantly higher in men than women (p<0.05) whereas, other stiffness parameters were similar in both men and women (p>0.1) (Table 2).

Body mass index, hip circumference, BF%, LDL, insulin and Apo-B which are not components of MS but are known as CVD risk factors were found to increase with presence of increasing number of MS components in both the genders (Table 3). Mean anthropometric parameters such as BMI, BF% and hip circumference were significantly higher in MS-3 group than in the normal group in both the genders (p<0.05). Also, mean blood levels of LDL and Apo-B were significantly higher in MS-3 group than in the normal group in both men and women (p<0.05). Mean levels of serum zinc in normal subjects was significantly higher than in the normal group in both men and women (p<0.05).

Association of metabolic syndrome with CIMT and arterial stiffness parameters

Mean CIMT in the MS-group was higher as compared to normal group and the difference was marginal significantly with 6% increase in CIMT in MS-group over normal group (p=0.08). Across the three MS categories, increasing trend in CIMT was seen, however, it was not significant (p>0.1) (Figure 1). Similarly, a gradual change was seen in arterial stiffness parameters, with increasing number of MS components (Figures 2).
of metabolic syndrome

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (without MS)</th>
<th>With MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIMT (mm)</td>
<td>ZnS (41) (group A)</td>
<td>ZnD (21) (group B)</td>
</tr>
<tr>
<td>β</td>
<td>0.42 ± 0.01*</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>Ep (Kpa)</td>
<td>6.2 ± 0.4</td>
<td>5.4 ± 0.2*</td>
</tr>
<tr>
<td>AC (mm²/ Kpa)</td>
<td>0.77 ± 0.04</td>
<td>0.82 ± 0.03e</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>5.4 ± 0.2*</td>
<td>5.1 ± 0.14*</td>
</tr>
</tbody>
</table>

*Adjusted means

Discussion

As there is limited data regarding the prognostic significance of sub-clinical atherosclerosis in Indian individuals with MS, our data provide estimates of CIMT and stiffness parameters which are early markers for structural and functional changes in the carotid artery of apparently healthy Indian adults. MS components are found to be adversely associated with the functional markers of the carotid artery in the study population. To the best of our knowledge, for the first time the present study has demonstrated the synergistic effect of zinc deficiency along with MS on carotid arterial stiffness in Indian adults.

All the study participants were apparently healthy without any complications of CVDs, however, when the diagnostic criteria of MS was used to classify adults on the basis of prevalence of risk factors, around 36.6% adults were normal without any risk factors, 34% had one risk of MS, 17.4% showed presence of two risk of MS and 12.1% of the adults had three risk factors for MS. Percentage of adult with presence of MS found in our study was lower than those reported in other Indian studies, with prevalence up to 35% depending upon the definition and cut-offs used.

Study results showed a percent increase of 11.6% in stiffness and 6% in CIMT for MS-3 group which is comparable with a longitudinal Study by Scuteri et al. Besides this, we have observed an increasing trend in almost all the stiffness parameters with presence of increasing number of MS risk factors. Our results are consistent with other cross-sectional studies showing close association of MS traits with an increased PWV, an indirect marker of sub-clinical atherosclerosis.

CIMT is a useful surrogate marker of cardiovascular disease which also correlates with MS and insulin resistance in type 1 and 2 diabetics. Previous cross-sectional studies have shown that subjects with MS are at increased risk for progressive carotid atherosclerosis. Aldolphe et al. have shown that carotid IMT increased with increasing numbers of metabolic syndrome components.
syndrome components. Ryuichi\(^9\) showed that patients with MS had an increased mean IMT and an increase in the number of components of the metabolic syndrome was associated with an increase in mean CIMT. Similar trend was observed in the present study for CIMT in MS group.

Results revealed inverse association of serum zinc with stiffness of the artery only in the presence of MS indicating the risk of atherosclerosis involved with zinc deficiency. Thus, zinc could be another risk factor for the risk of atherosclerosis. Most of the cohort studies in western adults have demonstrated an association between the uses of micronutrient supplementation especially zinc and change in metabolic profile\(^6\) however, such studies in Indian context are scarce. Our study is first of its kind to report association of zinc with arterial stiffness in Indian adults with MS.

In conclusion, our findings, pertaining to an apparently healthy population, demonstrate early arterial stiffening even with moderate increase in metabolic risk factors, and therefore emphasize the importance of primary prevention of MS to avoid further complications of atherosclerosis. An important implication is that all individuals with even one risk of MS need to receive assessment for atherosclerosis, especially at younger age where one or moderate risk factor is easily overlooked in clinical practice. Our study, therefore, emphasize the need of a more intensive awareness and early monitoring of risk factor clustering in adults, especially after 30 years of age.

**Conflict of interest**

Authors declare no conflict of interest.

**Acknowledgement**

We would like to express our profound gratitude to all the participants for their cooperation and contribution towards this study. Our sincere thanks to Director, Hirabai Cowasji Jehangir Medical Research Institute for funding this study.

**List of Abbreviations**

- **MS**: Metabolic syndrome
- **CIMT**: Carotid intima media thickness
- **β**: stiffness index
- **PWV**: Pulse wave velocity
- **Ep**: Elasticity modulus
- **AC**: Arterial compliance
- **MS-1**: Adults with one MS component
- **MS-2**: Adults with two MS component
- **MS-3**: Adults with three MS component
- **CVD**: Cardiovascular diseases
- **CAD**: Coronary artery disease
- **HCJMRI**: Hirabai Cowasji Jehangir Medical Research Institute
- **DM**: Diabetes Mellitus
- **BMI**: Body mass index
- **LDL**: Low density lipoprotein
- **HDL**: High density lipoprotein
- **DXA**: Dual energy X-ray absorptiometry
- **RMSD**: Root mean square standard deviation
- **ZnS**: Zinc sufficiency
- **ZnD**: Zinc deficiency

**References**