Food and Nutrition Sciences, **2013, 4, 1021-1027**
http://dx.doi.org/10.4236/fns.2013.410133 Published Online October 2013 (http://www.scirp.org/journal/fns)

Inflammatory Biomarkers in Asian Indian Women with Metabolic Syndrome

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Received May 22nd, 2013; revised June 22nd, 2013; accepted June 29th, 2013

ABSTRACT

Cardiovascular diseases (CVD) are the leading cause of mortality necessitating its early detection. The emergence of newer subclinical biomarkers in addition to the known cardiometabolic risk factors may play an important role in early detection of CVD risk. In the present study, 74 adult females (30 - 75 y) with metabolic syndrome (MS) were selected and additional biochemical parameters such as C-reactive protein (CRP) and Homocysteine (Hcy) levels were analyzed. The average body mass index (BMI) and waist circumference of subjects were found to be 30 kg/m^2 and 99 cm respectively. Mean LDL levels were found to be much higher than normal (139 mg/dl) while the HDL levels were low (41.5 mg/dl). The average fasting blood sugar and insulin levels were within the normal range. However, 40.5% females had serum Hcy levels >13.2 µmol/l and 59.5% women had CRP levels >3 mg/L indicating increased risk of CVD. Higher Hcy levels were associated with hyperinsulinemia ($p < 0.01$) and hyperglycemia ($p < 0.05$), indicating predilection for glucose intolerance. CRP levels showed significant negative correlation with HDL ($p < 0.05$), indicating a predilection for glucose intolerance. The present study reports overall more than 40% MS women are classified as high risk group using the Western standards. Limited data on normal levels of inflammatory biomarkers are available for Asian Indians. The study results indicate the importance of Hcy and CRP values among females having metabolic syndrome, known to be at a high risk of CVD.

Keywords: Metabolic Disorder; Inflammatory Biomarkers; Homocysteine; C-Reactive Protein; Asian Indians; Metabolic Syndrome

1. Introduction

Metabolic syndrome (MS) is defined as a cluster of the most dangerous heart attack risk factors: diabetes and prediabetes, abdominal obesity, high cholesterol and high blood pressure [1]. M. Thiruvagounder *et al.* (2010) [2] reported that people with MS are about twice as likely to develop cardiovascular disease (CVD) and over four times as likely to develop Type 2 diabetes compared to subjects without metabolic syndrome. Asian Indians have strong predisposition to MS and CVD [3], which has been shown through several studies on Indians living in India [4,5] and abroad [6,7].

Traditional CVD risk factors account for only around 50% of cardiovascular morbidity and mortality [8]. Thus, the importance and utility of newer predictors have been emphasized as an additional means of early detection of CVD risk [9]. These novel markers may be useful in predicting risk in addition to the conventional risk factors [10].

The 20th century has seen the emergence of substantial evidence to prove homocysteine (primarily atherogenic marker), C-reactive protein and fibrinogen (primarily thrombosis marker) [1,11] as the newer risk factors for CVD amongst Asian Indians.

Homocysteine (Hcy), a homologue of the naturally occurring amino acid cysteine, has been touted as one of the new-age inflammatory markers of CVD risk. In 1969, autopsy of children with homocystinuria revealed presence of severe atherosclerosis demonstrating pathogenic role of homocysteine [12]. Thereafter, the second phase of NHANES III (1991-1994) included study of homocysteine understanding its significance as more than just a marker of vitamin deficiency. A study by R. Carmel *et al.* (2002) [13] found that Asian Indian population residing in US have significantly higher Hcy levels compared

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to men from 4 other ethnic groups with 25.6% being hyperhomocysteinemic. They concluded that hyperhomocysteinemia is common among apparently healthy Asian Indian men accompanied by subclinical cobalamin deficiency.

Similarly, P.E. Szmitko *et al.* (2003) [14] reported that CRP may play a direct active role in the pathogenesis of atherosclerosis through its inflammatory action in blood vessels. C-reactive protein (CRP) is an acute phase reactant that increases in response to systemic inflammation such as infection or tissue injury [15]. Inflammation causes release of cytokines, especially Interlukin-6, which is largely responsible for triggering the production of CRP from the liver. Studies have shown that high sensitivity CRP consistently predicts new coronary events in patients with unstable angina and acute myocardial infarction [16,17].

The normal levels for the Homocysteine and CRP have been calculated for the Western population. The present study has assessed the Homocysteine and CRP level among Asian Indians with metabolic syndrome at risk for CVD and compared the value with the normal data.

2. Material and Methods

2.1. Sample

Female subjects in the age group of 30 - 75 yr were screened for the presence of 3 or more of the following symptoms to be classified as having metabolic syndrome [18]:

- Waist circumference > 80 cm in women
- Blood pressure (BP) ≥ 130/85 or medical treatment of previously diagnosed hypertension
- Triglycerides (TG) ≥ 150 mg/dL
- Low levels of High Density Lipoprotein (HDL) Cholesterol in women < 50 mg/dL
- Fasting blood sugar (glucose) ≥ 110 mg/dL

A total of 74 female were then enrolled based on written informed consent.

The proposal was approved by the Institutional Ethics committee of Department of Pharmacology, Grant medical College and Sir J.J. Group of Hospitals, Byculla, Mumbai.

The basic information of the subjects such as age, gender, caste was recorded. BMI and waist circumference were measured as per standard protocol.

2.2. Blood Sampling

5 ml of peripheral venous blood was collected by vein puncture using a dry, disposable syringe between 8 AM - 9 AM after an overnight fast (10.5 - 12 hours). Blood was collected in sterile tubes containing EDTA as anticoagulant for plasma separation. For serum separation, blood was collected without EDTA. Plasma and serum were separated by centrifugation at 3000 rpm for 15 min and were stored at 4˚C and used for various biochemical assays.

2.3. Biochemical-Analytical Methods

Glucose: Fasting blood glucose was estimated using the GOD-POD method [19].

Total cholesterol: Serum total cholesterol level was assayed by the kit method of CHOD-PAP with ATCS method [19-22].

Triglyceride: Serum triglyceride level was estimated by using the GPO-POD method with ESPAS [23-27].

HDL-cholesterol: HDL-cholesterol was estimated by the method of HDL precipitating reagent [28-33].

LDL-cholesterol: Serum LDL-cholesterol was estimated from the primary measurements by using the empirical equation of Friedewald *et al.* 1972 [34].

Serum LDL cholesterol = Total cholesterol – (HDL cholesterol – Triglyceride/5).

C-Reactive Protein: CRP Turbilatex with high sensitivity and specificity was used [35-41].

Homocysteine (*Hcy*): Automated enzymatic assay for homocysteine [42].

2.4. Statistical Analysis

Numerical data were presented as mean values ± S.D. Pearson's correlation co-efficient was used to test the magnitude of association between the inflammatory markers and different anthropometric and biochemical tests.

3. Results & Discussion

Subjects with metabolic syndrome were identified based on the guideline provided by modified National Cholesterol Education Program—Adult Treatment Panel III [18] for Asians. These include presence of higher abdominal waist circumference, high blood pressure, hypertriglyceridemia, low HDL cholesterol and. high blood glucose. Individuals exhibiting at least three or more of these parameters simultaneously were selected.

It has been noted that coronary artery disease occurs 10 years earlier among South Asian Indians compared to other populations across the world and develop metabolic abnormalities at a lower body mass index and waist circumference thereby leading to 25% to 50% underestimation of MS prevalence [43].

Table 1 shows the basic demographic profile of the subjects enrolled from the cosmopolitan city Mumbai, India. The average age of the women subjects was 50 years with majority being in obese category as indicated by a higher average BMI. BMI cut-offs for defining "overweight" and obesity in Asian Indians are 23 kg/m^2 and 25 kg/m^2 respectively [5].

3.1. Traditional Indicators for CVD Risk

3.1.1. Central Obesity and High Blood Pressure

Central obesity, high blood pressure and dyslipidemia are most common indicator for CVD risk. **Table 2** shows that female subjects had the typical signs of metabolic syndrome with higher waist circumference and higher mean systolic and diastolic blood pressure. A. Misra *et al.* (2006) [44] suggested revision of waist circumference cut-offs to above 80 cms among Asian Indians since metabolic abnormalities contributing to cardiovascular risk factors are detectable at a lower waist circumference in Asians.

3.1.2. Lipid Profile

Figure 1 shows that although the average total cholesterol was only slightly elevated as compared to the normal range, the increased LDL cholesterol and low HDL cholesterol levels independently may increase the risk of cardiovascular disease. Dyslipidemia among women subjects further increases the risk of atherosclerosis [45]. E. Dhanaraj *et al.* (2009) [46] noted that HDL-C was stronger predictor of MS among women compared to men.

3.1.3. Fasting Blood Sugar and Insulin Level

M. Thiruvagounder *et al.* (2010) [2] reported that people with MS are over four times as likely to develop type 2 diabetes compared to subjects without metabolic syndrome. As shown in **Table 3**, the average fasting blood sugar levels of the women was within normal range with only 8% women having levels greater than 100 mg/dL. The average fasting insulin levels were found to be within the normal range (**Table 3**). R. Mack *et al.* (2004) [48] reported significantly higher mean fasting insulin levels in individuals with diabetes (29.1 μIU/mL) compared to non-diabetic (15.8 μIU/mL) and only 9.2% non-diabetics having elevated insulin levels >27 μIU/mL compared to 29.7% diabetics. As the range for normal fasting insulin is very wide (2 - 25 μIU/mL), it may be difficult in predicting disease risk, especially with insulin resistant or insulin deficient diabetic condition.

In the present study, 72.9% women had serum insulin levels >10 μIU/mL. In a similar study on 45 non diabetic patients, fasting insulin level higher than 10 microIU/mL was associated with 11 fold increased probability of coronary artery disease [49].

Table 1. Demographic profile of the enrolled subjects (n = 74).

Parameter	Mean ± SD	Min	Max
Age (yrs)	50 ± 10	30	75
Weight (kg)	71.2 ± 12.4	50	105
BMI (kg/m^2)	29.9 ± 5	20.36	46.7

Table 2. Values of common indicators in selected subjects (n = 74).

Parameter	Mean ± SD	Min	Max	Ref values*
WC (cm)	98.7 ± 12	67	132	80 cm
BP (mmHg)	128/84	110/60	160/110	130/85
Systolic BP (mmHg)	128 ± 11.6	110	160	130
Diastolic (mmHg)	84 ± 8.4	60	110	85

*Modified NCEP ATP III 2006 [18].

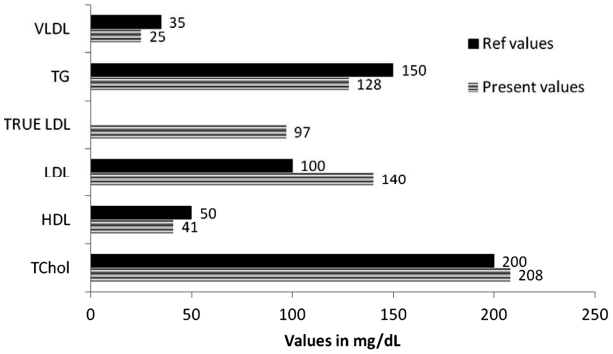

Figure 1. Lipid profile of metabolic syndrome subjects (AACE 2012 [45]).

Table 3. Blood sugar and insulin in women with metabolic syndrome (n = 74).

Parameter	Mean ± SD	Min	Max	Ref value
FBS (mg/dL)	86.6 ± 10.5	68	129	≥100[$]
Fasting Insulin (μIU/mL)	13.1 ± 6.9	4.21	47.7	NA
HbA1c (%)	6.01 ± 0.6	4.9	8.3	<6[&]

[$]NHLBI 2010, [&]I.A. Syed & W.A. Khan 2011 [47].

In the present study, the average fasting HbA1c levels were found to be within the normal range (**Table 3**).

3.1.4. Glycosylated Protein

HbA1C levels also have a strong association with metabolic syndrome that persists after adjusting for age and sex [50]. In the present study, the average fasting HbA1c levels were found to be within the normal range (**Table 3**).

3.2. New Indicators for CVD Risk

3.2.1. Homocysteine

Table 4 shows that the average Homocysteine (Hcy) levels of the female subjects were 14.4 μmol/l; higher than the range of normal serum homocysteine levels of 2.2 to 13.2 μmol/l [51]. 40.5% females had serum homocysteine levels greater than 13.2 μmol/l may indicate higher risk of cardiovascular disease.

Several studies among the Western population have

found that homocysteine levels up to 14 - 15 µmol/l was associated with significantly increased mortality rate [52-54]. Higher Hcy levels even at levels considered as normal are associated with increased health risks [55]. Experts suggest that target of <7 - 8 µmol/l is the optimal range that should be maintained in individuals over 30 years of age to prevent complications [56]. L. Broxmeyer (2004) [57] observed increased risk of atherosclerosis, heart attack and stroke in adults with Hcy values ≥ 6.3 µmol/l.

B.S. Raheja & M. Talim (2000) [58] have proposed that in Asian Indians, factors such as traditional cooking practices with prolonged heating of vegetables leading to destruction of folate, increased use of processed and refined cereal products deficient in vitamin B6 such as bread, cakes and cookies, and use of fibrates to lower triglycerides [59] can contribute to increased homocysteine levels in addition to faulty fat intake.

3.2.2. C-Reactive Protein

Figure 2 indicates that highly elevated CRP level (≥3 mg/L) was observed in 59.5% subjects. This supports the findings by A. Misra *et al*. [5] that adult Asian Indians have higher CRP levels than other populations with greater susceptibility to Type 2 diabetes mellitus and coronary heart disease. According to A. Misra *et al.* [5], elevated CRP in Asian Indians may be associated with excess body fat (subcutaneous fat) and physical inactivity but relationship with inflammation and protein deficiency need to be explored.

It was also observed that out of the 44 women with serum CRP levels ≥3 mg/L, 91% were in the obese category (BMI > 25 kg/m^2). Aronson *et al.* (2004) [60] have also reported significantly higher CRP levels in obese individuals compared to non-obese. Also, 79.5% of the women with elevated CRP levels were found to have waist circumference much higher than normal range (>89 cm).

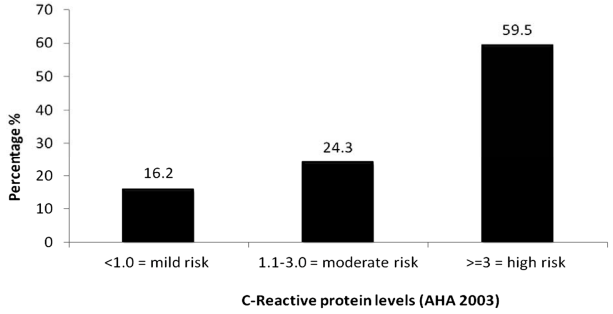

Figure 2. CRP risk stratification among the MS Subjects (n = 44).

Table 4. Inflammatory markers in metabolic syndrome subjects.

Parameter (n = 74)	Mean ± SD	Min	Max	Ref values
Hcy	14.4 ± 6	1.3	32.4	2 - 12 µmol/L
CRP	5.6 ± 4.5	0.3	18.3	<1 mg/L

The American Heart Association [61] suggested that hs-CRP may have a role in risk stratification of patients with established CVD, although more data are needed to compare the prognostic value of elevated levels of hs-CRP with other measures currently in use. Prospective data among apparently healthy men suggest that CRP levels may be useful in identifying those at an increased long-term risk of sudden cardiac death [62].

3.2.3. Correlation of Inflammatory Markers with Other Cardiometabolic Risk Factors

The association between the inflammatory markers such as Hcy and CRP and the known cardiometabolic risk factors (MS) was noted. The study attempts to validate the importance of these additional novel biomarkers in relation to CVD prevention.

In the present study, there was significant positive correlation noted between Hcy and fasting insulin and high blood glucose (**Table 5**), indicating that high Hcy levels may be associated with hyperinsulinemia ($p < 0.01$) and hyperglycemia ($p < 0.05$).

A weak positive correlation of CRP levels with BMI and fasting insulin levels was also noted. In a similar study on Indian males, N.K. Vikram *et al.* (2006) [63]

Table 5. Correlation of inflammatory markers with other cardiometabolic risk factors.

Parameters	CRP		HCY	
	r	p	r	p
Weight	0.187	0.116	−0.052	0.669
BMI	0.229	0.053	−0.003	0.979
WC	0.161	0.177	0.080	0.509
T-hol	−0.293*	0.012	−0.198	0.098
HDL-C	−0.281*	0.016	0.124	0.302
LDL-C	−0.197	0.099	−0.222	0.064
TG	0.012	0.922	0.050	0.677
ESR	0.346**	0.003	0.183	0.131
FBS	0.068	0.570	0.233*	0.050
Fasting Insulin	0.226	0.055	0.373**	0.001
HbA1c	0.171	0.148	−0.090	0.454
SysBP	0.131	0.296	−0.017	0.891
DiasBP	0.140	0.262	−0.065	0.602

*Significant at p value < 0.05, **Significant at p value < 0.01.

reported to have no correlation between markers of insulin resistance and hs-CRP levels.

It was observed that CRP levels negatively correlated with HDL-Cholesterol ($p < 0.05$) indicating that as CRP levels increase, the HDL-Cholesterol may decrease. But in 2007, J.S. Wasir *et al.* [64] reported no interrelation between CRP and low HDL levels in postmenopausal women, stating both as independent risk factors for cardiovascular disease risk.

In a recent study on 45 - 60 years old male patients suffering from CAD at Coimbatore, India, P. Aparna *et al.* (2010) [65] reported higher serum homocysteine and CRP levels as compared to controls.

4. Conclusion

While Asian Indians have greater genetic predisposition and susceptibility to metabolic diseases, there is a strong need to detect it in early stages to take appropriate preventive measures. Although, the significance and prognostic value of inflammatory markers such as Hcy and CRP have been studied and well established for assessing CVD risk in the Western population, we lack sufficient evidence to replicate the findings among Asian Indians. The risk stratification among Asian Indians may begin at much lower values than Western standards requiring us to revise and re-establish population-specific normal values. The present study showed significant positive correlation between Hcy and fasting insulin and high blood glucose. The monitoring and evaluation of inflammatory markers alongside the metabolic risk factors will enable better understanding of metabolic disease prevention and progression.

5. Acknowledgements

The report is a part of larger project on Subclinical Parameters of Patients with Metabolic Syndrome, sponsored by Tata Global Beverage Ltd. We greatly acknowledge the support from Tata Global Beverage Ltd.

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