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Original Article

Pathology Section

Enhanced Inflammatory Status in Patients with Simple Central Obesity in Absence of Metabolic Syndrome

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ABSTRACT

Introduction: Abdominal obesity is associated with cardiovascular risk factors. Metabolic Syndrome (MS) describes a constellation of metabolic abnormalities, including abdominal obesity. However, there is limited data on the comparison of inflammatory biomarkers in centrally obese subjects without MS.

Aim: To examine the biomarkers of inflammation in subjects with MS, central obesity without MS, and normal controls.

Materials and Methods: This was a cross-sectional study involving 501 subjects categorised into MS, central obesity without MS (COBXMS), and healthy controls (NC). Soluble biomarkers of inflammation {high sensitivity C-reactive protein (hs-CRP), Interleukin-6 (IL-6), Soluble Intercellular Adhesion Molecule-1 (sICAM-1), Soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) and soluble E-selectin} were measured.

Results: MS had significant elevation in all biomarkers, while COBXMS had higher concentrations of all biomarkers except sVCAM-1 compared to NC. COBXMS had elevated hs-CRP, IL-6 and sICAM-1 comparable with MS. Obesity categories of subjects (MS, COBXMS or NC) were associated with quartiles of each biomarker concentration ($p<0.001$). Waist circumference was significantly correlated and associated with all biomarkers, and was the independent predictor for sICAM-1 and E-selectin after correcting for confounding factors.

Conclusion: Centrally obese subjects without MS have elevated concentration of inflammatory biomarkers comparable to those with MS, suggesting the pivotal role of simple central obesity even in the absence of MS in inflammation and possible enhanced atherogenesis.

Keywords: E-selectin, Inflammation, sVCAM-1, sICAM-1

INTRODUCTION

The global epidemic of obesity is largely responsible for the increased prevalence of Metabolic Syndorme (MS) worldwide and it is projected that the incidence will continue to rise in the future. Globally, the number of overweight and obese individuals has increased from 857 million in 1980, to 2.1 billion in 2013 [1]. Malaysia is not spared from this epidemic and the prevalence of obesity is escalating at an alarming rate; from 5.8% in 1996 to 19.5% in 2011 [2,3]. Rapid urbanisation has seen obesity affecting the rural population as much as the urban dwellers, with an 11.2% prevalence of obesity reported in a rural population [4]. A nationwide survey revealed that the prevalence of MS in Malaysia ranged between 32.1-42.5% according to different definitions, which was higher than other Asian countries such as India, Hong Kong and China (6.1-25.8%) [5].

MS is a constellation of metabolic risk factors that increase the risk of developing cardiovascular disease and Diabetes Mellitus (DM). MS is associated with an approximate doubling of cardiovascular risk and more than five times higher risk for diabetes compared to those without the syndrome. In addition, MS is linked to a number of other co-morbidities such as non-alcoholic fatty liver disease, polycystic ovarian syndrome and obstructive sleep apnoea. Key components that make up MS are: central obesity, dysglycaemia or insulin resistance, elevated Blood Pressure (BP) and atherogenic dysglycaemia [6,7].

MS, central obesity, and insulin resistance have all been associated with a pro-inflammatory state and endothelial dysfunction, both of which are important factors in the pathogenesis of atherosclerosis and diabetes-related complications [8]. The chronic low-grade inflammation in these disorders characterised by abnormal production of cytokines. This is due to the excess adipose tissue, increased acute-phase reactants and inflammatory mediators such as high sensitivity C-Reactive Protein (hs-CRP), Tumour Necrosis Factor-alpha (TNF-α), Interleukin (IL)-1, IL-6 and IL-18, as well as activation of a network of inflammatory signalling pathways. Obese and MS subjects have been shown to have higher circulating concentrations of inflammatory cytokines than lean subjects [9], and increasing evidence indicates that this inflammatory response plays an important role in the development of associated co-morbidities such as atherosclerosis, insulin resistance, atherogenic dysglycaemia, hypertension, and a prothrombotic state [10-14].

Obesity, insulin resistance and endothelial dysfunction have been found to closely co-exist, but the mechanisms by which they are interrelated are complex and include visceral fat-derived metabolic products, hormones and cytokines [12]. Various metabolic abnormalities found in MS such as hypertension, dysglycaemia, obesity, insulin resistance, atherogenic dysglycaemia, excessive fatty acids and increased oxidative stress can individually impair endothelial function. Endothelial dysfunction is a pivotal early event in atherosclerosis, initiated by endothelial activation, contributing to plaque initiation and progression. Function of the endothelium can be assessed by evaluating endothelium-dependent vasodilation in response to pharmacological or mechanical stimuli, or by measuring circulating levels of endothelial activation biomarkers such as adhesion molecules [15,16].

Limited data are available on simultaneous comparison of inflammation biomarkers in MS, centrally obese subjects without MS, and normal controls to investigate the pattern of changes in these biomarkers with increasing clinical severity as the subject's progress from lean and healthy, towards central obesity and MS. Therefore, the present study was undertaken with the objectives to: (1) compare the soluble biomarkers of inflammation in drug-naive MS and centrally obese subjects without MS with normal controls; (2) investigate the association between biomarkers of inflammation

and obesity categories of subjects; (3) assess the correlation and association between these biomarkers with parameters of MS; and (4) evaluate whether any of the MS parameters are independent predictors for these biomarkers after correcting for various confounding factors.

MATERIALS AND METHODS

Study Design

This cross-sectional study involved 501 subjects aged 30-65-year-old, recruited from Specialist Clinics in Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForM) and Faculty of Medicine, Universiti Teknologi MARA, Sg. Buloh Campus, Jalan Hospital, 47000 Sg. Buloh, Selangor, Malaysia, and community health screenings programmes. The study was conducted in accordance with the Declaration of Helsinki. Institutional Research Ethics Committee approval {reference code: 600-RMI (5/1/6/01)} was obtained prior to the commencement of the study and all subjects gave written informed consent. For each subject, a set of questionnaire was completed and detailed history taking including smoking habits, alcohol intake and family history of premature Coronary Heart Disease (CHD) were documented.

Exclusion criteria for this study were those on oral hypoglycaemic agents or insulin, anti-hypertensive, or lipid-lowering medications, on long-term anti-oxidant or anti-inflammatory therapy, and subjects with chronic inflammatory disorders, malignancy or severe diseases that shorten life expectancy. The recruited subjects with DM, hypertension and/or dyslipidaemia were either newly diagnosed and/or drug-naive with regard to anti-diabetic, anti-hypertensive and lipid-lowering medications.

Recruited subjects were categorised into MS (n=223), central obesity without MS (COBXMS, n=182), and Normal Control (NC, n=96) groups.

MS was defined according to the 2006 International Diabetes Federation (IDF) definition [17]. Individuals were considered to have MS if they were centrally obese {Waist Circumference (WC) ≥90 cm in men, ≥80 cm in women} with at least 2 out of 4 of the following criteria: elevated Triglycerides (TG) ≥1.7 mmol/L; reduced High Density Lipoprotein cholesterol (HDL-c) <1.0 mmol/L in men, <1.3 mmol/L in women; elevated BP {systolic BP (SBP) ≥130 mmHg and/or Diastolic BP (DBP) ≥85 mmHg}; or elevated fasting plasma glucose (FPG) ≥5.6 mmol/L.

Subjects with WC of ≥90 cm for men and ≥80 cm for women of MS were grouped as COBXMS, while NC were those with WC <90 cm for men and <80 cm for women, BP <130/85 mmHg, FPG <5.6 mmol/L, TG <1.7 mmol/L and HDL-c ≥1.0 mmol/L in men and ≥1.3 mmol/L in women.

Anthropometric and Blood Pressure Measurement

Anthropometric measurements including height, weight, Body Mass Index (BMI), and WC were obtained using standardised techniques. Body weight and height (to the nearest 0.1 kg and 0.01 m, respectively) were measured in light clothing without shoes using a pre-calibrated Seca digital scale and height rod. BMI was calculated as weight (kg) divided by squared height (m^2). WC was measured to the nearest 0.5 cm using a measuring tape midway between the inferior margin of the last palpable rib and the top of the iliac crest. BP was taken using an automated BP monitor (Omron, USA) on the right arm with the subject in a seated position and the right arm supported at heart level, after at least five minutes rest. BP was measured three times for each subject and average of the last two readings was taken as the subject's BP [17].

Biochemical Analysis

Fasting 5 cc venous blood samples were collected and centrifuged at 3,500 rpm for seven minutes within two hours after collection. Serum and plasma were aliquoted and stored at -20°C until analysis. Fasting Plasma Glucose (FPG) was assayed using the hexokinase method, while Total Cholesterol (TC), TG, and HDL-c were measured by enzymatic reference methods on an automated analyser (Cobas Integra 400 plus, Roche Systems, Germany). Low-density lipoprotein cholesterol (LDL-c) concentration was calculated using Friedewald equation. The intra- and inter-assay coefficient of variation (CV) for FPG, TC, TG and HDL were 1.8% and 2.1%; 0.5% and 1.9%; 1.6% and 1.9%; and 1.1% and 1.0%, respectively.

Fasting insulin concentration was analysed using the electrochemiluminescence method (Elecsys 2010, Roche Systems, Germany) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the equation: {(Fasting insulin, μIU/mL×Fasting glucose, mmol/L) /22.5}. The intra- and inter-assay CV for fasting insulin were 1.9% and 2.6%, respectively.

Inflammatory biomarkers measured were hs-CRP, IL-6, soluble Intercellular Adhesion Molecule 1 (sICAM-1), soluble Vascular Cell Adhesion Molecule 1 (sVCAM-1) and soluble E-selectin.

hs-CRP measurement was based on the particle enhanced immunoturbidimetric method on an automated analyser (Cobas Integra 400 plus, Roche Systems, Germany); with intra- and inter-assay CV of 0.8% and 2.9%, respectively. IL-6, sICAM-1, sVCAM-1 and E-selectin were measured using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (eBioscience Bender MedSystems, Austria). The intra- and inter-assay CV of IL-6, sICAM-1, sVCAM-1 and E-selectin were 4.0% and 14.7%; 8.3% and 16.0%; 4.7% and 16.3%; and 5.9% and 14.2%, respectively.

STATISTICAL ANALYSIS

Statistical analyses were carried out using Statistical Package for Social Science Software (SPSS) version 16.0 (SPSS Inc. Chicago Il, USA 2008). Normality testing was determined by Kolmogorov Smirnov test. Data were expressed as mean±Standard Deviation (SD) or proportions (%). Data for fasting insulin, HOMA-IR and biomarkers of inflammation were $\log_{10}$ transformed to improve the normality. Comparison of continuous variables was performed using one-way Analysis of Variance (ANOVA), followed by post-hoc Scheffe or Dunnett's T3 test depending on the result of Levene statistics. Correlation between two continuous data was analysed by Pearson's correlation coefficient test. Association between categorical data was assessed using chi-square test. Multiple logistic regression analysis was used to identify the independent predictors for inflammatory biomarkers. Level of significance was set at $p<0.05$.

RESULTS

Baseline characteristics of the 501 study subjects are presented in [Table/Fig-1]. All three categories (MS, COBXMS and NC) were matched for age, gender, ethnicity and smoking status ($p>0.05$). The mean age (±SD) of the subjects were 47.6 (±8.4) years old; comprising of 336/503 (66.8%) females and 167/503 (33.2%) males.

Subjects with MS and COBXMS had higher BMI, WC, SBP and DBP compared to NC ($p<0.001$). With respect to serum biochemistry profile, MS and COBXMS had higher TG ($p<0.001$) and FPG ($p<0.001$ and $p<0.05$, respectively) with lower HDL-c concentration ($p<0.001$) compared to NC. Fasting insulin concentration and HOMA-IR were significantly higher in MS and COBXMS compared to NC, with higher values observed among the MS subjects.

With regards to biomarkers of inflammation, data analysis on all subjects showed that MS had significantly elevated concentrations of all biomarkers compared to NC; and higher sVCAM-1 and E-selectin compared to COBXMS. Meanwhile, COBXMS demonstrated significantly higher concentration of all biomarkers except sVCAM-1 compared to NC and comparable concentrations of hs-CRP, IL-6 and sICAM-1 with MS [Table/Fig-2].

Parameters	MS (n=223)	COBXMS (n=182)	NC (n=96)	p-value[1] (MS vs. NC)	p-value[2] (COBXMS vs. NC)	p-value[3] (MS vs. COBXMS)
Age (years)	48.7±8.4	47.0±8.5	46.2±7.8	NS	NS	NS
[b]Gender (% Male /Female)	(36.8/63.2)	(28.0/72.0)	(34.4/65.6)	NS	NS	NS
[b]Ethnicity (% Malay/Chinese/Indian/Bumiputera)	96.9/0.9/1.3/0.9	96.7/0.5/2.7/0.0	96.9/1.0/0.0/2.1	NS	NS	NS
[b]Current smoker (%)	12.1	8.8	14.3	NS	NS	NS
[a]BMI (kg/m^2)	29.5±4.1	28.9±4.3	21.9±2.5	***	***	NS
[a]WC (cm)	94.4±8.8	93.0±9.7	73.8±7.7	***	***	NS
[a]Systolic BP (mmHg)	138.7±22.4	125.4±16.5	112.8±10.0	***	***	***
[a]Diastolic BP (mmHg)	84.5±12.8	78.4±10.4	69.9±8.4	***	***	***
[a]TC (mmol/L)	6.0±1.1	5.6±0.8	5.6±1.0	*	NS	***
[a]TG (mmol/L)	2.3±1.2	1.3±0.4	1.0±0.3	***	***	***
[a]HDL-c (mmol/L)	1.1±0.3	1.4±0.3	1.6±0.4	***	***	***
[a]LDL-c (mmol/L)	3.8±1.0	3.6±0.7	3.5±1.0	NS	NS	*
[a]FPG (mmol/L)	7.4±3.3	5.1±0.6	4.9±0.5	***	*	***
[a]Log Insulin (µU/mL)	1.1±0.4	1.0±0.4	0.8±0.3	***	***	*
[a]Log HOMA-IR	0.7±0.4	0.5±0.3	0.3±0.3	***	**	***

[Table/Fig-1]: Baseline characteristics of subjects according to groups.
Data are expressed as amean±SD, or b percentage. * p<0.05, ** p<0.01, *** p<0.001, NS: not significant
MS: Metabolic syndrome; COBXMS: Central obesity without metabolic syndrome; NC: Normal controls; BMI: Body mass index; WC: Waist circumference; BP: Blood pressure; TC: Total cholesterol; TG: Triglycerides; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; FPG: Fasting plasma glucose; HOMA-IR; Homeostasis assessment of insulin resistance

Biomarker	MS (n=223)	COBXMS (n=182)	NC (n=96)	p-value[1] (MS vs. NC)	p-value[2] (COBXMS vs. NC)	p-value[3] (MS vs. COBXMS)
All subjects						
Log hs-CRP (mg/L)	0.54±0.3	0.49±0.3	0.27±0.2	***	**	NS
Log IL-6 (pg/mL)	0.65±0.2	0.69±0.2	0.55±0.2	**	***	NS
Log sICAM-1 (ng/mL)	2.75±0.2	2.76±0.2	2.62±0.2	***	***	NS
Log sVCAM-1 (ng/mL)	3.09±0.3	2.93±0.3	2.90±0.2	***	NS	***
Log E-selectin (ng/mL)	1.71±0.3	1.58±0.3	1.36±0.1	***	***	***
Males						
Log hs-CRP (mg/L)	0.47±0.3	0.46±0.3	0.31±0.2	NS	NS	NS
Log IL-6 (pg/mL)	0.66±0.2	0.67±0.2	0.51±0.1	**	**	NS
Log sICAM-1 (ng/mL)	2.75±0.2	2.77±0.2	2.61±0.2	**	**	NS
Log sVCAM-1 (ng/mL)	3.08±0.3	2.90±0.3	2.88±0.2	***	NS	**
Log E-selectin (ng/mL)	1.72±0.3	1.70±0.3	1.38±0.1	***	***	NS
Females						
Log hs-CRP (mg/L)	0.58±0.3	0.51±0.3	0.25±0.2	***	**	NS
Log IL-6 (pg/mL)	0.65±0.2	0.69±0.2	0.57±0.2	NS	**	NS
Log sICAM-1 (ng/mL)	2.75±0.2	2.75±0.2	2.62±0.3	**	**	NS
Log sVCAM-1 (ng/mL)	3.09±0.3	2.94±0.3	2.91±0.2	***	NS	***
Log E-selectin (ng/mL)	1.70±0.3	1.53±0.3	1.34±0.2	***	***	***

[Table/Fig-2]: Comparison of inflammatory biomarkers concentration between groups in all subjects and according to gender.
Data are expressed as mean±SD. *p<0.05, **p<0.01, ***p<0.001, NS: Not significant
MS: Metabolic syndrome; COBXMS: Central obesity without metabolic syndrome; NC: Normal controls; Hs-CRP: High sensitivity C-reactive protein; IL-6: Interleukin-6; Sicam-1: Soluble intercellular adhesion molecule-1; Svcam-1: Soluble vascular cell adhesion molecule-1

Data analysis performed after segregation of subjects according to gender showed that in males, hs-CRP and E-selectin concentrations were not significantly different across the three groups and between MS and COBXMS, respectively; and in females, the concentration of IL-6 in MS and NC was not significantly different. The rest of the biomarkers showed similar trends as observed in all subjects [Table/Fig-2].

Significant association was observed between obesity categories of subjects (MS, COBXMS or NC) with quartiles of each biomarker concentration (p<0.001). A higher proportion of MS subjects were found in the highest quartile of each biomarker compared to COBXMS and NC groups [Table/Fig-3].

Correlation and association analyses between MS parameters and biomarkers of inflammation showed that WC was strongly correlated and associated with all the biomarkers. Significant association was observed between WC and quartiles of each biomarker concentration (p<0.001). A higher proportion of centrally obese subjects (WC ≥90 cm for men, ≥80 cm for women) were found in the highest quartile of each biomarker compared to those without central obesity [Table/Fig-4].

Pertaining to the biomarkers, sVCAM-1 was significantly correlated and associated with all MS parameters except TG: WC (r=0.090, p<0.05; p<0.001, respectively), HDL-c (r=-0.142, p<0.01; p<0.01), SBP (r=0.200, p<0.001; p<0.001), DBP (r=0.176, p<0.001; p<0.01) and FPG (r=0.180, p<0.001; p<0.01). Meanwhile, E-selectin was significantly correlated and associated with all MS parameters: WC (r=0.332, p<0.001; p<0.001, respectively), HDL-c (r=-0.274, p<0.001; p<0.001), TG (r=0.240, p<0.001; p<0.001), SBP (r=0.262, p<0.001; p<0.001), DBP (r=0.229, p<0.001; p<0.001) and FPG (r=0.119, p<0.01; p<0.01). The quartiles of sVCAM-1 and E-selectin concentrations showed significant association with presence of MS parameters. A higher proportion of subjects with presence of MS risk factors were found in the highest quartile of sVCAM-1 and E-selectin compared to those without the risk factors [Table/Fig-5].

Biomarkers	MS n (%)	COBXMS n (%)	NC n (%)	p-value
Log hs-CRP (mg/L)				
Q1: <0.28	35 (20.5)	31 (23.7)	16 (53.3)	**<0.001**
Q2: 0.28-0.50	40 (23.4)	33 (25.2)	8 (26.7)	
Q3: 0.51-0.75	42 (24.6)	37 (28.2)	6 (20.0)	
Q4: ≥0.76	54 (31.6)	30 (22.9)	0 (0.0)	
Log IL-6 (pg/mL)				
Q1: <0.50	45 (21.8)	38 (21.6)	34 (38.2)	**<0.001**
Q2: 0.50-0.62	53 (25.7)	33 (18.8)	29 (32.6)	
Q3: 0.63-0.77	59 (28.6)	43 (24.4)	17 (19.1)	
Q4: ≥0.78	49 (23.8)	62 (35.2)	9 (10.1)	
Log sICAM-1 (ng/mL)				
Q1: <2.60	43 (20.2)	34 (19.1)	41 (43.6)	**<0.001**
Q2: 2.60-2.73	58 (27.2)	42 (23.6)	24 (25.5)	
Q3: 2.74-2.85	52 (24.4)	51 (28.7)	15 (16.0)	
Q4: ≥2.86	60 (28.2)	51 (28.7)	14 (14.9)	
Log sVCAM-1 (ng/mL)				
Q1: <2.81	38 (17.4)	52 (29.4)	28 (31.8)	**<0.001**
Q2: 2.81-2.96	40 (18.3)	49 (27.7)	29 (33.0)	
Q3: 2.97-3.16	56 (25.7)	45 (25.4)	24 (27.3)	
Q4: ≥3.17	84 (38.5)	31 (17.5)	7 (8.0)	
Log E-Selectin (ng/mL)				
Q1: <1.36	26 (11.8)	45 (25.0)	49 (60.5)	**<0.001**
Q2: 1.36-1.56	44 (20.0)	50 (27.8)	22 (27.2)	
Q3: 1.57-1.81	72 (32.7)	37 (20.6)	10 (12.3)	
Q4: ≥1.82	78 (35.5)	48 (26.7)	0 (0.0)	

[Table/Fig-3]: Association between obesity categories of subjects with quartiles of inflammatory biomarkers.
MS: Metabolic syndrome; COBXMS: Central obesity without metabolic syndrome; NC: Normal controls; Hs-CRP: High sensitivity C-reactive protein; IL-6: Interleukin-6; Sicam-1: Soluble intercellular adhesion molecule-1; Svcam-1: Soluble vascular cell adhesion molecule-1

Biomarkers	Waist circumference		p-value
	≥90 cm (♂); ≥80 cm (♀) n (%)	<90 cm (♂); <80 cm (♀) n (%)	
Log hs-CRP (mg/L)			
Q1: <0.28	66 (21.9)	16 (53.3)	**<0.001**
Q2: 0.28-0.50	73 (24.2)	8 (26.7)	
Q3: 0.51-0.75	79 (26.2)	6 (20.0)	
Q4: ≥0.76	84 (27.8)	0 (0.0)	
Log IL-6 (pg/mL)			
Q1: <0.50	83 (21.7)	34 (38.2)	**<0.001**
Q2: 0.50-0.62	86 (22.5)	29 (32.6)	
Q3: 0.63-0.77	102 (26.7)	17 (19.1)	
Q4: ≥0.78	111 (29.1)	9 (10.1)	
Log sICAM-1 (ng/mL)			
Q1: <2.60	77 (19.7)	41 (43.6)	**<0.001**
Q2: 2.60-2.73	100 (25.6)	24 (25.5)	
Q3: 2.74-2.85	103 (26.3)	15 (16.0)	
Q4: ≥2.86	111 (28.4)	14 (14.9)	
Log sVCAM-1 (ng/mL)			
Q1: <2.81	90 (22.8)	28 (31.8)	**<0.001**
Q2: 2.81-2.96	89 (22.5)	29 (33.0)	
Q3: 2.97-3.16	101 (25.6)	24 (27.3)	
Q4: ≥3.17	115 (29.1)	7 (8.0)	
Log E-Selectin (ng/mL)			
Q1: <1.36	71 (17.8)	49 (60.5)	**<0.001**
Q2: 1.36-1.56	94 (23.5)	22 (27.2)	
Q3: 1.57-1.81	109 (27.3)	10 (12.3)	
Q4: ≥1.82	126 (31.5)	0 (0.0)	

[Table/Fig-4]: Association between waist circumferences with quartiles of inflammatory biomarkers.
♂: Males; ♀: Females; hs-CRP: High sensitivity C-reactive protein; IL-6: Interleukin-6; sICAM-1: Soluble intercellular adhesion molecule-1; sVCAM- 1: Soluble vascular cell adhesion molecule-1

Parameters	Log sVCAM-1 (ng/mL)					Log E-Selectin (ng/mL)				
	Q1 <2.81	Q2 2.81-2.96	Q3 2.97-3.16	Q4 ≥3.17	p-value	Q1 <1.36	Q2 1.36-1.56	Q3 1.57-1.81	Q4 ≥1.82	p-value
HDL-c										
Low HDL-c	23 (16.5)	29 (20.7)	36 (25.9)	51 (36.7)	**0.001**	21 (14.9)	25 (17.7)	37 (26.2)	58 (41.1)	**<0.001**
Normal HDL-c	95 (27.6)	89 (25.9)	89 (25.9)	71 (20.6)		99 (28.3)	91 (26.0)	92 (26.3)	68 (19.4)	
TG										
≥1.7 mmol/L	41 (20.9)	37 (18.9)	48 (24.5)	70 (35.7)	**<0.001**	23 (11.7)	42 (21.4)	61 (31.1)	70 (35.7)	**<0.001**
<1.7 mmol/L	77 (26.8)	81 (28.2)	77 (26.8)	52 (18.1)		97 (34.0)	74 (26.0)	58 (20.4)	56 (19.6)	
SBP										
≥130 mmHg	39 (20.6)	34 (18.0)	47 (24.9)	69 (36.5)	**<0.001**	27 (14.1)	43 (22.5)	63 (33.0)	58 (30.4)	**<0.001**
<130 mmHg	78 (26.7)	83 (28.4)	78 (26.7)	53 (18.2)		92 (31.9)	73 (25.3)	55 (19.1)	68 (23.6)	
DBP										
≥85 mmHg	25 (18.0)	26 (18.7)	35 (25.2)	53 (38.1)	**<0.001**	24 (17.0)	20 (14.2)	47 (33.3)	50 (35.7)	**<0.001**
<85 mmHg	92 (26.9)	91 (26.6)	90 (26.3)	69 (20.2)		95 (28.1)	96 (28.4)	71 (21.0)	76 (22.5)	
FPG										
≥5.6 mmol/L	40 (22.5)	39 (21.9)	36 (20.2)	63 (35.4)	**0.001**	27 (15.2)	43 (24.2)	52 (29.2)	56 (31.5)	**0.001**
<5.6 mmol/L	78 (25.6)	79 (25.9)	89 (29.2)	59 (19.3)		93 (30.7)	73 (24.1)	67 (22.1)	70 (23.1)	

[Table/Fig-5]: Association between quartiles of sVCAM-1 and E-selectin with metabolic syndrome parameters.
sVCAM-1: Soluble vascular cell adhesion molecule-1; HDL-C: High density lipoprotein cholesterol; TG: Triglycerides; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose

In ascertaining which of the MS parameters were independent predictors for the biomarkers, this present study showed that WC was an independent predictor for sICAM-1 (p<0.001), FPG for sVCAM-1 (p=0.015), and WC and SBP for E-selectin (p<0.001), respectively, after correcting for various confounding factors i.e. age, gender, ethnicity, smoking status and lipid profile. Segregation according to gender showed that in men, DBP was an independent predictor for IL-6 (p=0.004), HDL-c and WC for sICAM-1 (p=0.017 and p=0.024, respectively) and SBP for E-selectin (p=0.005); whereas in women, WC was the independent predictor for sICAM-1 (p<0.001), after correcting for age, ethnicity, smoking status and lipid profile [Table/Fig-6].

Variables	Independent predictors	Constant	Beta	SE	Adjusted OR	95% CI	p-value
All subjects							
Log sICAM-1	WC	-3.966	0.045	0.008	1.046	1.029, 1.064	<0.001
Log sVCAM-1	FPG	-1.847	0.093	0.038	1.097	1.018, 1.183	0.015
Log E-Selectin	WC	-6.064	0.035	0.009	1.036	1.018, 1.055	<0.001
	SBP		0.021	0.005	1.021	1.011, 1.032	<0.001
Males							
Log IL-6	DBP	-3.861	0.042	0.015	1.043	1.013, 1.074	0.004
Log sICAM-1	HDL-c	-1.845	-1.791	0.752	0.167	0.038, 0.727	0.017
	WC		0.042	0.018	1.042	1.006, 1.081	0.024
Log E-selectin	SBP	-6.964	0.032	0.011	1.032	1.010, 1.055	0.005
Females							
Log sICAM-1	WC	-4.493	0.053	0.011	1.054	1.032, 1.077	<0.0001

[Table/Fig-6]: Independent predictors for the inflammatory biomarkers.
The model reasonably fits well. Model assumptions are met. There are no interaction and multicollinearity problem
Sicam-1: Soluble intercellular adhesion molecule-1; Svcam-1: Soluble vascular cell adhesion molecule-1; IL-6: Interleukin-6; WC: Waist circumference; FPG: Fasting plasma glucose; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HDL-C: High density lipoprotein cholesterol

DISCUSSION

Previous studies have reported significantly elevated biomarkers of inflammation in MS compared to subjects without MS [17,18], and in the centrally obese compared to lean subjects [19,20]. To the best of authors knowledge, this present study is the first study comparing the biomarkers of inflammation concurrently in MS vs. centrally obese subjects without MS vs. normal controls to investigate the pattern of changes in these biomarkers with increasing clinical severity as the subjects' progress from lean and healthy, towards central obesity and MS.

MS subjects have enhanced inflammation compared to NC, which was reflected by the significantly higher hs-CRP, IL-6, sICAM-1, sVCAM-1 and E-selectin concentrations. The present findings reinforced the results of previous studies associating MS with inflammation [17-20].

The COBXMS group demonstrated an augmented inflammatory state. They have significantly higher concentrations of all biomarkers except sVCAM-1 compared to NC. These findings are in accordance with previous studies which have reported a significant association between elevated hs-CRP [21-23], IL-6 [21,22] and E-selectin [24] with central obesity. However, these previous studies did not segregate between centrally obese subjects with and without MS to compare the status of inflammation between the two groups.

The present study highlighted the presence of enhanced inflammation in simple central obesity even in the absence of MS. This present study clearly demonstrated the COBXMS group had comparable concentrations of hs-CRP, IL-6 and sICAM-1 with those of MS subjects. To the best of knowledge, this is the first study to report that subjects with central obesity even without MS have enhanced inflammation comparable to those with MS. These findings suggest that central obesity even in the absence of MS may pose increased risk of atherosclerosis-related complications such as CAD, stroke and PVD.

Among the MS parameters, WC was found to be strongly correlated and associated with all inflammatory biomarkers. Significant association was observed between WC and quartiles of each biomarker concentration. A higher proportion of centrally obese subjects were found in the highest quartile of each biomarker compared to those without central obesity. Furthermore, WC was found to be an independent predictor for sICAM-1 and E-selectin after correcting for various confounding factors. The present findings are in parallel with those of Chen SJ et al., and Nishida M et al., which reported that WC was significantly correlated with and a significant determinant of hs-CRP and IL-6, respectively [20,22]. The association between increased WC with sVCAM-1 and E-selectin was previously described albeit in a different study population of elderly by Ingelsson E et al., while Moussavi N et al., found WC to be an independent predictor for sICAM-1 in type 2 DM patients and normal controls [23,24]. All these indicate that WC or central obesity plays a pivotal role in inflammation and needs to be the target of intervention in order to reduce inflammation.

With regards to the biomarkers, E-selectin was strongly correlated and associated with all MS parameters, while sVCAM-1 was correlated and associated with all parameters of MS except TG. The present data suggested that these two biomarkers have great potential for clinical utility as biomarkers of coronary risk in MS subjects. Miller MA and Cappuccio FP, studied the relationship between cellular adhesion molecules with obesity and MS, and found E-selectin to be significantly associated with measures of obesity, BP, HDL-c, TG and serum fasting insulin [25]. E-selectin showed the strongest and more robust association with measures of obesity and other cardiovascular risk factors compared to other adhesion molecules [25], which were in keeping with the present findings. E-selectin is rapidly synthesised in response to endothelial activation and its soluble plasma concentration may serve as an early marker of atherogenesis.

In this study, authors found that FPG was an independent predictor for sVCAM-1 and SBP for E-selectin after adjusting for age, gender, ethnicity, smoking status and lipid profile. Although the relationships between FPG and sVCAM-1 as well as BP and E-selectin have been described previously [26-28], they were for different study populations and not in the context of MS or central obesity. Osman MT et al., reported FPG as one of the independent factors related to sVCAM-1 levels in Type 2 diabetes patients apart from fasting C-peptide and TC [26]. Meanwhile, Miller MA and Cappuccio FP, reported that SBP, DBP and pulse pressure were significantly associated with E-selectin in women after adjustment for age, ethnicity, BMI and smoking [25]. Segregation according to gender revealed additional MS parameters that were independent predictors for the inflammatory biomarkers in men. DBP was the independent predictor for IL-6, and HDL-c for sICAM-1 in addition to WC. Chae CU et al., did report that all blood pressure measures including DBP were independent predictors for IL-6 levels [29]. However, their study involved the apparently healthy men population and not MS or centrally obese subjects [29]. Meanwhile, Calabresi L et al., reported that a low HDL-c level was the strongest independent predictor of a higher sICAM-1, though they did not find any significant difference in the level of cellular adhesion molecules between males and females [30].

The present findings suggest that each inflammatory biomarker is influenced differently by the MS parameters and intervention targets to reduce these biomarkers should be adjusted accordingly, with different emphasis on men and women.

It is important to note that all subjects recruited in this study were drug-naive and not subjected to any lifestyle or therapeutic intervention with anti-diabetic, anti-hypertensive and/or lipid-lowering medications, which are potential confounding factors with regards to inflammation. Lipid-lowering drugs such as statins have been shown to decrease inflammatory biomarkers such as hs-CRP, sICAM-1 or E-selectin [31,32], whilst anti-hypertensive and anti-diabetic medications such as glibenclamide have also been shown to reduce inflammation [33].

LIMITATION

Limitation of this study is the cross-sectional design, which can only prove association or correlation, but not cause and effect. Additional studies of a prospective, longitudinal nature would further shed light on the changes in inflammatory biomarkers as the subject's progress from lean and healthy, to central obesity, MS and development of complications such as CHD and Type 2 diabetes mellitus.

CONCLUSION

Centrally obese subjects without MS have elevated concentration of inflammatory biomarkers comparable to those with MS. WC was significantly correlated and associated with all biomarkers of inflammation; and was the independent predictor for sICAM-1 and E-selectin after correcting for the various confounding factors, suggesting the pivotal role of central obesity in inflammation. Strategies to reduce inflammation by lifestyle modifications or pharmacological interventions may be an important therapeutic target in centrally obese subjects even in the absence of MS. It is therefore important for future studies to examine the enhanced risk for coronary events in simple COB even in the absence of MS and its prevention with early strategic intervention.

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