Full Length Article

Chemerin is associated with markers of inflammation and predictors of atherosclerosis in Saudi subjects with metabolic syndrome and type 2 diabetes mellitus

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Abstract

Chemerin is a novel adipokine, suggested to be involved in insulin resistance in obesity and type 2 diabetes and may be an attractive candidate for assessing risk of atherosclerotic cardiovascular disease. The aim is to examine the relationship of chemerin and markers of inflammation, and predictors of atherosclerosis in the metabolic syndrome, and type 2 diabetes mellitus in Saudi Arabians. Twenty healthy control subjects (group I), 20 patients with type 2 diabetes mellitus (group II), in addition to 20 subjects with metabolic syndrome (group III) are examined by anthropometric and blood pressure measurement, laboratory investigations including fasting and post-prandial blood sugar, fasting serum insulin, lipid profile and serum chemerin and leptin levels. Moreover, tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)), high sensitivity C-reactive protein (hsCRP) levels, and interleukin 6 (IL6) are measured. Vascular health was assessed by the brachial–ankle pulse wave velocity (baPWV) and carotid intima-media thickness (IMT). Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR) and the cardio-vascular risk value were calculated. Our present study revealed a significant positive correlation between serum chemerin level and fasting and post-prandial blood sugar, IL6 and TNF-\(\alpha\) \(p<0.05\), respectively in group II and III. Moreover, IMT and baPWV are positively associated with chemerin in both groups \(p<0.01\), respectively but not with leptin. In group III, it was revealed that, chemerin, systolic blood pressure and waist circumference are risk factors determining baPWV values and the three variables together account

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1. Introduction

Adipose tissue is now considered as an active endocrine organ that secretes a large number of bioactive mediators (adipokines) that signal to brain, liver, skeletal muscle, and the immune system, the important metabolic organs in the body (Yan et al., 2012). These adipokines include adiponectin, leptin, omentin, resistin, retinol binding protein-4(RBP-4), tumor necrosis factor-α (TNF-α), interleukin-6 (IL6), vaspin, visfatin and chemerin (Yan et al., 2012). Dysregulation of pro-inflammatory and anti-inflammatory adipokines secretion in obesity may serve as a pathogenic link between obesity, insulin resistance and cardiovascular diseases (Roh et al., 2007; Goralski, 2007). Chemerin, also known as tazarotene induced gene 2 (TIG2) and retinoic acid receptor responder 2 (RARRES2), is a recently discovered adipokine that has been reported to act by its binding to the chemerin receptor (Chemerin R, chemokine like receptor 1, G protein-coupled receptor) (Roh et al., 2007). It is secreted as an 18 kDa inactive pro-protein and undergoes extracellular serine protease cleavage of the C-terminal portion of the protein to generate the 16 kDa active chemerin which is present in plasma and serum (Goralski et al., 2007). The functions of chemerin vary dependent on the ways it is cleaved. For example, chemerin 21−157 has a strong chemotactic effect and is responsible for an early inflammatory reaction of the immune cells, whereas chemerin 21−154 is anti-inflammatory via inhibiting macrophage activation (Du et al., 2009). Chemerin and its receptor/ChemR23 are expressed abundantly in adipose tissue, suggesting its function in autocrine/paracrine fashion (Ernst et al., 2012). Type 2 diabetes mellitus is a group of disorders characterized by hyperglycemia and associated with microvascular and macrovascular complications. Hyperglycemia results from lack of endogenous insulin or resistance to the action of insulin in muscle, fat and liver in addition to an inadequate response by the pancreatic beta cells (Wolfs et al., 2009).

It was originally reported to be present in circulation in plasma and serum, respectively, at 3.0 and 4.4 nM concentrations in humans, and 0.6 and 0.5 nM concentrations in mice (Goralski et al., 2007). Serum chemerin concentrations are elevated in obese, insulin-resistant, and inflammatory states in vivo and suggested to be an obvious cause of insulin resistance (Hart and Greaves, 2010).

It may link obesity and inflammation since chemerin is a pro-inflammatory cytokine that recruits and activates immune cells and contributes to inflammation by promoting macrophage adhesion to vascular cell adhesion molecule-1 (VCAM-1) and fibronectin (Ouchi et al., 2011). Pulse wave velocity (PWV) is a marker of arterial stiffness (Asmar et al., 1995; Lehmann, 1999 and Yufu et al., 2004) and a reliable indicator of vascular damage. It is not only a marker of vascular damage (Cohn, 1999; van Popele et al., 2001) but also a prognostic predictor (Cruickshank et al., 2002; Laurent et al., 2003; Pannier et al., 2005).

Obesity and atherosclerosis are increasingly viewed as inflammatory states. Biomarkers that integrate metabolic and inflammatory signals may be attractive candidates for assessing risk of atherosclerotic cardiovascular disease (Rajala et al., 2003).

The accumulation of chemerin in an atherosclerotic lesion could attract immune cells which add to the remodeling of the vessel wall, the alteration of insulin sensitivity and glucose uptake in adipocytes and skeletal muscle and the direct inflammatory effect on vascular endothelial cells all could contribute to development of atherosclerosis (Yamawaki, 2011).

A cluster of coronary heart disease (CHD) risk factors including high blood pressure, dyslipidemia, hyperglycemia and central obesity is known as the metabolic syndrome that is associated with decreased ability of insulin to stimulate glucose disposal on peripheral target tissues (Oluwafide and Byrne, 2008).

Obesity, particularly central obesity, is the prominent risk factor for insulin resistance and results in type 2 diabetes and metabolic syndrome (Zeyda and Stulnig, 2009). In addition to its immunomodulatory effects, chemerin was reported to be associated with components of the metabolic syndrome and the parameters of type II diabetes (Bozaoglu et al., 2007) including body mass index (BMI), plasma triglyceride (TG) levels, and blood pressure. Chemerin was shown to modulate the expression of adipocyte genes involved in glucose and lipid homeostasis such as glucose transporter-4, fatty acid synthase, adiponectin, and leptin (Goralski, 2007) and to enhance insulin signaling in 3T3-L1 adipocytes (Takahashi et al., 2008). Recently, it was reported that CMKLR1 is expressed in vascular endothelial cells and its expression is up-regulated by tumor necrosis factor (TNF)-α, interleukin (IL)-1β, or IL-6 (Kaur et al., 2010). However, mechanisms of actions of chemerin on vascular endothelial cells remain to be fully clarified. The known junction of adipocyte and macrophage function of chemerin may provide an interesting link between obesity, inflammation, and atherosclerosis and diabetes mellitus type 2 in humans (Lehrke et al., 2009).

As chemerin has a regulatory role in adipogenesis and adipocyte metabolism, and influencing chemerin and chemerin R signaling might to lead to novel therapeutic approaches in the treatment of obesity, diabetes mellitus type 2, and cardiovascular diseases (Goralski et al., 2007).

The aim of the present study is to examine the relationship of chemerin, markers of inflammation and predictors of atherosclerosis in the metabolic syndrome and type 2 diabetes, and to investigate its correlation with clinical and laboratory parameters of these conditions in Saudi Arabians.
2. Subjects and methods

2.1. Subjects

2.1.1. Control group I (n = 20)
This group included 15 healthy male subjects and 5 females. They were whose ages ranged from 45.00 to 68.00 year (52.15 ± 5.87).

2.1.2. Patients’ group (n = 40)
This group included forty patients attending to Internal Medicine Clinics for medical investigations in different hospitals in Taif KSA. They were 22 males and 18 females. They were further divided into two groups.

2.1.2.1. Group II; patients with type 2 diabetes mellitus (n = 20). This group included twenty patients, 12 males and 8 females, whose ages ranged from 41 to 56 years (Mean 49.25 ± 3.99). Diagnosis of diabetes was according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus in 1997 and the follow-up reports in 2003. We identified Type 2 diabetes mellitus by one of the following criteria: i) Symptoms of diabetes mellitus plus random blood sugar concentration ≥200 mg/dL. ii) Fasting blood sugar (FBS) ≥126 mg/dL on more than one occasion. iii) 2 h post prandial plasma glucose concentration ≥200 mg/dL during oral glucose tolerance test.

2.1.2.2. Group III: patients with metabolic syndrome (n = 20). This group included twenty non-diabetic patients. They were 10 males and 10 females, whose ages ranged from 45.00 to 61.00 years (Mean 51.20 ± 4.40). The metabolic syndrome was identified by the criteria of the National Cholesterol Education Program Adult Treatment Panel III (2002) (NCEP ATP III) guidelines, as described by Lin et al., 2009.

Hypertension was defined as a systolic blood pressure of ≥130 mmHg and/or diastolic blood pressure of ≥85 mmHg on repeated measurements, or the patient receiving antihypertensive treatment. (ii) Abdominal obesity was defined as waist circumference exceeding 102 cm and 88 cm in men and women, respectively. (iii) Fasting serum triglycerides more than 150 mg/dL (1.7 mmol/L) or patient receiving treatment for this lipid abnormality. (iv) Fasting serum HDL-C less than 40 mg/dL (1.0 mmol/L) and 50 mg/dL (1.3 mmol/L) in men and women, respectively or patient receiving treatment for this lipid abnormality. (v) Fasting blood sugar ≥110 mg/dL (6.1 mmol/L) Metabolic syndrome was diagnosed by the presence of three or more of the previous criteria (Grundy et al., 2004).

2.2. Exclusion criteria

A number of clinical conditions, were excluded e.g. chronic liver disease, hepatitis B or hepatitis C virus infection, liver cell failure as they were known to be associated with increased serum chemerin levels (Marra and Bertolani, 2009), chronic kidney disease and renal failure (Hu and Feng, 2011). All individuals included in this study were subjected to: Full history taking: focusing on family history of type 2 diabetes mellitus, hypertension, smoking and physical activity. All participants gave informed consent and the study followed the rules of the Medical National Committee for Medical and Bio-ethics. Thorough clinical examination including: waist circumference and blood pressure measurement. Mean arterial blood pressure (MAP) was also calculated. MAP = ([2 × diastolic] + systolic)/3. Laboratory investigations that included: fasting blood sugar, post-prandial blood sugar, fasting insulin, lipid profile and serum chemerin and leptin levels. The Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR) and the cardio-vascular risk value were calculated.

2.3. Sampling

Five ml of venous blood were collected after 12–14 h fasting under complete aseptic precautions in plain test tubes without anticoagulant. After coagulation, samples were centrifuged (at 1500 × g for 15 min). The separated serum was divided into three aliquots. One was designated for the immediate assay of fasting glucose and lipid profile. The other two aliquots were stored at ~20 °C for subsequent assay of insulin and chemerin. Hemolysed samples were discarded. Repeated freezing and thawing was avoided.

2.4. Methods

2.4.1. Analytical methods

2.4.1.1. Serum glucose level. It was assayed using Synchron CX-9 (Instruments Inc.; Scientific Instruments Division, Fillerton, CA 92634, 3100, USA.) system autoanalyzer applying enzymatic colorimetric method (Carroll et al., 1970).

2.4.1.2. Total cholesterol (TC). The analysis was done using the Synchron CX-9 system auto-analyzer applying enzymatic colorimetric method (Dietschy et al., 1976).

2.4.1.3. Triglycerides (TG). The analysis of TG was assayed using the Synchron CX-9 system auto-analyzer applying enzymatic colorimetric method (McGowan et al., 1983).

2.4.1.4. High density lipoprotein cholesterol (HDL-C). It was assayed on the Synchron CX-9 system auto-analyzer after precipitation of LDL and VLDL by dextran sulfate and magnesium in the separating reagent. The LDL and VLDL portions were then removed by centrifugation. The cholesterol in the HDL fraction which remains in the supernatant was assayed with an enzymatic timed endpoint method (Assman et al., 1983).

2.4.1.5. Low density lipoprotein cholesterol (LDL-C). It was calculated according to “Friedewald equation”:

\[
\text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{TG}/5)
\]

This equation was applied provided that serum TG level is <400 mg/dL (Friedewald et al., 1972 and Warnick et al., 1990).

2.4.1.6. CAD-risk percentage. Cardiovascular risk was calculated as HDL-C/TC% (Carl et al., 2006).

2.4.1.7. Insulin. Insulin was assayed by Micro-particle Enzyme Immunoassay (MEIA) on the AxsYM (Abbott...
Table 1 — Descriptive statistics of the studied parameters in the different studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Diabetic group</th>
<th>Metabolic syndrome</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52.15 ± 5.87</td>
<td>51.20 ± 4.40</td>
<td>49.25 ± 3.99</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>(45.00–68.00)</td>
<td>(45.00–61.00)</td>
<td>(41.00–56.00)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.55 ± 3.24</td>
<td>158.90 ± 2.36a</td>
<td>161.85 ± 3.22c</td>
<td>&lt;.05</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.41 ± 1.22</td>
<td>27.31 ± 2.27a</td>
<td>29.53 ± 2.04bc</td>
<td>&lt;.05</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>81.25 ± 6.21</td>
<td>101.35 ± 6.74a</td>
<td>118.1 ± 3.99bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120.80 ± 5.82</td>
<td>123.45 ± 2.82a</td>
<td>137.40 ± 2.93bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>68.00–91.00</td>
<td>89.00–115.00</td>
<td>112.00–125.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>76.30 ± 4.22</td>
<td>156.30 ± 9.24a</td>
<td>117.00 ± 4.69bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PPBS (mg/dL)</td>
<td>112.80 ± 6.78</td>
<td>176.85 ± 11.17a</td>
<td>126.40 ± 3.95bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>FSI (µU/mL)</td>
<td>4.70 ± 0.74</td>
<td>12.65 ± 1.27a</td>
<td>11.60 ± 1.31c</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>22.23 ± 2.43</td>
<td>3.69 ± 1.25a</td>
<td>3.74 ± 1.25a</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>NS</td>
</tr>
<tr>
<td>CHOL (mg/dL)</td>
<td>181.65 ± 11.20</td>
<td>195.05 ± 8.08a</td>
<td>229.55 ± 15.14bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>160.00–200.00</td>
<td>180.00–213.00</td>
<td>210.00–252.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>106.82 ± 12.17</td>
<td>125.04 ± 9.35a</td>
<td>161.85 ± 15.52bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>88.80–127.40</td>
<td>109.60–152.80</td>
<td>140.00–185.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD-RISK (%)</td>
<td>73.50 ± 3.88</td>
<td>45.90 ± 5.36a</td>
<td>30.50 ± 2.06bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CRP (mg/ml)</td>
<td>110.30 ± 5.16</td>
<td>120.50 ± 6.44a</td>
<td>186.00 ± 4.83bc</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IL6 ng/mL</td>
<td>3.60–15.80</td>
<td>9.20–25.80</td>
<td>11.51–16.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα (pg/L)</td>
<td>7.46 ± 2.98</td>
<td>15.13 ± 4.76a</td>
<td>13.25 ± 1.21b</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>NS</td>
</tr>
<tr>
<td>Carotid-IMT (mm)</td>
<td>1.50–3.40</td>
<td>3.40–6.70</td>
<td>2.30–5.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemerin (ng/dL)</td>
<td>.66 ± .042</td>
<td>.69 ± 0.3a</td>
<td>.63 ± .03b</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.00–3.50</td>
<td>1.50–2.50</td>
<td>1.50–2.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVV (m/sec)</td>
<td>12.98 ± 1.23</td>
<td>14.53 ± 2.52a</td>
<td>15.05 ± 2.34b</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>NS</td>
</tr>
</tbody>
</table>
| P1 a = Diabetic group compared to control group. 
P2 b = Metabolic syndrome compared to control group. 
P2 c = Metabolic syndrome compared to diabetic group.

2.4.1.9. Chemerin. Chemerin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by AVISCEMA BIOSCIENCE 2348 Walsh Ave. Suite C Santa Clara, CA 95051 USA.

2.4.1.10. Leptin. Plasma leptin concentration was measured by commercially available DSL Active TM Human Leptin Enzyme-Linked Immunosorbent ELISA kit (Diagnostic Systems Laboratories, USA (Hanigaya et al., 1997)). The interassay CV for this method was <$7%.

Ireland, Diagnostic Division-Lisnamuck, Longford Co. Longford, Ireland +353-43-31000 for the quantitative determination of insulin in human serum or plasma.

2.4.1.8. The homeostasis model assessment-insulin resistance index (HOMA-IR). It was calculated using the equation:

\[
\text{HOMA-IR} = \frac{\text{fasting glucose (mg/dL)}}{\text{fasting insulin (µU/mL)}} \times 405
\]

The cutoff point to define insulin resistance corresponds to HOMA-IR ≥3.8 (Shirai, 2004).
3. Measurement of pulse wave velocity

baPWV was measured using a volume plethysmographic apparatus (model BP-203RPE, Colin, Co.Ltd. Komaki, Japan). Details of this method, including the validity and reproducibility, have been described previously by Yamashina et al. The intra- and inter-observer reproducibility of this method was 10.0% and 8.4%, respectively. In the current study, the baPWV was calculated as the mean of the left and right baPWV values. Briefly, after 15 min of rest, the subjects were examined while resting in the supine position. Waveform data were obtained from a volume plethysmographic sensor in cuffs on both brachiums and both ankles, and time intervals (T) between the wave front of the brachiums and that of the ankles were calculated. The distance (L) between the heart and sampling points was calculated automatically according to the subject’s height. baPWV was calculated using the following formula: \[ \text{baPWV} = \frac{L}{T} \] (L = La–Lb, where La is the path length from the heart to ankle, and Lb is the path length from the heart to brachium). In the present study, the measurement of baPWV was performed on a different occasion from the blood collection so that the participant would be mentally relaxed.

4. Measurement of carotid IMT

The IMT of the common carotid artery was determined using high-resolution B-mode ultrasonography (EnVisor; Philips Medical Systems, Andover, MA, USA) with a 5–12 MHz transducer. Carotid IMT was measured using IMT measurement software (Intima scope; Media Cross Co., Tokyo, Japan) at 3 levels of the lateral and medial walls of the carotid artery, 1–3 cm proximal to the carotid bifurcation. The IMT was the average value of 99 computer-based points in the region. In the present study, the carotid IMT was calculated as the mean of the left and right IMT value. All measurements were recorded by one trained technician who was blinded to the subject’s anthropometric and laboratory data.

5. Statistical analysis

Data are expressed as the mean ± SD. Each variable was assessed for a normal distribution using the Kolmogorov–Smirnov test. Statistical differences between the groups were identified using one-way analysis of variance (ANOVA) followed by post-test. Pearson’s correlation test was performed to determine the relationships between serum chemerin levels and study variables, including the baPWV and mean carotid IMT values. Multiple linear step-wise regression analysis with baPWV as a dependent variable was performed to identify the risk factors which determined the baPWV. All statistical results were based on two-sided tests. Data were analyzed using Package for Social Sciences (SPSS) software for Windows (version 12.0; SPSS Inc., Chicago, IL, USA). P < 0.05 was regarded as significant.

6. Results

The results obtained in the present study in Table 1, showed that weight, BMI, WC, Systolic blood pressure, diastolic blood pressure, FBS, PPBS, Cholesterol, TG, Ldl, IL6, TNF-α, carotid IMT, serum chemerin levels and serum leptin were significantly higher in metabolic syndrome group when compared to type 2 diabetes mellitus and control groups (p < 0.001, p < 0.05 respectively). All the above parameters are significantly higher (p < 0.001) in type 2 diabetes mellitus group when compared to control group. Moreover, height was significantly higher in metabolic syndrome group when compared to type 2 diabetes mellitus group (p < 0.001) but not significantly different from control group (p > 0.05).

Fasting serum insulin was significantly higher (p < 0.001) in type 2 diabetes mellitus group and metabolic syndrome group than in the control group but it was significantly higher (p < 0.05) in type 2 diabetes mellitus group than in metabolic syndrome group. HOMA-IR was significantly higher (p < 0.001) in type 2 diabetes mellitus group and metabolic syndrome group than in the control group but it was not significantly different in type 2 diabetes mellitus group compared to metabolic syndrome group (p > 0.05). HDL was significantly lower (p < 0.001) in metabolic syndrome group than in diabetic and control groups, also it was significantly lower in type 2 diabetes mellitus group than in control group.

CAD-risk percentage, hs-CRP and baPWV were significantly higher (p < 0.001) in metabolic syndrome group and in type 2 diabetes mellitus group when compared to control group. However, they were not significantly different in type 2 diabetes mellitus group compared to metabolic syndrome group (Table 1). The correlation study between serum

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**Table 2 – Correlation analysis between serum chemerin level and other studied parameters in diabetic and metabolic syndrome groups.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum chemerin level in DM</th>
<th>Serum chemerin level in MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>R² 0.329</td>
<td>0.439</td>
</tr>
<tr>
<td>WC</td>
<td>R² 0.658*</td>
<td>0.706*</td>
</tr>
<tr>
<td>SBP</td>
<td>R² 0.511*</td>
<td>0.440*</td>
</tr>
<tr>
<td>DBP</td>
<td>R² 0.504*</td>
<td>0.461*</td>
</tr>
<tr>
<td>FBS</td>
<td>R² 0.536*</td>
<td>0.454*</td>
</tr>
<tr>
<td>PPBS</td>
<td>R² 0.496*</td>
<td>0.423*</td>
</tr>
<tr>
<td>FSI</td>
<td>R² 0.314</td>
<td>— 0.140</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>R² 0.532*</td>
<td>0.636*</td>
</tr>
<tr>
<td>CHOL</td>
<td>R² 0.468*</td>
<td>0.751*</td>
</tr>
<tr>
<td>Ldl</td>
<td>R² 0.314</td>
<td>— 0.152</td>
</tr>
<tr>
<td>HDL</td>
<td>R² 0.202</td>
<td>— 0.577*</td>
</tr>
<tr>
<td>TG</td>
<td>R² 0.154</td>
<td>0.308</td>
</tr>
<tr>
<td>CAD-risk</td>
<td>R² 0.177</td>
<td>0.475*</td>
</tr>
<tr>
<td>CRP</td>
<td>R² 0.386*</td>
<td>0.539*</td>
</tr>
<tr>
<td>IL6</td>
<td>R² 0.746*</td>
<td>0.550*</td>
</tr>
<tr>
<td>TNF</td>
<td>R² 0.804*</td>
<td>0.527*</td>
</tr>
<tr>
<td>IMT</td>
<td>R² 0.821*</td>
<td>0.678*</td>
</tr>
<tr>
<td>Leptin</td>
<td>R² — 0.008</td>
<td>— 0.025</td>
</tr>
<tr>
<td>PWV</td>
<td>R² 0.486*</td>
<td>0.808*</td>
</tr>
</tbody>
</table>

*a Correlation is significant at the 0.05 level (2-tailed).

*b Correlation is significant at the 0.01 level (2-tailed).
chemerin level and other studied parameters in (Table 2) revealed a significant positive correlation ($p < 0.05$) between serum chemerin level and both FBS and PPBS in type 2 DM and MS group. Moreover, a significant positive correlation was found between serum chemerin levels and each of IL6 and TNF-$\alpha$. Moreover, it is positively associated with carotid IMT and baPWV in both groups in (Fig. 1) compared to leptin which showed no significant correlation ($p < 0.01$, respectively) with IMT and baPWV. Our correlation study also revealed a significant negative correlation between serum chemerin level and HDL-C and a significant positive correlation ($p < 0.05$) with CAD-risk in metabolic syndrome group. Moreover, a significant positive correlation ($p < 0.05$) was found between serum chemerin levels and each of systolic blood pressure, diastolic blood pressure, waist circumference, HOMA-IR and total cholesterol in both groups. However, a non-significant correlation ($p > 0.05$) was observed between serum chemerin and the rest of other parameters in both groups, (Table 2).

Stepwise multiple regression analysis in metabolic syndrome group revealed that chemerin, SBP and WC are risk factors in determining baPWV values. Chemerin alone in model 1, chemerin together with SBP in model 2 and chemerin, SBP and WC in model 3 account for 46.0% ($F = 15.308, p = 0.001$) and 69.7% ($F = 19.586, p < 0.001$) and 76.9% ($F = 17.713, p < 0.001$) of the variance in baPWV values respectively (Table 3).

### 7. Discussion

Metabolic syndrome (MetS) is a collection of physical and laboratory abnormalities, including hypertension, hyperglycemia, hyperlipidemia and abdominal obesity. MetS is now considered as a significant risk factor for cardiovascular disease and mortality in the general population (Isomaa et al., 2001). Early prediction of the risk for CAD in patients with MetS is important for prevention strategies. Serum biomarkers are important tools for prediction, diagnosis, risk stratification and therapeutic decision-making for patients with cardiovascular disease (Corson, 2009). The results obtained in the present study showed that weight, BMI, WC, Systolic blood pressure, diastolic blood pressure, FBS, PPBS, Cholesterol, TG, IL6, TNF-$\alpha$, carotid IMT, chemerin, leptin and baPWV were significantly higher in metabolic syndrome group when compared to type 2 diabetes mellitus and control groups ($p < 0.001$ and $p < 0.05$ respectively). Moreover, height was significantly higher ($p < 0.001$) in metabolic syndrome group when compared to type 2 diabetes mellitus group but not significantly ($p > 0.05$) different from control group (Table 1). Our results are consistent with the study of Osman et al. (2012), who revealed that the systolic blood pressure, diastolic blood pressure, waist circumference, fasting serum insulin, fasting serum glucose, HOMA-IR, total cholesterol, LDL-C, triglycerides and serum chemerin levels were significantly higher in metabolic syndrome group when compared to control group. However, Bozaoglu et al. (2007) described that circulating chemerin levels in type 2 diabetes human subjects were not significantly higher than those in normal control subjects, this may be due to taking anti-diabetic drugs by a proportion of their type 2 diabetic study subjects. In addition, Blüher et al. (2012) proved that insulin, triglycerides and chemerin are biomarkers that tightly correspond to changes in body weight. Moreover, Ernst et al. (2012) stated that CMKLR1(–/–) mice had lower food consumption, total body mass, and percent body fat compared with wild-type controls. These findings suggested that chemerin may play a role in the pathophysiology of obesity and metabolic syndrome Fig. 2.

HDL in the present study was significantly ($p < 0.001$) lower in metabolic syndrome group than in diabetic and control groups also it was significantly lower in 2 diabetes mellitus group than in control group. These results were in agreement with the results of Osman et al. (2012) who revealed that HDL-C was significantly lower in metabolic syndrome group compared to control group.

In the present study, CAD-risk percentage was significantly higher ($p < 0.001$) in metabolic syndrome group and in type 2 diabetes mellitus group when compared to control group.
However, CAD-risk percentage was significantly not significantly different in type 2 diabetes mellitus group compared to metabolic syndrome group (Table 1). These results were in disagreement with the results obtained by the study of Osman et al. (2012) who found that CAD-risk percentage was significantly lower in metabolic syndrome group when compared to type 2 diabetes mellitus and control group in addition, they revealed that CAD-risk percentage was also significantly lower in type 2 diabetes mellitus group compared to control group.

Our correlation study also revealed a significant negative correlation between serum chemerin level and HDL-C and a significantly positive correlation ($p < 0.05$) with CAD-risk in metabolic syndrome group. Our results were in consistent with the results of Osman et al. (2012) who revealed a significant negative correlation between serum chemerin levels and HDL-C but we disagree with the results of the same study regarding CAD-risk in metabolic syndrome patients. Also, our results agreed with Wang et al. (2009), Dong et al. (2011), Yoo et al. (2012) and Yan et al. (2012) who revealed that serum chemerin levels were significantly elevated in metabolic syndrome patients with CAD compared to those without CAD and healthy subjects and were associated with several cardiovascular risk factors. Also, it was negatively correlated with HDL-C levels. Moreover, a significant positive correlation was found between serum chemerin levels and each of systolic blood pressure, diastolic blood pressure, waist circumference, HOMA-IR, FBS and PPBS in both groups ($p < 0.05$, respectively). Our results concerning blood pressure were in agreement with studies done by Stejskal et al. (2008) and Bozaoglu et al. (2009). They revealed that chemerin serum levels correlated positively with systolic and diastolic blood pressure. Chemerin may also be a novel regulator of blood pressure because of good correlations with both systolic and diastolic pressure.

This hypothesis is supported by the fact that chemerin is highly expressed in the kidney, a key site of blood pressure regulation. Chemerin is structurally related to other circulatory factors, as kininogens, whose proteolytic product is the vasoactive peptide bradykinin. Sell et al. (2009) findings can explain our findings regarding the highly significant increase in FBS, PPBS and HOMA-IR and their correlation to chemerin levels in metabolic syndrome group and type 2 diabetes mellitus by the fact that higher chemerin release is associated with insulin resistance by decreasing the rate of auto-

### Table 3 – Coefficients of the predictors of baPWV values in metabolic syndrome group.

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
</tr>
<tr>
<td>1</td>
<td>(Constant) 1.227E1 0.039</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemerin 0.6671 0.023</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(Constant) -1.254E0 0.203</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemerin 0.6091 0.023</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(Constant) -1.381E0 0.183</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemerin 0.6331 0.023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SBP 0.401 0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WC 0.282 0.002</td>
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</tr>
</tbody>
</table>

Dependent Variable: IMT.

Fig. 2 – Correlation between circulating leptin levels and mean carotid IMT (A), and baPWV (B).
phosphorylation and subsequent downstream intracellular signaling cascades of insulin receptor-tyrosine kinase in peripheral tissues. Chemerin also inhibits glycogen synthase kinase phosphorylation, an enzyme necessary for glycogen synthesis and storage, and thus inhibits glucose uptake. In addition, chemerin activates extracellular signal-regulated kinase (ERK). Inhibition of ERK prevents chemerin-induced insulin resistance, pointing to participation of this pathway in chemerin action. Moreover, Takahashi et al. (2011) showed that chemerin-deficient mice are glucose intolerant and glucose intolerance was mainly due to increased hepatic glucose production and impaired insulin secretion. They suggested that chemerin and its receptor were expressed in β-cell and chemerin regulates β-cell function and plays an important role in glucose homeostasis in a tissue dependent manner. In adipocytes, chemerin stimulates the differentiation and insulin sensitivity (Takahashi et al., 2008), whereas it induces the insulin resistance in skeletal muscle, suggesting that the effects of chemerin are different dependent on the tissue types (Sell et al., 2009). A possible relation of chemerin to inflammatory proteins and insulin resistance in obesity and type 2 diabetes is suggested as muscle insulin resistance is a major risk factor for the pathogenesis of type 2 diabetes (Lambernd et al., 2012). Chemerin downregulation during adipocyte maturation results subsequently in lower expression of perilipin, GLUT4 (insulin-regulated glucose transporter), adiponectin and leptin by mature adipocytes (Goraliski et al., 2007). Our results are also supported by a study done by Ernst et al. (2012) who revealed that recombinant chemerin administration exacerbated glucose intolerance in obese and diabetic mice. This study provided evidence that serum chemerin levels are elevated in obesity and diabetes and that chemerin exacerbates glucose intolerance in these models by decreasing serum insulin levels and glucose uptake in liver tissue. Our results agree with results of El-Mesallamy et al. (2011) and Hu and Feng (2011) who proved that serum chemerin levels were significantly increased in patients with type 2 diabetes and in patients with type 2 diabetes with ischemic heart disease compared with healthy control subjects. Our study revealed a significant positive correlation between serum chemerin levels and each of systolic blood pressure, diastolic blood pressure, waist circumference, HOMA-IR, FBS, PPBS and total cholesterol in both type 2 DM and MS group (p < 0.05, respectively). Our study agrees in some findings and disagrees in other ones with the study of Osman et al. (2012) who revealed that serum chemerin was correlated positively with each of systolic blood pressure, diastolic blood pressure, waist circumference, fasting serum insulin, HOMA-IR, total cholesterol, LDL-C and triglycerides in metabolic syndrome patients, and they revealed no correlation with either FBS or PPBS. Moreover, a significant positive correlation was found between serum chemerin levels and each of IL6, TNF, CRP and carotid–IMT and baPWV in both groups (p < 0.01, respectively). These results agree with the study of Dong et al. (2011) who proved that serum chemerin in MetS subjects was positively correlated with CRP who demonstrate the association of serum chemerin levels with the development of CAD in patients with MetS. Previous studies have suggested that elevated serum chemerin levels are strongly related to inflammatory markers such as high sensitivity CRP, interleukin-6 and tumor necrosis factor-α (Weigert et al., 2010; Lehrke et al., 2009). TNF-α treatment of 3T3-L1 adipocytes increased bioactive chemerin levels, suggesting that inflammatory cytokines contribute to the up-regulation of chemerin in obesity (Parlee et al., 2010). Thus, it is possible that adipocyte-derived chemerin may be involved in the pathogenesis of obesity-related inflammatory disorders, including atherosclerosis. These results are also in consistence with the study of Yoo et al. (2012) who demonstrated that the circulating chemerin level was positively associated with arterial stiffness, as represented by the baPWV, and the serum chemerin level was an independent determining factor for the baPWV, even after adjusting for other cardiovascular risk factors but our results disagree with this study regarding IMT which revealed non significant correlation with chemerin levels.

McCarthy et al. (2008) explained our findings by the ability of chemerin for chemotactic recruitment for macrophages and dendritic cells expressing CMKLR1, as well as its ability to promote cholesterol uptake and foam cell formation, suggests a role of chemerin in inflammatory states and possibly atherosclerosis. In addition, Lehrke et al. (2009) proved that chemerin is strongly related to markers of inflammation as tumor necrosis factor-α, interleukin-6 and CRP. Thus, it is conceivable that chemerin may be up-regulated in states of inflammation such as obesity, metabolic syndrome and atherosclerosis to dampen inflammatory processes and to improve metabolic regulation.

Stepwise multiple regression analysis in metabolic syndrome group revealed that chemerin, SBP and WC are risk factors in determining baPWV values. Chemerin alone in model 1, chemerin together with SBP in model 2 and chemerin, SBP and WC in model 3 account for 46.0% (F = 15.308, p = .001) and 69.7% (F = 19.586, p = <.001) and 76.9% (F = 17.713, p = <.001) of the variance in baPWV values respectively. Our results agree with the results of Yoo et al. (2012) who proved that age, waist circumference, systolic blood pressure, and serum fasting glucose and chemerin levels were definitive risk factors for arterial stiffness. Lehrke et al. (2009) showed that circulating chemerin had a positive correlation with atherosclerotic plaque burden assessed by multislice CT angiography (Mark et al., 2010). Arterial stiffness is recognized as a result of structural and functional changes of the vascular tree (O’Rourke and Mancia 1999).

Carotid IMT quantitatively measures the arterial morphology consisting of intimal lesions and medial hypertrophy (Simon et al., 2002). Many studies have shown that PWV is an independent risk factor for cardiovascular disease; Cruickshank et al. (2002) during a 10-year follow-up period, reported that arterial stiffness independently predicted all-cause and cardiovascular mortality in 397 diabetic patients for each 1 m/s increase in PWV (hazard ratio, 1.08; 95% confidence interval, 1.03–1.14); therefore, our results suggest that circulating chemerin may directly mediate the process of cardiovascular disease. Hart and Greaves 2010, showed that chemerin rapidly stimulated the adhesion of macrophages to the extracellular matrix protein, fibronectin, and to the adhesion molecule, vascular cell adhesion molecule-1, suggesting that chemerin might promote the progression of atherosclerosis. Kaur et al. (2010) demonstrated the novel presence of a G-protein coupled...
chemerin receptor (CMKLR1) in human endothelial cells and its significant up-regulation by pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6). Thus, the altered expression of chemerin and its receptors during an inflammatory process may cause dysregulated angiogenesis, leading to the development of cardiovascular disease. In conclusion, the present study confirmed that the circulating chemerin level is significantly elevated in metabolic syndrome and type 2 diabetes mellitus individuals and has a close correlation with various metabolic risk factors. It can be also suggested that chemerin is associated with markers of inflammation, and predictors of atherosclerosis in the metabolic syndrome, and type 2 diabetes and we revealed that chemerin, systolic blood pressure and waist circumference are important risk factors determining arterial stiffness in Saudi Arabians. The influence of chemerin on the predictors of atherosclerosis and the control of diabetes and metabolic syndrome opens a new era in the field of prevention of atherosclerosis and cardiovascular diseases that accompany these conditions. Further experimental studies are warranted to clarify the role of chemerin in the atherosclerotic process.

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