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Utility of Serum Inflammatory Chemokines as Markers of Metabolic Syndrome and Type II Diabetes Mellitus

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Abstract: Background: Inflammation has repeatedly been demonstrated to be associated with the metabolic syndrome (MS) and type II diabetes mellitus (type II DM), but the relative importance of different aspects of inflammatory process is largely unexplored. **Subjects:** Circulating adipokine (chemerin) and cytokines (TNF-α and IL-6) were evaluated in 56 age matched males, that are classified into three groups.Group1 (healthy controls, n=16), Group2 (non-diabetic MS patients, n=20) and Group3 (non-MS patients with overt type II DM, n=20). These biomarkers were related to MS and type II DM calculating cardiovascular risk (CAD-risk) and the homeostasis model assessment insulin resistance index (HOMA-IR). **Results:** Comparing to control, subjects with MS and type II DM had higher levels of chemerin, TNF-α and IL-6. For MS patients, HDL-C and CAD-risk levels were lowered and were negatively correlated to serum chemerin. A significant positive correlation was found between serum chemerin levels and each of waist circumference (WC), blood pressure (systolic and diastolic), LDL-C, TGand total cholesterol. **Conclusions:** High serum chemerin, TNF-α levels were strongly associated with MS disorders and type II DM and high IL-6 in MS, and assessment of their levels could be beneficial in diagnosis, early detection and prevention of these pathophysiological states.

Key words: Inflammation, metabolic syndrome, type II Diabetes Mellitus, chemerin, TNF-α, IL-6.

INTRODUCTION

Type II diabetes mellitus (Type II DM) is considered as the predominant form of diabetes worldwide, accounting for 90% of cases globally (Buse *et al*., 2008). Patients with type II DM usually have insulin resistance and relative insulin deficiencyand often have a strong genetic component (Stumvoll *et al*., 2005). In addition, many studies have identified that insulin resistance is commonly associated with several metabolic derangements as hypertension, central obesity and dyslipidemia collectively described as insulin resistance syndrome (Deedwania, 2004).

Metabolic syndrome (MS) is characterized by a clustering of metabolic risk factors, including obesity, hypertension, impaired glucose metabolism and dyslipidemia.Adipose tissue produces and secretes adipokines, and their dysregulation in visceral obesity, may play a role in the development of MS (Bozaoglu *et al*., 2007). The roles of adipokines released from visceral adipose tissue have been increasingly emphasized and may play a role in the pathophysiology of the metabolic syndrome (Suzuki *et al*., 2010). Patie nts with MS have an increased risk of developing diabetes mellitus and cardiovascular morbidity and mortality (Stefikova *et al*., 2004).

Low grade inflammation has been associated with central obesity, cardiometabolic and type II DM (Grundy *et al*., 2004). Under normal conditions, the vessel wall has its own machinery to maintain vascular homeostasis. However, the balance is broken when repetitive metabolic stimuli resulting from hypertension, insulin resistance or obesity strike the vessel wall. Most of these metabolic stimuli disturb homeostasis through the initiation of inflammation that is the recruitment of inflammatory cells, the increased adhesion molecules, secretion of chemo-attractants and proinflammatory cytokines from endothelial cells and the migration and proliferation of smooth muscle cells from media (Libby, 2002). Adipokines have diverse autocrine, paracrine and endocrine actions and have been implicated in the pathogenesis of MS and cardiovascular diseases (Ernst *et al*., 2010). Furthermore, the concept that heightened inflammation is important in the pathogenesis of type II DM is supported by evidence that inflammation in islets, adipose tissue, liver and muscles may provoke insulin resistance and β- cell dysfunction and may therefore antedate the diagnosis of diabetes (Jager *et al*., 2007).

Chemerin is a recently described adipokine which has dual roles in adipose tissue metabolism and regulation of immune response (Parlee *et al*., 2010). Chemerin and its receptor, CMKLR1, are highly expressed in adipose tissue (Bozaoglu *et al*., 2007). Serine proteases and cysteine proteases contribute in the conversion

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of prochemerin, the inactive form of chemerin, to chemerin (Yoshimura and Oppenheim, 2008).

Other adipokines associated with MS and insulin resistance include the circulating inflammatory markers TNF-α and IL-6,wherethe effect of these adipokines on MS and type II DM seems to stem from the influence of a combination of adipokines rather than from the effect of a single adipokine(Xydakis*et al*., 2004).TNF-α is produced primarily by activated monocytes and/or macrophages. Two different TNF receptors of 55 and 75 kDa, each encoded by a separate gene, bind both TNF-α and TNF-β. TNFs activate a sphingomyelinase, resulting in release of ceramide from sphingomyelin, which in turn activates a Mg^{+2}-dependent protein kinase.On the other hand, IL-6 is secreted by a wide variety of cells, including T and B lymphocytes, monocytes and/or macrophages. IL-6 binds to the receptor IL-6Rα and the IL-6-IL-6Rα complex, which then binds two gp130 molecules to form a gp130 disulfide-linked homodimer, resulting in tyrosine phosphorylation of gp130 and signal transduction (Callard and Gearing, 2001).

Therefore, the present studywas conducted to evaluate the level of some inflammatory chemokines such as chemerin, TNF-αand IL-6 and detect their implication in diagnosis, prognosis and severity of MS and type II DM as well as their role in obesity.

Subjects and Methods:

A total of 56 male subjects aged (32-60) years were included in this study. They obeyed for clinical manifestations.Many blood parameters, inflammatory chemokines such as serum chemerin, TNF-α and IL-6 levels were evaluated.

Subjects were classified into three groups;Group 1 (healthy controls; n=16), Group2(MS group: non-diabetic patients with metabolic syndrome; n=20)andGroup3(Type II DM group: non- metabolic syndrome patients with overt type II diabetes mellitus; n=20).All patients were investigated in the morning after an overnight fast, with no medication or smoking allowed after midnight. After recording of WC, blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm.

Serum samples were used for measuring the following:total cholesterolaccording to the method of Dietschy *et al*., (1976), triglycerides according to the method of McGowan *et al*., (1983) using Elitech kits, France, HDL- C(Assman *et al*., 1983) using Bicon kits, Germany,LDL-C was calculated using the equation [Total cholesterol

– (Triglycerides/5) – HDL-C] (Warnick *et al*., 1990; Friedwald *et al*., 1972),fasting blood sugar and postprandial blood sugar(Carl *et al*., 2006)using Diamond Diagnostics kits, Hannover, Germany. Whole blood wasused for measuring glycosylated hemoglobin (HbA1C)(Gonen and Rubenstein. 1978) using Teco Diagnostics kits, U.S.A.

Serum insulin(Smith *et al*., 1993) was assayed by Micro-particle Enzyme Immunoassay (MEIA) on the AxSYM (Abbott Ireland).Homeostasis model assessment insulin resistance index (HOMA-IR) was calculated by multiplying fasting blood glucose (mg/dl) × fasting insulin (IU/ml) / 405Cutoff point to define insulin resistance corresponds to HOMA-IR≥ 3.8 (Shirai, 2004);not evaluated in subjects on insulin treatment.CAD-risk was calculated according to the equation [HDL-C/Total cholesterol %] (Carl *et al*., 2006).

MS was defined according to the modified National Cholesterol Education Program (NCEP) Adult Treatment Panel III criteria (Grundy *et al*., 2005)by the presence of three or more of the followings: increased WC(≥102 cm for men, ≥88 cm for women); hypertriglyceridaemia(≥150 mg/dL) orpatient receiving treatment for this lipid abnormality; or low high-density lipoprotein (HDL-C)(<40 mg/dL for men and <50 mg/dL for women); elevated blood pressure (≥130 mmHgsystolic or ≥85 mmHg diastolic or treatment for hypertension); hyperglycaemia (fasting blood glucose ≥ 110 mg/dL) ortreatment for elevated glucose.

Inflammatory marker chemerin concentrations(Roh *et al*., 2007) were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by Biovendor laboratory Medicine, Germany. It provides a principle that the surface of wells in microtitration plate was coated with polyclonal anti-human chemerin specific antibody. Standards, Quality Controls (QC) and diluted samples were pipetted into the wells.

TNF-α (Thomson, 1994) and IL-6 concentrations (Ali and Rizvi,2007),were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by Anogen Inc, Canada. Both provide the principle that the microtitre plate was precoated with specific monoclonal antibody. Standards and samples were then added to the microtitre plate wells with a specific biotin-conjugated polyclonal antibody preparation and incubated.

Statistical Analysis:

All statistical analyses were conducted using SPSS (Statistical Package for Social Sciences) program for

Windows, version 7.0. The Student's t-test was used to compare the parametric data and Mann Whitney test was carried out to compare the non- parametric data. Spearman correlation analysis was used to assess the univariat associations of metabolic variables with serum chemerin, TNF-α and IL-6. All tests were considered statistically significant when ($p< 0.05$). The sensitivity, specificity and Area under ROC were calculated. To

assess the predictive value of the biomarkers, all variables were entered into a conditional logistic regression model and cumulative AUC was calculated using the predictive probability values.

Results:

Clinical Characteristics Of Patients:

A total of 56 male subjects aged (32-60) years were included in this study; their clinical and demographic data were summarized in **Table (1)**. Patients with MS and type II DM showed a highly significant elevation ($p<0.001$) in WC when compared to control subjects.

Blood Parameters:

Comparative statistics regarding fasting blood sugar, postprandial blood sugar, HbA1C, fasting insulin and HOMA-IR were summarized in **Table (2)** expressed as range and median. **Table (2)** demonstrated that patients with type II DM showed a highly significant elevation ($p<0.001$) in FBS and PPBS when compared to patients with MS and those of control group. Also it showed a significant elevations ($p<0.001$) and ($p<0.05$) in HOMA- IR when compared to control group and MS group, respectively. Moreover, the same group showed a significant increase ($p<0.05$) in HbA1C and fasting insulin compared to control group.

Figures (1) showed the significant variations in the mean value of blood sugar levels (FBS and PPBS). It showed the significant increase ($p<0.001$) in fasting blood sugar levels in type II DM group compared to control group. Meanwhile, there was a significant increase ($p<0.001$) in the level of the same parameter in type II DM group compared to MS group. In addition, type II DM group showed a highly significant increase ($p<0.001$) in the level of PPBS either in comparison to control or to MS group. **Figure (2)** indicated that, glycosylated hemoglobin level was significantly increased ($p<0.05$) in type II DM subjects when compared with the control with no significant changes comparing to MS group. Moreover, **Figure (3)** showed a significant increase ($p<0.05$) in fasting insulin level in both type II DM and MS subjects with no significant changes in type II DM subjects compared to those of MS.

In addition, comparative statistics among studied groups regarding lipid profile and CAD-risk were summarized in **Table (3)**. Patients with metabolic syndrome showed a highly significant elevation ($p<0.001$) in serum triglycerides and CAD-risk when compared to patients of type II DM and those of control group. Also, cholesterol was significantly increased in MS patients comparing to control while type II DM patients recorded a significant elevation in the same parameter comparing to MS subjects.

Chemokine Levels:

Comparative statistics among studied groups regarding chemerin, TNF-α and IL-6 were summarized In **Table (4)**. Patients with metabolic syndrome and type II DM showed a highly significant elevation ($p<0.001$) in serum TNF-α when compared to control subjects. Serum chemerin and IL-6 were significantly increased($p<0.001$) and ($p<0.05$) respectively, in cases of metabolic syndrome when compared to control subjects. Also, chemerin showed a highly significant elevation ($p<0.001$) in type II DM group compared to MS group.

Table 1: Comparative statistics among studied groups regarding clinical and demographic data using Student's t- test for parametric data and Mann-Whitneys U-test for non-parametric data.

		Control [n=16]	(MS) group [n=20]	(Type II DM) group [n=20]
Age (years)	Range	32 - 60	32 – 60	35 - 60
	Median (interquartile range)	44.0 (37.5 - 48.8)	51.0 (40.5 - 56.8)	50.5 (46.3 - 55.3)
	*p value	-	NS	S
	#$p$value	-	NS	-
Systolic Blood Pressure (mmHg)	Range	100 – 140	110 -170	110 - 135
	Median (interquartile range)	120 (112.5 -133.8)	142.5 (125 -130)	120 (112.5 - 130)
	*p value	-	S	NS
	#p value	-	HS	-
Diastolic Blood Pressure (mmHg)	Range	70 – 90	70 -120	70 – 95
	Median (interquartile range)	80 (70 – 80)	95 (82.5 -100)	80 (80 – 90)
	*p value	-	S	NS
	#p value	-	S	-
Waist Circumference (cm)	Range	76 – 96	90 -134	80 - 130
	Median (interquartile range)	87 (81 – 90.5)	108.5 (98.8 -115.8)	99.5 (93.5 - 109)
	*p value	-	HS	HS
	#p value	-	S	-

*p :Significant difference comparing to the control group..

#p :Significant difference comparing to type II DM group at p <0.05.

NS: Non-Significant; S: Significant (p<0.05); HS: Highly Significant (p<0.001).

Table 2: Comparative statistics among studied groups regarding fasting blood glucose, postprandial blood glucose, glycosylated hemoglobin, fasting insulin and HOMA-IR using Student's t- test for parametric data and Mann-Whitneys U-test for non-parametric data.

		Control [n=16]	(MS) group [n=20]	(Type II DM) group [n=20]
Fasting Blood Sugar (mg/dL)	Range	70 - 116	80 - 108	87 - 369
	Median (interquartile range)	96 (83.5 - 106.3)	92.0 (89.3 - 99.8)	143.5 (130 - 195.5)
	*p value	-	NS	HS
	#p value	-	HS	-
2 Hours Postprandia Blood Sugar (mg/dL)	Range	81 - 135	98 - 202	61 - 216
	Median (interquartile range)	109.5 (99.3 - 117)	111 (100 - 120.8)	173 (137 - 187.3)
	*p value	-	NS	HS
	#p value	-	HS	-
Glycosylated Hemoglobin (%)	Range	4.5 - 7.2	4.5 - 8.0	4.7- 8.8
	Median (interquartile range)	6.0 (5.1 - 6.7)	6.4 (5.5 - 7.0)	7.0 (6.0-7.9)
	*p value	-	NS	S
	#p value	-	NS	-
Fasting Insulin (IU/ml)	Range	2.7 - 19.2	4.1 - 21.0	3.3 - 22.9
	Median (interquartile range)	4.8 (3.0 - 8.2)	8.7 (7.2-12.3)	9.0 (6.1 - 13.8)
	*p value	-	S	S
	#p value	-	NS	-
HOMA-IR	Mean±SD	1.5 ± 1.0	2.4 ± 1.2	4.6 ± 3.9
	Range	0.6 -3.8	0.8 -4.9	80 – 130
	Median (interquartile range)	1.1 (0.7 - 2.0)	2.1 (1.6 -3.2)	99.5 (93.5 - 109)
	*p value	-	S	HS
	#p value	-	S	-

*p : Significant difference comparing to the control group..

#p : Significant difference comparing to type II DM group at p<0.05.

NS: Non-Significant; S: Significant (p<0.05); HS: Highly Significant (p<0.001).

Table 3: Comparative statistics among studied groups regarding total cholesterol, triglycerides, HDL-C, LDL-C and CAD-risk using Student's t- test for parametric data and Mann-Whitneys U-test for non-parametric data.

		Control [n=16]	(MS) group [n=20]	(Type II DM) group [n=20]
Total cholesterol (mg/dL)	Range	152 - 220	150 - 466	87 - 247
	Median (interquartile range)	187 (164.8 - 202.3)	221.5 (182 - 241.8)	192 (157 - 204.3)
	*p value	-	S	NS
	#p value	-	S	-
Triglycerides (mg/dL)	Range	65 - 127	106 - 308	40 - 182
	Median (interquartile range)	93 (76.8 - 113.8)	169.5 (122.5 -213)	116.5 (75.3 - 129.8)
	*p value	-	HS	NS
	#p value	-	HS	-
HDL-C (mg/dL)	Range	38-69	12-53	24-64
	Median (interquartile range)	48.5 (42.3 - 54.3)	39 (35.3-43.8)	50 (37.8 - 55.8)
	*p value	-	S	NS
	#p value	-	S	-
LDL-C (mg/dL)	Range	70 - 157	71 - 357	42 - 209
	Median (interquartile range)	120.5 (95.3 - 131.8)	133 (110.3 -185.5)	115 (97 - 137.3)
	*p value	-	NS	NS
	#p value	-	S	-
CAD-risk (%)	Mean±SD	1.5 ± 1.0	18.2 ± 6.1	27.0 ± 4.0
	Range	17.7 -45.1	3.9 - 33.3	18.2 - 35.8
	Median (interquartile range)	25.0 (20.8 - 33.7)	18.1 (14.8 - 21.7)	26.2 (24.9 - 30.0)
	*p value	-	HS	NS

	#p value	-	HS	-

*p : Significant difference comparing to the control group..

#p : Significant difference comparing to type II DM group at p<0.05.

NS: Non-Significant; S: Significant (p<0.05); HS: Highly Significant (p<0.001).

Table 4: Comparative statistics among studied groups regarding chemerin, TNF-α and IL-6 using Student's t-test for parametric data and Mann-Whitney's U-test for non-parametric data.

		Control [n=16]	(MS) group [n=20]	(Type II DM) group [n=20]
Serum chemerin (ng/ml)	Range	25 - 125	140 - 800	30 – 200
	Median (interquartile range)	67 (39.5 - 116)	450 (275 - 725)	112.5 (80 - 172.5)
	*p value	-	HS	S
	#p value	-	HS	-
Serum TNF-α (pg/ml)	Range	3.8 - 15.4	18.7 - 32.2	18.7 - 31.2
	Median (interquartile range)	7.9 (5.8 - 11.0)	26.1 (22.6 - 28.2)	23.5 (21.6 - 28.3)
	*p value	-	HS	HS
	#p value	-	NS	-
Serum IL-6 (pg/ml)	Range	1.3 - 13.5	1.7 - 39.8	1.5 - 23.9
	Median (interquartile range)	2.1 (1.8 - 3.7)	3.3 (2.0 - 10.9)	2.0 (1.8 - 4.3)
	*p value	-	S	NS
	#p value	-	NS	-

*p : Significant difference comparing to the control group..

#p : Significant difference comparing to type II DM group at p<0.05.

NS: Non-Significant; S: Significant (p<0.05); HS: Highly Significant (p<0.001).

Correlation Of Chemokines With All Studied Parameters:

A correlation study between serum chemerin and all studied parameters was summarized in **Table (5)**, which revealed that there was a highly significant positive correlation between serum chemerin level and each of serum total cholesterol (r= 0.845, p<0.001) and serum LDL-C (r= 0.815, p<0.001).In addition, there was a significant positive correlation between serum chemerin level and each of serum triglycerides (r= 0.72, p<0.05), waist circumference (r=0.658, p<0.05), HbA1C (r= 0.65, p<0.05), SBP (r= 0.61, p<0.05) DBP (r= 0.594, p<0.05) and FBS (r=0.504, p<0.05). There was a significant negative correlation between serum chemerin level and each of CAD-risk (r= -0.724, p<0.05) and serum HDL-C (r= 0.596, p<0.05). Our study failed to give a significant correlation between serum chemerin level and each of serum TNF-α, IL-6, PPBS, fasting insulin and HOMA-IR.

Table 5: Correlation study between chemerin and all studied parameters using Spearman's rank correlation coefficient in all patient groups.

	Chemerin		
	r* / rho	p value	significance
TNF-α	0.188	0.485	NS
IL-6	0.234	0.382	NS
FBS	0.504	0.047	S
PPBS	0.487	0.056	NS
HBA1C	0.65	0.006	S
FBI	0.251	0.349	NS
HOMA-IR	0.38	0.147	NS
HDL-C	-0.596	0.015	S
LDL-C	0.815	0	HS
Triglycerides	0.72	0.002	S
Total cholesterol	0.845	0	HS
CAD risk	-0.724	0.002	S
Waist Circumference	0.658	0.006	S
Systolic blood pressure	0.61	0.012	S
Diastolic blood pressure	0.594	0.015	S

* Spearman's rank correlation coefficient

NS: Non-Significant; S: Significant (p<0.05); HS: Highly Significant (p<0.001)

Fig. 1: Changes in the levels of Fasting Blood Sugar (FBS) (mg/dL) and postprandial blood sugar (PPBS) (mg/dL). Values are expressed as means±SD.
Significant difference from the control group at **p*<0.05.
Significant difference from the type II DM group at #*p*<0.05

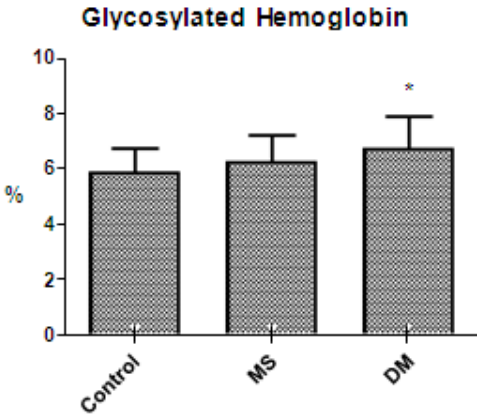

Fig. 2: % Changes in the levels of Glycosylated Hemoglobin (%). Values are expressed as means±SD.

Significant difference from the control group at **p*<0.05. Significant difference from the type II DM group at #*p*<0.05.

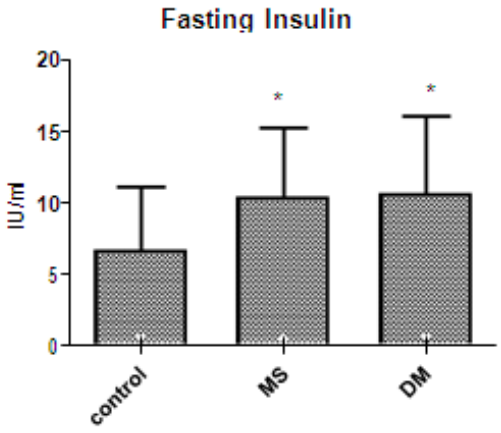

Fig. 3: Changes in the levels of Fasting Insulin (IU/ml). Values are expressed as means±SD.

Significant difference from the control group at **p*<0.05. Significant difference from the type II DM group at #*p*<0.05.

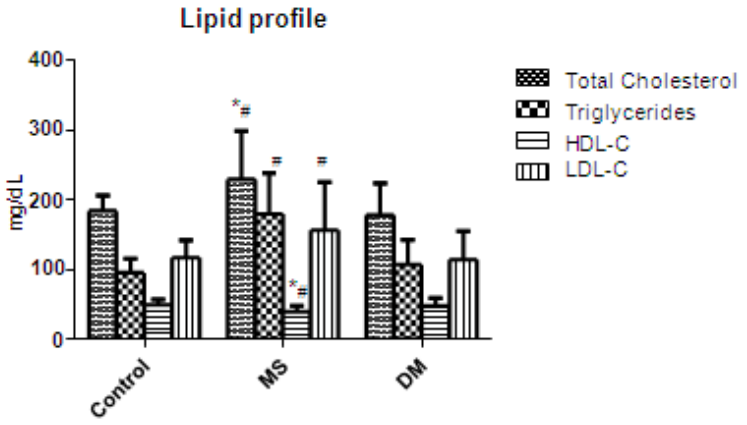

Fig. 4: Changes in the levels of lipid profile (mg/dL). Values are expressed as means±SD.

Significant difference from the control group at **p*<0.05. Significant difference from the type II DM group at #*p*<0.05.

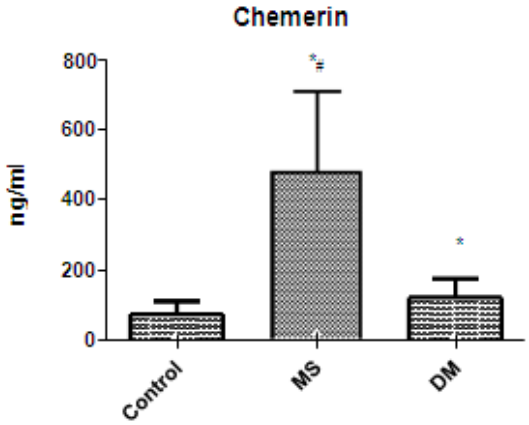

Fig. 5: Changes in the levels of Chemerin (ng/ml). Values are expressed as means±SD.

Significant difference from the control group at **p*<0.05. Significant difference from the type II DM group at #*p*<0.05.

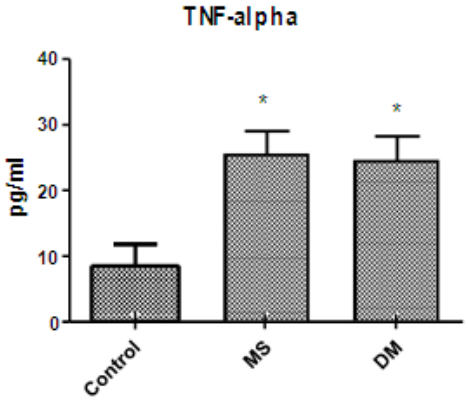

Fig. 6: Changes in the levels of TNF-alpha (pg/ml). Values are expressed as means±SD.

Significant difference from the control group at **p*<0.05. Significant difference from the type II DM group at #*p*<0.05.

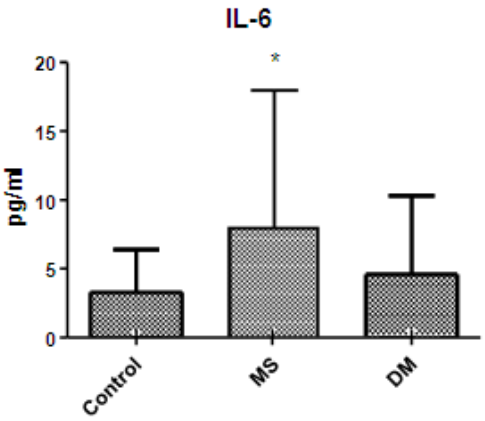

Fig. 7: Changes in the levels of IL-6 (pg/ml). Values are expressed as means±SD.

Significant difference from the control group at $*p<0.05$. Significant difference from the type II DM group at $^{\#}p<0.05$.

Discussion:

Type II DM is considered the predominant form of diabetes worldwide, accounting for 90% of cases globally. It represents a major public health threat, and constitutes an important contributor to the predicted decline in life expectancy (Buse *et al*., 2008). Metabolic syndrome (MS) is characterized by a clustering of metabolic risk factors, including obesity, hypertension, impaired glucose metabolism and dyslipidemia. Patients with MS have an increased risk of developing diabetes mellitus and cardiovascular morbidity and mortality (Stefikova *et al*., 2004). The roles of adipokines released from visceral adipose tissue have been increasingly emphasized and may play a role in the pathophysiology of the metabolic syndrome and type II DM (Suzuki *et al*., 2010).Chemerin is a recently described adipokine which has dual roles in adipose tissue metabolism and regulation of immune response (Parlee *et al*., 2010). Xydakis *et al*., (2004***),*** demonstrated that other adipokines associated with metabolic syndrome and insulin resistance include the circulating inflammatory markers TNF-α and IL-6 and the effect of adipokines on metabolic syndrome and insulin resistance seems to stem from the influence of a combination of adipokines rather than from the effect of a single adipokine.

On assessment of the different studied parameters carried out on a total of 56 male subjects the results of their clinical characteristics revealed that the WC showed a highly significant elevation ($p< 0.001$) in MS group and Type II DM group when compared to control group. Also, SBP was significantly increased ($p< 0.001$) in MS group compared to both Type II DM group and control group.

Blood parameters results revealed that the FBS and PPBS showed a highly significant elevation ($p< 0.001$) in Type II DM group when compared to both MS group and control group. In addition, HOMA-IR was significantly increased ($p< 0.001$) and ($p< 0.05$) in Type II DM group when compared to control group and MS group, respectively. Moreover, HbA1C and fasting insulin were significantly increased ($p< 0.05$) in Type II DM group when compared to control group. Also, serum TG showed a highly significant elevation ($p< 0.001$) in MS group when compared to both Type II DM group and control group. A significant increase ($p< 0.05$) was showed in serum total cholesterol and HDL-C in MS group when compared to both Type II DM group and control group. Serum LDL-C was significantly increased ($p< 0.05$) in MS group compared to Type II DM group. Furthermore, CAD-risk showed a highly significant decrease($p< 0.001$) in group2 (MS group) when compared to both Type II DM group and control group.

The lack of significance between patients with Type II DM and control subjects regarding lipid profile results was due to that patients with Type II DM were under insulin therapy with or without oral hypoglycemic drugs. This was in agreement with (McCopy *et al*., 2012), who stated that interventions to improve insulin sensitivity may be considered as therapeutic options to improve HDL-cholesterol, TG and TNF-α; without changes in IL-6, weight, blood pressure, or body composition.

Moreover, the results revealed that serum TNF-α was significantly increased ($p< 0.001$) in MS group and Type II DM group when compared to control group. In addition, serum chemerin and IL-6 were significantly increased ($p< 0.001$) and ($p< 0.05$), respectively, in MS group when compared to Type II DM group and control group, respectively. Also, IL-6 was significantly increased ($p< 0.05$) in MS group when compared to both Type II DM group and control group.

These findings were in agreement with studies previously performed by (Stejskal *et al*.,2008 and Bozaoglu *et al*.,2009), who reported that higher serum chemerin concentration have a strong and independent

association with aspects of the metabolic syndrome including WC, fasting serum insulin and lipid profile independent of age and sex in non-diabetic subjects. Furthermore, higher chemerin release is associated with insulin resistance at the level of lipogenesis. It contributes to the pathophysiology of insulin resistance by its reversible bindingto the extracellular domain of insulin receptor-tyrosine kinasein peripheral tissues and decreases the rate of auto- phosphorylationand subsequent downstream intracellular signaling cascades. Chemerin also induces insulin resistance in human skeletal muscle cells at the level of insulin receptor substrate, 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 (Sell et al., 2009).

In addition, Grunnet et al., (2006) reported that neither TNF-α nor IL-6 levels were independently associated with hepatic or peripheral insulin action. Nevertheless, in people with Type II DM, the circulating IL-6 concentration is correlated with adipose tissue mass, rather than with whole-body insulin sensitivity (Carey *et al*., 2004).

Concerning about Type II DM, the present study reported that there was a highly significant elevation in FBS, PPBS, HOMA-IR, WC, and TNF-α. In addition, there was a significant elevation in chemerin levels, HbA1C, and fasting insulin and a significant decrease in CAD-risk. These findings confirm the fact that insulin resistance promotes the development of visceral adiposity and hypertension (Ginsberg, 2000; Kopp, 2005). Furthermore, another study performed by (Ernst *et al*., 2010) 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.

There is also consistent with a recent study done by (Pfau *et al*., 2010) that proved that chemerin serum levels show a strong, positive and independent association with insulin resistance assessed as fasting insulin or HOMA-IR. These results indicate that the adipokine chemerin is linked to insulin resistance in DM but is probably not a causal factor in the pathogenesis of DM independent of insulin resistance.

The correlation of the present study between serum chemerin level and other studied parameters revealed that there was a significant negative correlation between serum chemerin level and both HDL-C and CAD-risk. Moreover, a significant positive correlation was found between serum chemerin levels and each of SBP, DBS, LDL-C, TG, total cholesterol and WC.This correlation is in agreement with previous studies done by (Stejskal *et al*.,2008), which stated that chemerin is a novel adipokine associated with key aspects of metabolic syndrome as disturbed lipid profiles, hypertension, increased waist circumference. Accordingly, chemerin could play a role in the pathogenesis of metabolic syndrome. Thus, the resultant dyslipidemia is highly atherogenic and accounts for the increase in cardiovascular complications.

The lack of significant correlation between serum chemerin levels and PPBS in this study could be attributed to the anti-diabetic drugs taken by the diabetic patients beside that the cases of metabolic syndrome group are selected carefully to be non-diabetic with normal fasting glucose levels. In addition, it is conceivable that other factors such as the level of glycemic control, medication history, duration of diabetes, and the presence of complications such as renal disease may have an impact on the relationship between circulating chemerin levels and fasting blood sugar (Bozaoglu *et al*., 2009).

On the other hand, Takahashi *et al*., (2008) disagreed with the present finding and postulated that, in adipocytes, chemerin has the opposite effect, where it increases insulin-stimulated glucose uptake, and so, it

stimulates insulin sensitivity. Hence, the increase in the levels of circulating chemerin is a compensatory mechanism in patients with insulin resistance. Thus, chemerin may exert different actions in endocrine and paracrine/autocrine ways.

However, this study failed to give a significant correlation between the cytokines TNF-α and IL-6 and the rest of the studied parameters. Regarding TNF-α, the results were in agreement with those of (Koistinen *et al*.,2000) who stated that TNF-α is poorly expressed in human adipose tissue, and no differences between control subjects and patients with metabolic syndrome have been found. In addition, the results concerning IL-6 were in accordance with those of (Ingelsson *et al*.,2008) who found that serum inflammatory markers were in relation to insulin resistance and metabolic syndrome with no significant difference was observed for IL-6 when compared to those of healthy subjects.

In conclusion, high serum TNF-α and chemerin levelswere strongly associated with MS disorders and Type II DM and assessment of their levels could be beneficial in diagnosis, early detection and prevention of these pathological states and their unfavorable consequences especially the cardiovascular complications and atherosclerosis. Hence, this study introduces serum TNF-α and serum chemerin as a novel markers for diagnosis of the metabolic syndrome to be added to the panel of laboratory parameters of this metabolic abnormality.

Abbreviations:

MS: Metabolic syndrome

Type II DM: Type II diabetes mellitus **TNF-α**: Tumor necrosis factor- alpha **IL-6**: Interleukin- 6

WC: waist circumference

HOMA-IR: Homeostasis model assessment insulin resistance index

CAD-risk: Cardiovascular disease- risk

HDL-C: High density lipoprotein- cholesterol **LDL-C**: low density lipoprotein- cholesterol **TG**: Triglyceride

AUC: Area under curve

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