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ORIGINAL ARTICLES

Role of serum chemerin level in atherosclerosis in Egyptian patients with Type 2 Diabetes Mellitus

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ABSTRACT

Vascular diseases, particularly atherosclerosis, are major causes of disability and death in patients with diabetes mellitus. Chemerin is a novel adipokine that is highly expressed in obese and insulin-resistant subjects. Chemerin may play an important role in linking metabolic syndrome and inflammation. The study was conducted to determine serum chemerin levels in diabetic patients, and its relation to BMI, disease duration, and CRP, also to assess the role of serum chemerin level in atherosclerosis. A total of 40 Egyptian patients with type 2 diabetes mellitus (20 diabetics without hypertension, 20 diabetics with hypertension), and 15 age and sex matched healthy control subjects were included in this study. Serum chemerin level was determined by ELIZA. Its level was compared between both diabetic patients and controls, between dyslipidemic and non-dyslipidemic patients, and between diabetic patients with hypertension and those without hypertension. Also to detect the correlation of serum chemerin level with CRP, BMI; also to assess the relation of carotid intima media thickness (CIMT) to chemerin level. There was statistically highly significant increase in mean serum chemerin in diabetic patients (316.34±92.85 ng/ml) compared with control group (130.56± 19.52 ng/ml) (P: 0.000). Chemerin level was not significantly correlated with BMI, CRP, FBG,2 hours postprandial blood glucose, lipid profile. There was a statistical significant increase in mean right and mean left carotid intima media thickness in diabetic patients (0.08 ± 0.02cm, 0.09 ± 0.019 respectively) compared with the control group (0.07 ± 0.12, 0.06± 0.01) (P:0.008, 0.000 respectively). There was highly significant increase in mean serum chemerin level in diabetic patients with hypertension (399.35 ± 72.0) compared with the diabetic patients without hypertension (234.6 ± 63.95) (P: 0.000). There was also no statistical significant difference in serum chemerin level between dyslipidemic diabetic patients and non dyslipidemic diabetic patients (P > 0.05). Conclusion: High levels of serum chemerin in our diabetic patients indicate the activation of immune response in these patients. High carotid intimal media thickness in diabetic patients was not related to chemerin denoting an underlying combining factor for atherosclerosis in diabetic patients other than chemerin. High chemerin in diabetic hypertensive patients denote additive role of hypertension to diabetic in beginning of immune response in those patients.

Key words: Serum chemerin-diabetes mellitus- hypertension-dyslipidemia.

Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases in nearly all countries, and continues to increase in numbers and significance, as changing lifestyles lead to reduced physical activity, and increased obesity. The global prevalence of DM in the year 2010 among adults has been estimated to be 6.4%. It is estimated that by the year 2030, Egypt will have at least 8.6 million adults with diabetes (Shaw, *et al*., 2010).

Diabetes is the eleventh most important cause of premature mortality in Egypt, and is responsible for 2.4% of all years of life lost. Similarly, diabetes is the sixth most important cause of disability burden in Egypt (NICHP, 2005).

The endothelium is a complex organ with a multitude of properties essential for control of vascular functions. Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of diabetic micro- and macro-angiopathy. Endothelial dysfunction in type I and II diabetes complicated by micro- or macro-albuminuria is generalized in that it affects many aspects of endothelial function and occurs not only in the kidney. The close linkage between micro albuminuria and endothelial dysfunction in diabetes is an attractive explanation for the fact that micro albuminuria is a risk marker for atherothrombosis (Schalkwijk, and Stehouwer, 2005).

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Hypertension also is a major determinant of micro angiopathy and atherothrombosis in diabetes (Stas, *et al*., 2004). Hypertension causes endothelial activation as indicated by elevated levels of soluble adhesion molecules (Boulbou, *et al*., 2005).

Inflammation plays a central role in the pathophysiology of atherosclerosis, starting from initiation, through progression and ultimately the thrombotic complications of atherosclerosis. Diabetes mellitus is a major risk factor for atherosclerosis. Hyperglycemia-induced endothelial dysfunctions, along with hypercoagulable potential of diabetes mellitus, accelerate the process of atherothrombotic complications. Therefore, clinically feasible markers to monitor subtle systemic inflammatory burden and specific add-on therapy for the same constitute need of the present day. The understanding of the concept of inflammation in diabetes-accelerated atherosclerosis can be used practically to predict future cardiovascular risk by evaluating inflammatory biomarkers and to design clinical trials making inflammation as a therapeutic target (Maiti, and Agrawal, 2007).

Among the top contributors of inflammatory stimuli are adipokines secreted from adipose tissue, which is now considered not as a mere mass of fat tissue, but an active organ that acts as a reservoir for energy in the energy excess state and as an active supplier of energy when the body runs short of it. Adipokines have diverse autocrine, paracrine and endocrine actions and have been implicated in the pathogenesis of metabolic syndrome and cardiovascular disease. Chemerin, also known as tazarotene-induced gene 2 protein (TIG2) or retinoid acid receptor responder 2 (RARRES2), was a recently identified novel adipokine that has a role in adaptive and innate immunity (Ernst, and Sinal, 2010). Chemerin is also recently discovered metabolic regulator hormone. The pathophysiologic role of this hormone in humans remains unknown (Yang, *et al*., 2010).

The aim of the study was to determine serum chemerin levels in diabetic patients, and its relation to BMI, diseases duration, and CRP, also to assess the role of serum chemerin level in atherosclerosis. Its level was compared between dyslipidemic and non-dyslipidemic patients, and between diabetic patients with hypertension and those without hypertension. Also to assess the relation of carotid intima media thickness to chemerin level

Material and Methods

Forty consecutive Egyptian patients with type 2 diabetes mellitus from outpatient Internal medicine clinic of Cairo University were studied (20 with and 20 without hypertension).

All patients were informed about the procedure and a verbal consent was taken. The local ethical committee at Cairo university faculty of medicine also approved this study. Forty patients with diabetes, and 15 age and sex matched healthy control subjects, all were from a similar ethnic background were included in this study.

All patients were subjected to full clinical history and thorough examination, including BMI, laboratory investigations in the form of CBC, CRP, fasting and 2 h postprandial blood glucose, serum creatinine, serum cholesterol, serum triglyceride, LDLc, HDLc, 24 hour urinary protein, serum chemerin level. We also assessed the association of plasma chemerin with body composition and metabolic parameters in these subjects.

All patients were subjected to imaging procedure in the form of carotid Doppler to measure intima media thickness of the distal common carotid, and the PSV, EDV, RI of the internal carotid artery by carotid ultrasound. B (brightness)-mode grey scale, color, spectral Doppler techniques were used to investigate the carotid arteries according to standardized protocol. The same operator interpreted all studies in a blind fashion, and the same ultrasound unit HD 5000 was used for scanning all participants.

About 5 ml of fasting (12-14 hours) venous blood samples were drawn from each subject in the study. Two ml was added to a tube containing EDTA for determination of complete blood picture on Coulter Counter T890 (Coulter Counter, Harpenden, UK). The rest of the blood (about 3 ml) was left to clot and the serum was separated by centrifugation and fasting blood glucose was determined immediately on Hitachi auto analyzer (Hitachi 736, Japan) by glucose oxidase method. The rest of the serum was stored at -20°C for determination of the followings: ALT, AST, albumin, uric acid, creatinine, cholesterol, triglyceride and chemerin.

The determination of serum ALT, serum AST, serum albumin, serum uric acid, serum creatinine, serum cholesterol and serum triglyceride which were carried out on Hitachi 736 (Roche Diagnostics GmbH, D-68298 Mannheim, USA) by colorimetric techniques.

For determination of HDL-cholesterol, phosphotungestic acid and magnesium ions are used for precipitating all lipoproteins except HDL fraction that was present in the supernatant and measured by auto analyzer. LDL cholesterol was measured by Friedwald formula (Friedwald, *et al*., 1972).

About 2 ml of venous blood samples were taken from all patients 2 hours after meals for determination of postprandial blood glucose on Hitachi 736 auto analyzer.

Direct detection of C-reactive protein (CRP) in serum using rapid latex agglutination procedure (Wadsworth, and Wadsworth, 1984).

The determination of serum chemerin was carried out by sandwich enzyme immunoassay (ELISA) (Pfau, *et al*., 2010) and the kit was supplied from R&D system Europe, Ltd., 19 Barton Lane, Abingdon Science Park, Abingdon, United Kingdom.

All patients were instructed to collect 24 hours urines for determination of urinary proteins by ponceau S-TCA method (Pesce, and Strande, 1973).

Statistical analysis: Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing 2 groups when normally distributed and Mann Whitney *U* test for independent samples when not normally distributed. Comparison of numerical variables between more than two groups was done using one way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. $P < 0.05$ was considered statistically significant. All statistical calculations were done using computer programs SPSS version 15 for Microsoft Windows.

Results:

Thirty out of the 40 diabetic patients were female (75%), 10 patients were male (25%), the age group of our patients were ranged from 40 to 55 years old (46.5±5.9). 25 patients present with peripheral neuritis (62.5%), BMI in our patients ranged from 23 to 29 with a mean of 25.47 ± 1.95. Disease duration in our patients ranged from 1 to15 years table (1), 13 patients were on insulin (32.5%), 16 patients were on sulphonylurea (40%), 18 patients were on metformin (45%).

Table 1: Demographic data and laboratory parameters of the studied groups

Groups	Cases		DM		DM&HTN		CONTROL	
Parameters	Mean	±SD	Mean	±SD	Mean	±SD	mean	±SD
Age (years)	46.50	5.948	45	4.923	49.5	6.557	47	5.181
SBP (mmHg)	130	13.63	120	8.645	140	9.248	120	7.480
DBP (mmHg)	80	7.355	72.5	6.781	80	5.955	80	9.037
Pulse (bpm)	70	8.921	70	9.475	80	8.248	70	8.381
BMI (Kg/m^2)	30	3.236	29.50	2.929	30	3.541	25	1.959
RCM (cm)	0.085	0.16	0.08	0.025	0.080	0.0146	0.06	0.008
RPSV (cm/sec)	75.2	15.94	75.50	16.63	72.55	14.860	52.4	2.7570
REDV (cm/sec)	28.6	6.269	28.70	6.486	28.55	5.719	23.6	1.3652
RRI	0.63	0.682	0.615	0.070	0.640	0.0653	0.55	0.02789
LCM (cm)	0.09	0.019	0.09	0.023	0.0850	0.0141	0.06	0.01033
LPSV (cm/sec)	76.7	13.24	79.20	11.08	75.70	15.171	63.5	1.2955
LEDV (cm/sec)	29.2	8.552	30.75	8.775	28.150	7.8014	26.6	1.0825
LRI	0.64	0.116	0.620	0.065	0.665	0.1516	0.54	0.0252
TLC (10^3/cmm)	7.60	2.103	7.0	2.500	8.20	1.665	7.30	0.819
Hemoglobin(g/dl)	12.90	1.261	12.55	1.456	13	1.068	12.80	0.787
Platelets(10^3/cmm)	308	64.77	295	66.19	316.5	57.968	345	53.469
24h UB (mg/24h)	86	18.23	97.5	22.98	81	10.465	17.47	5.15
FBG(mg/dl)	179	20.66	180	19.32	176.50	20.756	85	10.756
2HPPBG(mg/dl)	221	27.57	209	47.37	228	48.801	124	8.259
Creatinine (mg/dl)	0.7	0.123	0.7	0.125	0.7	0.1257	0.7	0.1580
Uric acid (mg/dl)	6.7	1.170	6.7	1.282	6.750	1.0598	6.4	1.4207
AST(U/l)	28	4.966	28	3.552	29	5.924	25	2.699
ALT(U/l)	29	4.819	28.5	3.048	34	5.650	28	4.704
Albumin (g/dl)	3.9	0.413	3.9	0.350	3.9	0.4740	3.9	0.3312
Cholesterol (mg/dl)	199.5	57.42	190	60.09	211.50	56.061	96	12.775
Triglyceride (mg/dl)	118	48.64	118	32.42	118.50	61.120	64	9.627
LDLC(mg/dl)	136	30.41	127.5	24.17	136	35.600	45	10.467
HDLC (mg/dl)	42.50	21.82	44.50	14.25	42	27.674	55	9.523
Chemerin (ng/ml)	307.5	92.85	234.6	63.95	399.35	72.002	131.70	19.529

RCM: Right carotid intima media thickness LCM: Left carotid intima media thickness
RPSV: Right peak systolic velocity LPSV: Left peak systolic velocity
REDV: Right end diastolic velocity LEDV: Left end diastolic velocity
RRI: Right resistivity index LRI: Left resistivity index
TLC: Total leucocytic count 24h UB: 24 hour urinary proteins
2HPPBG:2 hours postprandial blood glucose.

Laboratory investigations: are shown in tables below

Dyslipidemia presents in 57.5% of our patients. Hypertension was present in 20 patients (50%). There was highly significant increase in mean serum chemerin in diabetic patients compared with control group (P: 0.000) table (2). There was a highly significant increase in total serum cholesterol level, and serum triglyceride level, LDL in diabetic patients compared with control (P: 0.000). There was highly significant increase in right, and left carotid intima media thickness in diabetic patients (0.08 ± 0.02, 0.09±0.019 respectively compared with

control group (0.07 ± 0.012, 0.06± 0.01) (P : 0.008, 0.000 respectively), and there was a highly significant increase in PSV,EDV,RI in diabetic patients compared with control (P<0.05) (table2).

There was statistical significant difference in mean serum chemerin level between diabetic patients with hypertension (379.59 ± 72.0) and the diabetic patients without hypertension (234,6± 63.95) with higher values detected in the first group(P:0.000)(tables 3, 4 and 5).

Table 2: Comparison of different demographic, laboratory, and Doppler findings between patients and control.

	Cases		CONTROL		P	SIGN
	Mean	±SD	mean	±SD		
Age (years)	46.50	5.948	47	5.181	0.537	NS
SBP(mmHg)	130	13.63	120	7.480	0.006	S
DBP(mmHg)	80	7.355	80	9.037	0.625	NS
Pulse(bpm)	70	8.921	70	8.381	0.656	NS
BMI(Kg/m^2)	30	3.236	25	1.959	0.000	S
RCM(cm)	0.08	0.020	0.07	0.01254	0.027	S
RPSV(cm/sec)	75.2	15.94	52.4	2.7570	0.000	S
REDV(cm/sec)	28.6	6.269	23.6	1.3652	0.005	S
RRI	0.63	0.682	0.55	0.02789	0.000	S
LCM(cm)	0.09	0.019	0.06	0.01033	0.000	S
LPSV(cm/sec)	76.7	13.24	63.5	1.2955	0.000	S
LEDV(cm/sec)	29.2	8.552	26.6	1.0825	0.005	S
LRI	0.64	0.116	0.54	0.0252	0.003	S
TLC(10^3/cmm)	7.60	2.103	7.30	0.819	0.567	NS
Hemoglobin(g/dl)	12.90	1.261	12.80	0.787	0.466	NS
Platelets(10^3/cmm)	308	64.77	345	53.469	0.045	S
24h UB(mg/24H)	86	18.23	17.4	5.15	0.000	NS
Creatinine(mg/dl)	0.7	0.123	0.7	0.1580	0.967	NS
Uric acid(mg/dl)	6.7	1.170	6.4	1.4207	0.367	NS
AST(U/l)	28	4.966	25	2.699	0.002	S
ALT(U/l)	29	4.819	28	4.704	0.350	NS
Albumin(g/dl)	3.9	0.413	3.9	0.3312	0.454	NS
Cholesterol(mg/dl)	199.5	57.42	96	12.775	0.000	S
Triglyceride(mg/dl)	118	48.64	64	9.627	0.000	S
LDLC(mg/dl)	136	30.41	45	10.467	0.000	S
HDLC(mg/dl)	42.50	21.82	55	9.523	0.512	NS
Chemerin(ng/ml)	307.5	92.85	131.70	19.529	0.000	S

Table 3: Comparison of different demographic, laboratory, and Doppler findings between diabetic patients without hypertension and control

	DM without HTN		CONTROL		P	SIGN
	Mean	±SD	mean	±SD		
Age (years)	45	4.923	47	5.181	1.000	NS
SBP(mmHg)	120	8.645	120	7.480	1.000	NS
DBP(mmHg)	72.5	6.781	80	9.037	0.894	NS
Pulse(bpm)	70	9.475	70	8.381	1.000	NS
BMI(Kg/m^2)	29.50	2.929	25	1.959	0.001	S
RCM(cm)	0.08	0.025	0.07	0.01254	0.098	NS
RPSV(cm/sec)	75.50	16.63	52.4	2.7570	0.000	S
REDV(cm/sec)	28.70	6.486	23.6	1.3652	0.002	S
RRI	0.615	0.070	0.55	0.02789	0.010	S
LCM(cm)	0.09	0.023	0.06	0.01033	0.002	S
LPSV(cm/sec)	79.20	11.08	63.5	1.2955	0.000	S
LEDV(cm/sec)	30.75	8.775	26.6	1.0825	0.028	S
LRI	0.620	0.065	0.54	0.0252	0.086	NS
TLC(10^3/cmm)	7	2.500	7.30	0.819	1.000	NS
Hemoglobin(g/dl)	12.55	1.456	12.80	0.787	1.000	NS
Platelets(10^3/cmm)	295	66.19	345	53.469	0.017	S
Creatinine(mg/dl)	0.7	0.125	0.7	0.1580	1.000	NS
Uric acid(mg/dl)	6.7	1.282	6.4	1.4207	1.000	NS
AST(U/l)	28	3.552	25	2.699	0.106	NS
ALT(U/l)	28.5	3.048	28	4.704	1.000	NS
Albumin(g/dl)	3.9	0.350	3.9	0.3312	1.000	NS
Cholesterol(mg/dl)	190	60.09	96	12.775	0.000	S
Triglyceride(mg/dl)	118	32.42	64	9.627	0.002	S
LDLC(mg/dl)	127.5	24.17	45	10.467	0.000	S
HDLC(mg/dl)	44.50	14.25	55	9.523	1.000	S
Chemerin(ng/ml)	234.6	63.95	131.70	19.529	0.000	S

Table 4: Comparison of different demographic, laboratory, and Doppler findings between diabetic patients with hypertension and control

	DM&HTN		CONTROL		P	SIGN
	Mean	±SD	Mean	±SD		
Age (years)	49.5	6.557	47	5.181	0.508	NS
SBP(mmHg)	140	9.248	120	7.480	0.000	S
DBP(mmHg)	80	5.955	80	9.037	0.152	NS
Pulse(bpm)	80	8.248	70	8.381	1.000	NS
BMI(Kg/m^2)	30	3.541	25	1.959	0.000	S
RCM(cm)	0.080	0.0146	0.07	0.01254	0.232	NS
RPSV(cm/sec)	72.55	14.860	52.4	2.7570	0.001	S
REDV(cm/sec)	28.55	5.719	23.6	1.3652	0.269	NS
RRI	0.640	0.0653	0.55	0.02789	0.000	S
LCM(cm)	0.0850	0.0141	0.06	0.01033	0.000	S
LPSV(cm/sec)	75.70	15.171	63.5	1.2955	0.002	S
LEDV(cm/sec)	28.150	7.8014	26.6	1.0825	1.000	S
LRI	0.665	0.1516	0.54	0.0252	0.006	S
TLC(10^3/cmm)	8.20	1.665	7.30	0.819	1.000	NS
Hemoglobin(g/dl)	13	1.068	12.80	0.787	1.000	NS
Platelets(10^3/cmm)	316.5	57.968	345	53.469	1.000	NS
Creatinine(mg/dl)	0.7	0.1257	0.7	0.1580	1.000	NS
Uric acid(mg/dl)	6.750	1.0598	6.4	1.4207	0.775	NS
AST(U/l)	29	5.924	25	2.699	0.001	S
ALT(U/l)	34	5.650	28	4.704	0.155	NS
Albumin(g/dl)	3.9	0.4740	3.9	0.3312	0.967	NS
Cholesterol(mg/dl)	211.50	56.061	96	12.775	0.000	S
Triglyceride(mg/dl)	118.50	61.120	64	9.627	0.000	S
LDLC(mg/dl)	136	35.600	45	10.467	0.000	S
HDLC(mg/dl)	42	27.674	55	9.523	1.000	NS
Chemerin(ng/ml)	399.35	72.002	131.70	19.529	0.000	S

Table 5: Comparison of different demographic, laboratory, and Doppler findings between diabetic patients without hypertension and those with hypertension

	DM without HTN		DM&HTN		P	SIGN
	Mean	±SD	mean	±SD		NS
Age (years)	45	4.923	49.5	6.557	0.236	NS
SBP(mmHg)	120	8.645	140	9.248	0.000	S
DBP(mmHg)	72.5	6.781	80	5.955	0.005	S
Pulse(bpm)	70	9.475	80	8.248	0.737	NS
BMI(Kg/m^2)	29.50	2.929	30	3.541	1.000	NS
RCM(cm)	0.08	0.025	0.080	0.0146	1.000	NS
RPSV(cm/sec)	75.50	16.63	72.55	14.860	0.358	NS
REDV(cm/sec)	28.70	6.486	28.55	5.719	0.150	NS
RRI	0.615	0.070	0.640	0.0653	0.724	NS
LCM(cm)	0.09	0.023	0.0850	0.0141	1.000	NS
LPSV(cm/sec)	79.20	11.08	75.70	15.171	0.920	NS
LEDV(cm/sec)	30.75	8.775	28.150	7.8014	0.110	NS
LRI	0.620	0.065	0.665	0.1516	0.850	NS
TLC(10^3/cmm)	7	2.500	8.20	1.665	1.000	NS
Hemoglobin (g/dl)	12.55	1.456	13	1.068	1.000	NS
Platelets(10^3/cmm)	295	66.19	316.5	57.968	0.110	NS
Creatinine(mg/dl)	0.7	0.125	0.7	0.1257	1.000	NS
Uric acid(mg/dl)	6.7	1.282	6.750	1.0598	1.000	NS
AST(U/l)	28	3.552	29	5.924	0.293	NS
ALT(U/l	28.5	3.048	34	5.650	0.058	NS
Albumin(g/dl)	3.9	0.350	3.9	0.4740	1.000	NS
Cholesterol(mg/dl)	190	60.09	211.50	56.061	1.000	NS
Triglyceride(mg/dl)	118	32.42	118.50	61.120	1.000	NS
LDLC(mg/dl)	127.5	24.17	136	35.600	0.798	NS
HDLC(mg/dl)	44.50	14.25	42	27.674	1.000	NS
Chemerin(ng/ml)	234.6	63.95	399.35	72.002	0.000	S

There was no statistically significant correlation in diabetic patient with or without hypertension between serum chemerin level and BMI, diabetes duration, drug therapy, left and right carotid intima media thickness, total cholesterol level, triglyceride level, and LDLc level (table 6). There was also no statistical significant difference in serum chemerin level between dyslipidemic diabetic patients and non dyslipidemic diabetic patients (table 7).

Table 6: Correlation of serum chemerin to different demographic, laboratory and imaging data in DM without and with hypertension groups.

	Serum chemerin			
	DM without hypertension		DM with hypertension	
	r	P	r	P
BMI	0.197	0.406	-0.010	0.966
Disease duration	-0.123	-0.123	0.444	0.050
Insulin	-0.164	0.490	0.416	0.068
Sulphonylurea	-0.109	0.647	-0.392	0.087
Metformin	-0.105	0.661	0.064	0.790
Peripheral neuropathy	-0.127	0.593	0.310	0.184
RCM	0.139	0.559	0.155	0.513
RPSV	-0.159	0.502	0.139	0.558
REDV	-0.075	0.752	0.037	0.876
RRI	-0.151	0.526	-0.242	0.304
LCM	0.157	0.510	-0.226	0.338
LPSV	0.003	0.989	0.015	0.948
LEDV	-0.068	0.776	0.190	0.423
LRI	0.045	0.851	-0.198	0.402
24h urinary protein	0.048	0.840	-0.089	0.710
FBG	0.252	0.284	0.100	0.676
2HPPBG	0.035	0.883	0.082	0.730
Cholesterol	0.440	0.052	-0.337	0.146
Triglycerides	0.015	0.951	-0.162	0.496
LDLc	0.157	0.508	-0.077	0.746
HDLc	0.243	0.302	0.362	0.116
Plaque	0.058	0.809	0.364	0.114
Stenosis	-1.000	1.000	-0.500	0.667

Table 7: Correlations of serum chemerin to different imaging data in all diabetic patients without dyslipidemia.

	Serum chemerin	
	r	p
RCM	0.271	0.293
RPCV	-0.392	0.120
REDV	-0.137	0.601
RRI	-0.092	0.725
LCM	0.134	0.607
LPSV	-0.221	o.394
LEDV	0.026	0.922
LRI	0.158	0.544

Discussion:

Vascular diseases, particularly atherosclerosis, are major causes of disability and death in patients with diabetes mellitus. Diabetes mellitus substantially increases the risk of developing coronary, cerebrovascular, and peripheral arterial disease. The pathophysiology of vascular disease in diabetes involves abnormalities in endothelial, vascular smooth muscle cell, and platelet function (Creager*, et al*., 2003).

In this study, we tried to assess the relationship between serum chemerin level and atherosclerotic vascular disease in diabetic patients, to highlight the different etiology of atherosclerosis in diabetic patients, and to demonstrate any early changes in the carotid arteries in diabetic patients. We tried also to find any predictor of vascular affection in diabetic patients as compared to controls.

Adipokines are cytokines, chemokines and hormones secreted by adipose tissue that couple the regulation of lipid accumulation, inflammation, and atherogenesis, and therefore serve to link obesity with cardiovascular disorders. Obesity-related disorders including metabolic syndrome, diabetes, atherosclerosis, hypertension, and coronary artery disease are associated with dysregulated adipokine(s) expression. Recent studies demonstrate the proinflammatory effects as well as atherogenic properties of adipokines. Adipokines also participate in the regulation of endothelial function, which is an early event in atherosclerosis (Zhang, H., J. Cui, C. Zhang, 2010).

Chemerin, also known as tazarotene-induced gene 2 protein (TIG2) or retinoid acid receptor responder 2 (RARRES2), was a recently identified novel adipokine that has a role in adaptive and innate immunity (Ernst, and Sinal, 2010). Chemerin is associated with inflammation, adipogenesis, glucose, and lipid metabolism, all of which may contribute to the development of diabetic cardiovascular complications, especially atherosclerosis (Goralski, *et al*., 2007)

In our study there was a statistical significant increase in serum chemerin level in all the studied diabetic patients(with and without hypertension) with mean 316.34±92.85 SD ug/l compared with control with a mean 130.56±19.52 SD ug/l (P:0.000).

This was in agreement of Mesallamy *et al*., (2012) results who revealed that serum chemerin levels were found to be significantly increased in patients with type 2 diabetes (347 ± 14 ng/ml) compared with control subjects (281 ± 13 ng/ml) (P < 0.01).

Also in our study there was a highly significant increase in serum chemerin level in diabetic patients with hypertension(379.5 ± 63.95 ng/ml) compared with those without hypertension (253.09 ± 14 ng/ml) (P:0.000).

This was in agreement of Yang *et al*., (2010) results who found that plasma chemerin levels were found to be markedly increased in patients with type 2 diabetes mellitus with hypertension as compared with patients with type 2 diabetes mellitus and normal controls (P < 0.01).

Carotid-wall intima–media thickness (IMT) is a surrogate measure of atherosclerosis (Hodis, *et al*., 1996) associated with cardiovascular risk factors (O'Leary, D.H., *et al*., 1996) and with cardiovascular outcomes (O'Leary, *et al*., 1999).

Increased intima–media thickness of the common carotid artery represents a form of atherosclerosis that is manifested as diffuse arterial-wall thickening (Pignoli, *et al*., 1986). Although measurement of intima–media thickness is promoted as a tool for cardiovascular risk assessment (Greenland, *et al*., 2010) in primary prevention, the incremental predictive value of the intima–media thickness of either the common carotid artery or the internal carotid artery, over and above the value of traditional cardiovascular risk factors, is questionable (Nambi, *et al*., 2010).

In our study there was highly significant increase in right, and left carotid intima media thickness in diabetic patients compared with control groups, (P:0.027, 0.000 respectively), and this signifies early atherosclerotic changes in diabetic patients as compared to age and sex matched healthy control, whom has lower values of carotid intima media thickness. All our patients had no clinical manifestation of atherosclerosis (no macro vascular complication), so our finding highlight an important area; the importance of intima media thickness as a predictor of atherosclerosis, to be one of the subclinical parameter of atherosclerosis.

In our study there was no statistically difference between serum chemerin and BMI, CRP, cholesterol, triglyceride, LDL and HDL in all groups of our patients; (diabetic patient with and without hypertension, diabetics with hypertension, diabetics without hypertension and this was in contrast to Lehrke, *et al*., (2009) results who revealed that chemerin was associated with components of the metabolic syndrome including body mass index (r=0.23, P=0.0002), triglycerides (r=0.29, P<0.0001), HDL-cholesterol (r=−0.18, P=0.003), and hypertension (P<0.0001). In bivariate analysis, chemerin levels were weakly correlated with coronary plaque burden (r=0.16, P=0.006) and the number of non-calcified plaques (r=0.14, P=0.02). This may be different in patients selection as Lehrke, *et al*., (2009) choose patient with cardiac symptoms, while our entire patient had no cardiac symptoms. Also our results may signify a multi-factorial causes for atherosclerosis in type II diabetic patients and not a single agent can precipitate atherosclerosis in diabetics.

Also Bozaoglu *et al*., (2007), identified significant associations between circulating chemerin levels and characteristics of the metabolic syndrome in a relatively small sample of human subjects from Mauritius. He also conducted a second survey of circulating chemerin levels in a population that was genetically and geographically distinct from their initial study. In these studies, they had measured circulating chemerin levels in a large sample (n=1142) of Mexican-American individuals, they have found significant associations between plasma chemerin levels and a number of metabolic syndrome, and they concluded that circulating chemerin levels were associated with metabolic syndrome phenotypes in a second unrelated human population (Bozaoglu, *et al*., 2009). This may elicit another cause for the difference of our results and the other studies result, and this may be due to the difference in the ethnic background.

Conclusion and limitation of the study: High levels of serum chemerin found in our diabetic patients indicate the activation of immune response in these patients. High carotid intimal media thickness in diabetic patients was not related to chemerin denoting an underlying combining factor for atherosclerosis in diabetic patients other than chemerin. High chemerin in diabetic hypertensive patients denote additive role of hypertension to diabetic in beginning of immune response in those patients.

We also assessed the association of serum chemerin with body composition and metabolic parameters in these subjects. Limitation of our study may be in the small number and the selection of the studied patients, thus further studies using a large patient sample may prove that chemerin may be involved in the development of the metabolic syndrome, and to confirm the associations which were previously observed in several other studies. We cannot suggest that chemerin association with type 2 diabetes and metabolic parameters is population-specific.

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