Adipokines at the crossroad between obesity and type 2 diabetes mellitus

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Abstract

Objective: Investigate serum levels of chemerin, apelin, betatrophin and leptin adipokines and HsCRP in obese and non-obese patients either prediabetic or diabetic and correlate their levels with other biochemical parameters of IR. Additionally, studying the effect of their combinations on the sensitivity of investigation for the development and/or progression of T2DM.

Subjects and Methods: Serum levels of adipokines were analyzed using ELISA technique in 160 subjects.

Results: Serum Chemerin, and HsCRP levels were significantly higher in all obese groups compared to non-obese of the same group.Serum levels of both betatrophin & HsCRP were positively correlated with glycemic indices (FPG, A1c, HOMA-IR). Serum levels of chemerin were positively correlated with FPG, A1c. Serum Chemerin, betatrophin, HsCRP all showed negative correlation with HOMA-β level. The combination of different markers improved their sensitivity of detection, with the highest level of sensitivity shown between betatrophin with HsCRP.

Conclusion: These results indicate crucial roles played by the studied adipokines and HsCRP in IR caused by obesity and consequently T2DM development. Combination of Betatrophin and HsCRP can be an early sensitive predictor for IR occurrence in obese patients. These adipokines may be targets for the development of therapies to treat or inhibit IR associated to obesity.

Keywords: Obesity, Insulin resistance, T2DM, Adipokines, Combined sensitivity.

Introduction

Obesity is a chronic disorder characterized by genetic, environmental and hormonal origin.[1] The increase in the obesity rate has been paralleled by a reduction in life expectancy and an increase in various obesity-related comorbidities and many metabolic disorders including type 2 diabetes mellitus (T2DM).[2] It was suggested that obesity prompt insulin resistance and so develop T2DM.[3] Diabetes mellitus (DM) considered as a major public health problem. In 2010, it was estimated that 4.787 million Egyptians had DM and this number may increase to 8.615 million Egyptians by the year 2030.[4] Adipose tissue depots are the most susceptible target to mediate significant immune cells infiltration and inflammation contributing to systemic inflammation and IR in obese humans.[3] Additionally, adipose tissue secretes many bioactive peptides or hormones, collectively named "adipokines". They play a central role in energy and vascular homeostasis and are fundamental to the pathogenesis of the metabolic syndrome (MS).[1] Dysregulation of adipokines (pro-inflammatory/ anti-inflammatory) secretion in obesity may serve as a pathogenic link between obesity, IR and cardiovascular diseases.[5] Chemerin is an adepokine secreted as a 18KD inactive pro-protein and undergoes activation through an extracellular serine protease cleavage of the C-terminal portion of the protein to generateing the active 16 kDa chemerin.[5] According to the cleavage process the function of the formed chemerin will vary, for example, chemerin 21e157 is responsible for an early inflammatory reaction of the immune cells due to its strong chemotactic effect, while chemerin 21e154 act as anti-inflammatory though inhibition of macrophage activation.[6] In adipose tissue, chemerin and its receptor are plentifully expressed, suggesting its role in autocrine/ paracrine fashion.[7] Apelin is a peptide acting as a ligand of the G-protein-coupled receptor APJ. It is found in several active forms such as apelin-36, apelin-17, apelin-13, and the pyroglutamated form of apelin-13. Apelin and APJ are expressed in the CNS, especially in the hypothalamus as well as in many peripheral tissues. Apelin was shown to be involved in the regulation of food intake, cardiovascular and fluid homeostasis, cell proliferation, and angiogenesis. Apelin is produced and secreted by adipocytes and thus considered as an adipokine.[1,8] Betatrophin, known also as lipasin[9] is a 22-kDa hormone that is primarily expressed in liver and adipose tissue. It promotes pancreatic β-cell proliferation, expand β-cell mass, and improve glucose tolerance in a mice model of IR.[10] It has been reported that liver betatrophin expression was increased after administration of an insulin receptor antagonist in mice, leading to a compensatory increase in β-cell replication.[10] Moreover, serum level of betatrophin levels is found to be positively associated with type 1 DM (T1DM) and T2DM,[11] hyperlipidemia, and indices of IR.[12] Leptin is a 16-kDa protein secreted by adipocytes - inhibits glucose uptake, impairs lipogenesis, glycogen synthase and inhibits lipolysis,[13] while, in hepatocytes, it triggers insulin-like effects through regulating insulin-signaling pathway.[14] Thus, there is a possible link between leptin and insulin signaling, that make hepatocytes more sensitive to insulin.

Hereafter, the current study aimed to investigate the serum levels of chemerin, apelin, betatrophin and leptin in obese and non-obese patients either prediabetics or those

having T2DM. Besides, correlating their levels with other biochemical parameters of IR, and studying the effect of their combinations on the sensitivity of exploration of the development and/or progression of T2DM.

Subjects and Methods

Participants: The present study comprised one hundred and sixty subjects (49 male & 111 female) categorized into six groups: Group 1: healthy non obese group (n = 23); healthy subjects with body mass index (BMI) < 30 Kg/m^2, group 2: healthy obese group (n =24); healthy subjects with BMI > 30 Kg/m^2, group 3: prediabetic non obese group (n = 20); patients with BMI < 30 Kg/m^2 and with impaired glucose levels, group 4: prediabetic obese group (n=17); patients with impaired glucose having BMI > 30 Kg/m^2, group 5: Diabetic non obese group (n= 37); patients with T2DM having BMI <30 Kg/m^2, and finally group 6: Diabetic obese group (n= 39); patients withT2DM having BMI > 30 Kg/m^2. Patients were enrolled from outpatients Clinic of National Institute for Diabetes and Endocrinology, Cairo, Egypt, fulfilling the diagnostic criteria defined by the ADA.[15] All patients were also taking the same type of antidiabetic therapy. A self-made questionnaire was given to each subject for recording the demographic data. The study was approved by the Research Ethics Committee of the General Organization for Teaching Hospitals and Institutes. The study was performed according to the regulations and recommendations of the Declaration of Helsinki and all subjects gave written informed consent prior to participation in the study. For each subject, a detailed history was obtained, BMI was calculated [the weight (in kilograms) divided by the square of the height (in meters)] measured on the day of sample collection. Individuals having BMI > 30 were considered as obese subjects and those having BMI < 30 were non-obese. The exclusion criteria were: any history of smoking, alcohol habits, respiratory disorder, clinical or laboratory signs of liver disease, thyroid function impairments, chronic inflammation, patients who were receiving any medications other than antidiabetic drugs or those complaining from other chronic or acute diseases as well as any significant infectious diseases were excluded from the study

Methods: An overnight fasting blood samples were withdrawn from every participant, divided into three different tubes: I-0.5 M EDTA-containing tubes for glycated heamoglobin (A1c) determination, II- fluoride-containing tubes for fasting plasma glucose (FPG) determination and III- serum separating tubes. The serum tubes were centrifuged at 3000 rpm for 20 min, sera were separated and divided into several aliquots; one aliquot was used for lipid profile determination and serum creatinine and the other aliquots were kept at -80°C for the determination of serum Chemerin, Apelin, Betatrophin, leptin and hsCRP and insulin.

Laboratory Measurements

Fasting plasma glucose (FPG) was analyzed using glucose oxidase method (Siemens healthcare diagnostic, USA).[16] Serum triacylglycerol (TAG) and serum Total cholesterol (TC) were assayed by enzymatic-colorimetric end point methods.[17] Serum high-density lipoprotein cholesterol (HDL-C) was assayed by enzymatic method[18] and serum low-density lipoprotein cholesterol (LDL-C) was measured using precipitation, Heparin/Citrate method.[19] The lipid profile parameters were measured using commercially available kits from Spectrum Diagnostics, Egypt. HPLC fully automated system was used for the determination of serum A1c with G8 instruments (Tosoh, Tokyo, Japan).[20] Serum creatinine was assayed using enzymatic colorimetric method.[21] Sampling, reagent delivery, mixing, processing, calculating and printing were full automatically performed by BT3500 chemistry system (Biotecnica, Instruments Inc, ITALY). Serum insulin was estimated using ELISA kit (DRG International Inc., USA) according to the manufacturer's procedure. Evaluation of IR degree was done using the homeostasis model of assessment (HOMA-IR) using the formula: [FPG (mg/dL) × fasting insulin (μ IU/mL)]/405, and (HOMA- β) was used for evaluation of Beta-cell function by using the formula: [360 × fasting insulin (μIU/mL)]/ [FPG (mg/dL) – 63].[22] Serum Chemerin, Apelin, Betatrophin (Glory Science Co., USA), leptin (DRG Co., Germany) and hsCRP (Immunospec Corp, USA) concentrations were determined using commercially available ELISA kits that had been conducted according to the manufacturer's instructions using Absorbance Microplate Reader ELx808TM, Biotek, USA.

Statistical Analysis

Data analysis was done using IBM SPSS statistics 22 package program (For Windows, © 2013, IBM software Inc., V 22, USA). Graphs were plotted using GraphPad Prism 5 (For Windows, © 2007, Graphpad software Inc., V 5.01, USA). Results were expressed as mean ± SEM. Ordinary one-way analysis of variance (ANOVA) for parametric data was used to analyze more than two sets of data, followed by Tukey-Kramer multiple comparisons test. The difference between groups was considered significant if the probability (P value) $\leq$ 0.05. The correlations between variables were determined by Pearson's correlation coefficient. Sensitivity calculations were determined according to lows of accuracy indices documented by [23] which were verified Chi-square test.

Results

The clinical and laboratory parameters for all study subjects are summarized in Table 1. Concerning age and sex, the six groups of the study were matched as no significant difference was found among them. The mean BMI of all obese groups (normal, prediabetic and diabetic) were significantly higher compared to normal non-obese groups, and the mean BMI of non-obese (prediabetic and diabetic) groups were significantly lower than that of obese normal group. Regarding lipid profile, no significant difference was verified (P> 0.05) in the mean serum levels of TC, HDL-c and LDL-c upon comparing the studied groups to control group while serum TAG level showed

significant increase in the diabetic group - either obese or non-obese - compared to both obese or non-obese healthy groups. Studying glycemic control indices showed a significant increase in the mean FPG level in diabetic non-obese and obese groups compared to both control and prediabetic groups (either non-obese or obese). Also a significant difference was found between obese and non-obese diabetic groups. With respect to A1C, its mean level was noted to be gradually increased from control to prediabetic till reach significant highest value in diabetic groups (obese & non-obese) compared to the other four groups. Serum insulin level showed no significant difference among the six studied groups. Yet, HOMA-IR showed gradual increase in its value through the studied groups till showed significant high level in diabetic obese group compared to all other studied groups. On the other hand, HOMA- β showed marked decrease in both prediabetic and diabetic groups but significance is only noted in obese diabetic group compared to all other study groups and also in non-obese diabetic group compared to non-obese control group. Notably, serum Chemerin concentration was significantly increased in all obese groups (diabetic, prediabetic & control) compared to the non-obese of the same group with the higher value reached in the diabetic obese group (Fig. 1A). The same results obtained with serum leptin level although they did not reach significant values, but its level showed notable significant different values between obese and non-obese of the diabetic group (Fig. 1B). Serum Apelin and serum HsCRP levels showed significant increase in the obese groups of both prediabetic and control groups compared to the non-obese groups of the comparable groups (Fig. 1C & 1D respectively). Although serum betatrophin levels revealed slight increase in the obese groups of control, prediabetic & diabetic groups compared to corresponding non-obese cohorts, the only significant increase of betatrophin's level was seen in diabetic groups (obese & non-obese) compared to all other four groups (Fig. 1E). Upon testing available correlations, it is found that betatrophin & HsCRP serum levels were positively correlated with glycemic control indices (FPG, A1C, HOMA-IR) and also with each other, while both showed negative correlation with HOMA-β levels. Also, serum levels of chemerin were positively correlated with FPG and A1C while it showed a negative correlation with HOMA-β. Serum levels of Apelin correlated only with serum Chemerin level while serum level of Leptin did not show any correlation with the other parameters (Table 2). Evaluation of the sensitivity levels of the investigated markers either alone or in different combinations with each other's showed that combinations of apelin, chemerin, betatrophin and leptin with HsCRP increase the sensitivity of these markers with the highest sensitivity shown between betatrophin and HsCRP (Table 3).

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

Parameter	Control (*n* = 47)		Prediabetic group (*n* = 37)		Diabetic group (*n* = 76)	
	Non-obese (n=23)	Obese (n=24)	Non-obese (n=20)	Obese (n=17)	Non-obese (n= 37)	Obese (n=39)
Age (years)	42.09 ± 1.2	44.08±1.45	43.65±1.67	45.82±1.74	45.89±0.99	45.31±1.1
Gender M/F	8/15	6/18	9/11	4/13	9/28	13/26
Duration of disease	-----	--------	---------	---------	7.86±0.75	7.49±0.8
BMI (kg/m2)	24.65±0.69	35.53±0.8[a]	25.85±0.52[b]	35.53±1.17[a]	26.26±0.32[b]	35.16±0.68[a]
FPG (mg/dL)	88.04±1.19	96.04±2.29	106.7±5.97	104.65±3.18	194.95±12.7 [a, b, c, d]	239.79±17.71[a, b, c, d, e]
A1c (%)	5.32±0.05	5.51±0.03	5.88±0.05	6.05±0.05	9.17±0.28 [a, b, c, d]	9.86±0.34 [a, b, c, d]
TC (mg/dL)	187.3±6.07	197.58±10.46	192.65±7.09	206.35±9.42	206.43±6.54	211.28±8.01
TAG (mg/dL)	103.74± 9.18	104.83±7.93	117.1±11.43	140.59±7.71	148.3±9.45 [a, b]	154.36±12.27 [a, b]
HDL-c (mg/dL)	42.17±1.69	40.08±1.54	39.5±2.22	42.35±1.76	41.62±1.2	40.64±1.23
LDL-c (mg/dL)	123±6.11	133.75±9.00	120.85±7.44	133.12±8.97	134.81±8.25	134.08±6.56
Cr (mg/dl)	0.99±0.05	0.97±0.04	0.91±0.04	0.96±0.03	0.94±0.04	1.04±0.05
Insulin (µIU/mL)	11.09±1.87	9.86±1.05	13.88±2.01	12.45±1.59	15.93±1.95	17.12±2.06
HOMA-IR	2.43±0.43	2.25±0.21	3.85±0.69	3.87±1.03	7.15±0.95	10.61±1.88 [a, b, c, d]
HOMA- β	155.74±23.83	119.71±15.37	134.07±23.97	119.28±17.45	69.70±13.67 [a]	44.2±4.93 [a, b, c, d]
Leptin	20.87±3.2	28.1±2.23	15.48±2.32 [b]	27.19±3.46	14.53±1.54[b,d]	26.21±3.01[e]
betatrophine	11.44±1.57	15.7±2.01	13.77±2.09	17.23±2.42	28.78±1.68[a, b, c, d]	37.07±1.85[a, b, c, d]
Apelin	1388.4±53.07	2143.8±198.63[a]	1250.9±53.14[b]	1972.2±224.64[c]	1762.1±115.69	1751.9±73.25
Chemerin	968.71±80.31	1547.09±94.49 [a]	876.44±79.44 [b]	1408.12±100.94 [c]	1202.57±62.71	1767.67±105.62 [a, c, e]
HsCRP	60.61±8.36	103.34±5.65 [a]	73.79±9.74 [b]	110.47±6.85[a, c]	114.74±3.84 [a, c]	109.46±4.04 [a, c]

Results are represented as mean ± SEM.

[a]:compared to control non-obese group. [b]:compared to obese control group. [c]:Compared to prediabetic non-obese group. [d]:Compared to prediabetic obese group. [e]:Compared to Diabetic non-obese group.

BMI: body mass index; FPG: fasting plasma glucose; Cr: serum creatinine; TC: total cholesterol; TAG: triacylglycerol; LDL-c: low-density lipoprotein-cholesterol; HDL-c: high-density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment of β cell function; hsCRP: hi sensitive C- reactive protein.

Table 2: Correlations between Leptin, Apelin, Betatrophin, Chemerin, hsCRP and other studied parameters.

Variable	Leptin		Betatrophine		Apelin		Chemerin		hsCRP	
	r	p	r	p	r	p	r	p	r	p
FPG	0.014	0.865	0.349	0.000*	0.045	0.571	0.240	0.002*	0.280	0.000*
A1c	-0.064	0.418	0.452	0.000*	0.099	0.211	0.278	0.000*	0.327	0.000*
F. insulin	-0.044	0.577	0.060	0.447	0.116	0.143	0.006	0.944	0.134	0.092
HOMA-IR	-0.059	0.459	0.177	0.025*	0.095	0.234	0.118	0.137	0.206	0.009*
HOMA-β	-0.053	0.509	-0.304	0.000*	0.000	0.996	-0.223	0.005*	-0.168	0.034*
Betatrophine	0.028	0.724			0.043	0.591	0.096	0.228	0.264	0.001*
Apelin	0.094	0.236					0.196	0.013*	0.055	0.490
Chemerin	0.096	0.227							0.107	0.178
HsCRP	-0.032	0.686								

FPG: Fasting plasma glucose; F. insulin: fasting insulin; hsCRP: hi sensitive C- reactive protein.
r: Pearson rank correlation coefficient
*: significant correlations.
P < 0.05 is considered significant

Table 3: Combined sensitivity for the studied parameters as early markers for development of T2DM

Adipokine	Apelin (30.95%)	Chemerin (51.91%)	Betatrophin (81.54%)	Leptin (39.39%)
Chemerin *(51.91%)*	62.32%**			
Betatrophin *(81.54%)*	85.51%***	87.23%***		
Leptin *(39.39%)*	56.12%^{NS}	68.09%^{NS}	86.71%***	
HsCRP *(81.00%)*	85.11%***	88.19%***	93.15%***	87.76%^{NS}

- NS: p>0.05, **: p < 0.01, ***: p < 0.001 using Chi-square test.
- All data are in percent and values were approximated to the second digit.
- Individual sensitivity for each marker was shown in ***(bold italic)*** between parenthesis.

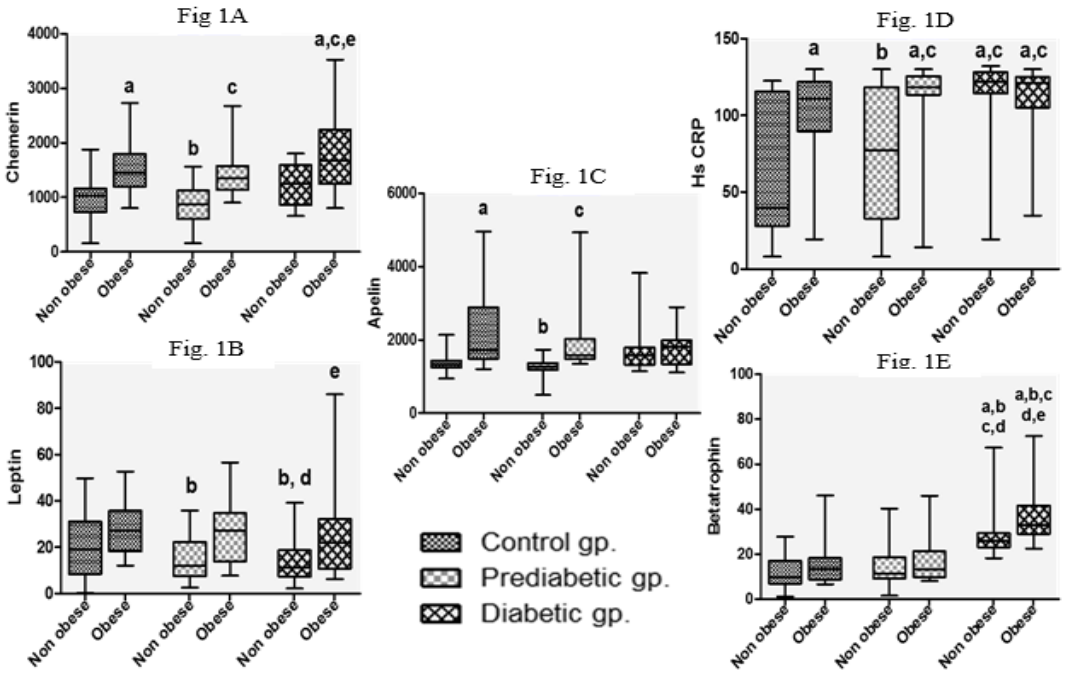

Fig. (1): Serum Chemerin (1A), Serum Leptin (1B), serum Apelin (1C), serum HsCRP (1D) and serum Betatrophin (1E) concentrations in all the studied groups.

a: Compared to control non-obese group. b: Compared to obese control group. c: Compared to prediabetic non-obese group. d: Compared to prediabetic obese group. e: Compared to Diabetic non-obese group.

Discussion

Obesity and T2DM, the two main components of metabolic syndrom, are the major non-communicable public health problems of the 21st century. The best way to tackle these problems is to develop strategies to prevent/treat obesity.[24] The increased prevalence in obesity is associated also with increasing prevalence of developing a variety of pathological conditions, including IR, T2DM, dyslipidemia and hypertension.[25] The importance of adipose tissue comes from its ability act as endocrine system secreting various bioactive molecules known collectively as adipokines[26] which regulate different physiological functions such as insulin sensitization, inflammatory response, vascular homeostasis and many others.[27] The findings of this study showed that serum Chemerin levels were elevated significantly in all obese groups compared to corresponding non-obese cohorts with the higher value reached in the diabetic obese group, this revealed that its level affected by both obesity and IR, which comes in agreement with previous reports of Verrijn Stuart et al, who showed that chemerin had elevated levels in obese patients compared to controls.[28] The present finding has also been confirmed by a study done by Henrike Sell et al, who reported that Adipocyte-derived secretion of chemerin contribute to the negative relationship between obesity and insulin sensitivity,[29] and also with agreement with a recent study done by Habib et al, who demonstrated that serum chemerin levels are elevated in patients with T2DM compared to healthy control subjects.[30] The strong relationship between chemerin levels and obesity as demonstrated here, could be considered a major risk factor for T2DM development. Being chemerin concentration is found in its highest value in the diabetic obese group confirms this hypothesis. The current study also demonstrated a significant direct correlation between serum chemerin level and glycemic indices (FPG & A1c). On contrary, a significant negative correlation is ensured with the index of β-activation, HOMA-β. These results came in accordance with Habib et al., who showed that serum chemerin is positively correlated with adiposity and IR in patients with T2DM.[30]

Regarding betatrophin, its serum levels were significantly elevated only in diabetic groups (obese & non-obese) compared to all other four groups although its level showed marked increased in the obese groups of both of control, prediabetic groups compared to the non-obese groups of the same category. These results came in agreement with a study done on Chinese people which showed that serum betatrophin levels were increased significantly in overweight subjects in both healthy and T2DM groups,[31] also in accordance with a meta-analysis study done which demonstrate the presence of a relationship between serum betatrophin levels and T2DM.[32] Also, this study demonstrated a positive correlation between betatrophin levels and glycemic indices (FPG, Ac1, HOMA-IR). Also a positive correlation with serum HsCRP level was found. On the other hand, betatrophin levels were negatively correlated with HOMA-β levels. This came in accordance with Guo et al., who showed that serum betatrophin concentrations were positively correlated with HOMA-IR in all subjects.[31]

The present study showed that serum leptin level was higher in obese groups compared to non-obese groups of the same category with the value reached significance between the diabetic subgroups only. This result came in consistence with a study done by Zimmet et al who reported that the strong relation of leptin with obesity is consistent with leptin production being proportional of mass to adipose tissue.[33] Interestingly, presence of higher leptin levels especially in obese subjects makes them even more resistant to insulin-like effects thus mediating T2DM in obese subjects. This has been well confirmed in studies showing rise in circulating leptin levels in relation to BMI and percentage total body fat.[34,35]

Also, the results matched with a study in which authors showed that serum leptin level decreased in obese individuals after they subjected to weight reduction.[36] In contrast with Tsu-Nai Wang et al who showed a presence of correlation between serum leptin level and IR,[36] serum leptin level in this study did not show any correlations with the glycemic indices nor the IR parameters.

Because of its close relationship with insulin,[37] recently, there is a growing appreciation that apelin has a crucial role in pathogenesis of IR and T2DM.[8,38] Our results came supporting and demonstrated that serum apelin concentration increases with dysregulation of the degree of IR, however the significance was only shown in prediabetic groups compared to corresponding healthy controls. On the other hand, no member of the diabetic group adopts treatment with insulin, and since insulin treatment is a strong positive trigger for apelin's expression and secretion,[8,37] this may elucidate the lack of the significance in apelin's concentration in the overt diabetic group.

The small number of the study group members may be considered another obstacle. During adipogenesis, apelin's gene is upregulated[37] and since there is also a triggered upregulation parallel to the expression of inflammatory markers elevated during obesity-associated IR,[8,39] our results came in consistence demonstrating a significant increase in apelin's concentration in the obese controls and prediabetics compared to non-obese individuals of the same group. Notably, this follows the pattern of increase in the inflammatory marker hsCRP in the control and prediabetic groups. However, due to the lack of increase in hsCRP in obese diabetics compared to non-obese individuals, the apelin's concentration fails to get significance between the two diabetic subgroups. Many previous studies support this link with chronic inflammatory markers and agree with us regarding the same pattern of increase in adiposity.[40]

Although these findings, it's not a fact that obesity is the main determinant for increased levels of apelin since its circulating concentrations are not always correlated with BMI in a significant manner. This is also a result in our study which came in accordance with many previously published data.[41,42] On contrary to the findings of other studies, changes in serum apelin level in this study fails to

correlate significantly with glycemic indices,[41,43] and HOMA-IR.[44]

HsCRP level in this study showed higher levels in obese groups compared to non-obese groups, in agreement with Uemura et al who demonstrates that elevated systemic inflammation, measured by serum hs-CRP, was associated with increased IR in a Japanese population.[45] Concomitant obesity and systemic inflammation might synergistically contribute to IR and subsequently T2DM.

This study for the first time, studied the effect of combination of different markers on the sensitivity of detection compared to that of each alone, and showed that combination causes increase the sensitivity significantly compared to individual markers with the highest level of sensitivity shown between betatrophin with HsCRP.

Conclusion

Our study supports the hypothesis that deregulated production of hsCRP and adipokines; chemerin, leptin, betatrophin, apelin owing to adipose tissue dysfunction can contribute to the pathogenesis of obesity-linked complications that may lead eventually to T2DM. It is apparent that the role of adipokines in obesity and its associate metabolic complications is complex, involving numerous proteins that may act independently or in consonance. A rather complicated interplay between a huge number of adipokines and their overlapping physiological effects adds to other environmental or genetic factors to decide the development of T2DM.

These results indicating crucial role for chemerin, leptin, betatrophin, apelin and hsCRP in IR caused by obesity and that these adipokines may be targets for the development of therapies to treat IR associated with obesity to prevent the development of T2DM in those patients. Betatrophin showed the greatest interest in this study as it showed the highest level of correlations with the glycemic indices and also showed increase sensitivity when combined with other markers especially when combined with HsCRP. However, the small number of patients is one of the limitations of the current study.

Any of the studied markers combined with either HsCRP or betatrophin, the sensitivity level will be significantly ameliorated, and this will be a good impact in early detection of T2DM even in prediabetes stage before development of overt diabetes and as a result this may be a good facility to protect the patient from serious complications. However, further studies on large scale should be conducted to confirm our findings and identify suitable strategies to prevent or at least retard the development of T2DM.

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