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ORIGINAL ARTICLE

Determination of Leptin Adiponectin (L/A), LDL/HDL ratio with substantial role of Adiponectin and its Receptor in Elderly Population as Marker of Dyslipidemia and Disease Severity

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ABSTRACT

Aim: To determine Leptin/Adiponectin ratio, LDL/HDL ratio and AdipoR1 in obese and healthy subjects along with their respective lipid status.

Methods: This cross-sectional comparative study was conducted in Sialkot city. One hundred and thirty-two participants took part in this research. Participants were equally divided into two groups containing non-obese and obese subjects. Mean age was 39.6±0.97 years. Mean BMI for obese subjects was 31.55±0.6 while non-obese group BMI was 20.5±0.2. Individuals with conditions and history of drugs were excluded. Informed and written consent was obtained prior to fasting blood sampling. Serum extraction and proper storage for later testing was carried out. ELISA method used for Adiponectin, AdiopR1 and leptin estimations while lipid profile was determined by Randox Diagnostics kits, using micro lab. SPSS v. 26. was used for comparison between by Mann-Whitney U tes.

Results: Higher levels of Leptin/Adiponectin ratio(0.85±0.1) and LDL/HDL ratio (3.39 ± 0.1), serum Adiponectin (545± 73.3 ug/L), leptin (320.7±50.3 pg/mL) and AdipoR1 (28.9± 2.8 ng/mL) in obese when compared with healthy individuals, Leptin/Adiponectin ratio (0.44 ± 0.07) and LDL/HDL ratio (2.56 ± 0.08) Adiponectin (834± 70.6 ug/L), AdiopoR1 (17.8± 1.97 ng/mL), leptin (224.4±168.7 pg/mL). Correlation of adiponectin found positive for AdipoR1($r=0.336, p<0.05$) and Leptin($r=0263, p<0.05$) in obese subjects. L/A ratio correlated positive with leptin ($r=0.644, p<0.05$) in obese while in healthy subjects (leptin $r=0.409, p<0.05$,adiponectin $r=-0.408, p<0.05$ and HDL $r=0.266, p<0.05$)..

Conclusion: The Leptin/Adiponectin ratio was found higher in obese subjects 0.85 as compared to healthy ones 0.44. Also the LDL/HDL ratio was found higher (3.39) when compared to non-obese (2.56), suggesting these ratios as a suitable marker to estimate metabolic disturbances and underlying dyslipidemia in the obese subjects.

Key Words: Adiponectin, LDL/HDL ratio, Leptin, Leptin/Adiponectin ratio, Obesity

INTRODUCTION

Adiponectin is a protein hormone encompassing 244 amino acids secreted by adipose tissue. It maintains different metabolic functions in body. In obese individuals the levels of adiponectin become reduced[1]. Increase in the levels of adiponectin is beneficial for healthy homeostatic environment. Elevated levels not only decrease oxidative stress environment in cellular compartments but also decline the glucose load by facilitating gluconeogenesis and increase glucose uptake from the cells. Apart from affecting the endothelial cells, adiponectin also mediate lipogenesis and the triglyceride contents inside the cell. The most useful function is cytoprotection and reduces inflammation inside the cells.

Primarily two operative receptors through which adiponectin meditates and perform its function are AdipoR1 and AdipoR2. In skeletal muscle the abundant form is AdipoR1, while in liver AdipoR2 is more abundant[2]. The execution of specific role which include restraining down the glucose production is done by inhibiting the genes involved in gluconeogenesis[3]. Moreover, it also applies a strong support in favor of some pathological events by slowing down cell death and inflammatory responses and enhancing cells ultimate survival[4]. The usefulness of adiponectin mimetics as therapeutic target in diabetes and related metabolic disorders has laid foundation towards revealing the structure of adiponectin as well as its receptor proteins[5]. Besides oncoming studies on adiponectin not only explain the molecular mechanisms but also help us understand the concept that adipose tissue serves as a glandular organ[6].

Determination of adiponectin receptor proteins AdipoR1 and AdipoR2 which have structural resemblance, share a common feature of transversing the cell membrane structure in correspondence to the location of the receptors. They also contribute in sharing a remarkable difference with G protein receptors of inverted membranous structure, and with amino terminal in cytoplasm and carboxyl terminal towards exterior of the cell. Both of these receptors are twenty five amino acids in length. There is a high affinity of globular adiponectin towards AdipoR1 expressing itself in muscles while full length adiponectin is more attracted towards AdipoR2 and more specifically expressed in liver[7].

There are diverse functions of adiponectin on activation. These include suppression of inflammatory response, inhibition of death in cells and most importantly making the cells more sensitive to the metabolic actions of insulin. However in recent studies it has been noted that there occurs a considerable degree of improvement in insulin signaling and energy homeostasis by augmenting the role of this enzyme genetically. This is done through its overexpression in liver and adipose tissues[8].

In vascular endothelial cells it causes increased production of nitric oxide by activation of endothelial nitric oxide synthase enzyme. This gets activated again by adenosine monophosphate kinase. The increased levels of adiponectin reduce tumor necrosis factor-alpha mediated response of vascular cell adhesion protein 1 and Il-8, by

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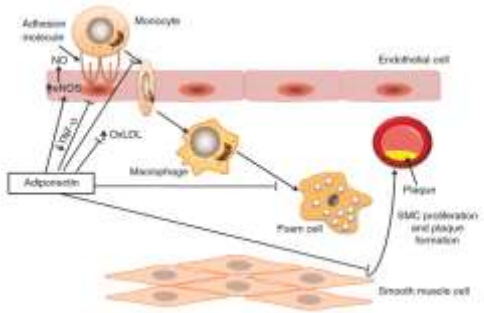
suppressing activity of nuclear factor kappa-b in endothelial cells. In addition, activated adenosine monophosphate kinase by adiponectin inhibits apoptosis triggered by these factors[9].

In adipocytes, there occurs promotion in activity of adipocyte formation ultimately leading to increase in fat content by activating C/EBP-alpha, PPAR-gamma, and sterol regulatory element binding protein 1c. Making adipose tissue more flexible and preventing against lipotoxic effects of high fat diet[11]. In cardiomyocytes in a specific condition of heart named as myocardial infarction, it has been studied that adenosine monophosphate kinase activation and raising the expression of cyclooxygenase 2 could prevent pressure overload and systolic diastolic dysfunction in this high risk event of cardiac injury. Thus by giving adiponectin as treatment improves endothelial function in myocytes by utilizing same cascade of events[12].

Figure 1: Protection of vascular endothelium by the adiponectin and preventing the formation of plaque in these cells[10]



In the above diagram the serum concentrations of 132 individuals with mean age group 40 have been calculated. The mean adiponectin concentrations performed are found to be 545 ug/L and 834 ug/L in obese and healthy subjects. The L/A ratio estimated is found to be 0.85 in obese and 0.44 in healthy subjects. The LDL/HDL ratio is found to be 3.39 in obese and 2.56 in healthy subjects

METHODOLOGY

We identified 66 obese individuals through the obesity clinic outdoor department of Medical unit of Bithania Hospital with 66 controls having healthy physique including all the paramedical staff over duration of 1 year. The basis of BMI according to Asian standards was set as criteria to recruit the individual subjects. Before taking the fasting samples written consent was taken and subjects were counseled about proper fasting before one night of taking sample. The age range was categorical between 40 to 60 years. Subjects having BMI range 25 to ≥ 29.9 kg/m^2 were categorized as obese while BMI range 18.5 to 22.9 kg/m^2 as non-obese normal subjects were included in this study[13]. The weight & height of all the subjects were measured in kilograms and centimeters respectively, using weighing with height scale machine (MIC Health Scale made in China). Height in centimeters was converted into meters for BMI calculations.

Calculation of BMI was done according to formula; Body mass index (BMI) = Weight in kilograms divided by (Height in Meters)^2.

Serum Measurements: The fasting samples were taken from patients after ethical approval and written consent of the individuals. The samples were centrifuged and serum separated and stored at -70°C. The blood samples were collected from each subject (control and patient) after an overnight fast (12 – 14 hours). Total 5ml of blood was taken from the antecubital vein and transferred to a gel containing serum separation tube with yellow cap. After 1 hour, gel containing serum separation tube was centrifuged for 5 minutes at a speed of 3000rpm. The serum was separated with the help of micropipettes and transferred to labeled sterilized eppendorf tubes (1.5ml). The serum containing labeled Eppendorf tubes were placed in boxes and stored at -70°C in laboratory refrigerator at University of Health Sciences until analyzed. Before subsequent biochemical analysis, each serum containing Eppendorf tube was placed at room temperature to thaw the serum sample. The bio data of all these subjects including weight in kilograms and height in meter square were obtained. After that the serum were transported to university of health sciences while maintaining cold chain and analyzed by performing ELISA on these samples using ELISA kit Sinogen Clone. Furthermore, results were analyzed using micro plate reader 5.2. The samples were further analyzed for fasting lipid profile; Total Cholesterol, HDL, and LDL lipoproteins with kits of RandoxDiagnostics.

RESULTS

Table 1: Biochemical parameters in obese and healthy subjects

Parameters	Obese $\bar{X} \pm SE$	Non- Obese $\bar{X} \pm SE$	U Test p value
Serum Adiponectin ug/L	545± 73.3	834 ± *70.6*	0.00**
Serum AdipoR1 ng/mL	28.9±2.8	17.8± 1.97	0.000**
Serum Leptin pg/mL	320.7±50.3	219 ± 21	0.197
Leptin/Adiponectin Ratio(LA)	0.85± 0.1	0.44±0.07	0.000**
Serum Cholesterol mg/dl	216 ± 5.3	180 ± 3.4	0.000**
Serum Triglyceridesmg/dl	177.8 ± 4.9	149.8 ± 3.1	0.000**
Serum HDL mg/dl	41.3 ± 0.3	42.3 ± 0.42	0.082
Serum LDL mg/dl	139.3 ± 4.5	107.4 ± 3.1	0.000**
LDL/HDL ratio	3.39 ± 0.1	2.56± 0.08	0.000**

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed)

Table 2 Correlation of Adiponectin with its receptor AdipoR1, Leptin and lipid profile in Obese and Healthy Subjects

	Adiponectin in Obese	Adiponectin in Healthy
AdipoR1		
Correlation	0. 336	0.094
Significance	0.006**	0.452
Leptin		
Correlation	0.263	0.047
Significance	0.033*	0.707
Cholesterol		
Correlation	-0.078	-0.121
Significance	0.532	0.332
Triglyceride		
Correlation	-0.121	-0.253*
Significance	0.332	0.040
LDL		
Correlation	-0.065	-0.068
Significance	0.603	0.588
HDL		
Correlation	0.003	-0.096
Significance	0.981	0.441

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

A positive correlation exists in obese subjects with its relevant receptor AdipoR1 and also with serum leptin levels ($p<0.05$).

The correlation when studied for adiponectin in healthy subjects it was found that only serum leptin showed significant positive correlation ($p<0.05$). The correlation when studied between individual parameters it was observed that in obese individuals leptin also correlated positively with AdipoR1 and Triglyceride correlated positively with LDL ($p<0.05$). However Healthy subjects displayed a negative correlation of adiponectin and TAG but was statistically significant ($p<0.05$).

Table 3: Correlation of L/A ratio with individual adipokines, AdipoR1 and lipid profile in obese and healthy Subjects

	L/A ratio in Obese	L/A ratio in Healthy
Leptin		
Correlation	**0.644****	**0.409****
Significance	**0.000**	**0.001**
Adiponectin		
Correlation	-0.066	**-0.408****
Significance	0.598	**0.001**
AdipoR1		
Correlation	0.159	-0.025
Significance	0.201	0.844
Cholesterol		
Correlation	0.177	0.067
Significance	0.156	0.591
Triglyceride		
Correlation	0.032	-0.014
Significance	0.799	0.912
LDL		
Correlation	0.203	0.040
Significance	0.102	0.748
HDL		
Correlation	-0.056	**0.266***
Significance	0.656	**0.031**

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 3 shows that L/A ratio correlated positively with leptin in obese subjects and shows statistically significant results ($p<0.05$). However in healthy subjects leptin adiponectin and HDL levels showed statistically significant results where L/A ratio correlated positively with serum leptin and HDL and negatively with serum adiponectin ($p<0.05$)

Table 4: Correlation of LDL/HDL ratio with Adiponectin, Leptin and Adiponectin Receptors and lipid profiles levels in Obese and Healthy Subjects

	LDL/HDL ratio in Obese	LDL/HDL in Healthy
Adiponectin		
Correlation	-0.117	0.317
Significance	0.348	0.232
Leptin		
Correlation	0.044	0.092
Significance	0.728	0.464
AdipoR1		
Correlation	-0.160	-0.018
Significance	0.200	0.884
Serum cholesterol		
Correlation	**0.877**	-0.003
Significance	**0.000****	0.980
Triglyceride		
Correlation	**0.642**	0.127
Significance	**0.000****	0.310
LDL		
Correlation	**0.901**	-0.142
Significance	**0.000****	0.257
HDL		
Correlation	**-0.248**	-0.010
Significance	**0.045***	0.936

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 4 displays LDL/HDL ratio when correlated in obese subjects showed significant results with serum Cholesterol, LDL and Triglycerides demonstrating positive correlation ($p<0.05$) and with HDL showing negative correlation ($p<0.05$). Healthy subjects show weak correlation of LDL/HDL ratio with individual parameters ($p>0.05$)

DISCUSSION

Obesity is one of the most imperative health problems throughout the world. Obesity leads to several health complications including heart diseases, musculoskeletal, nervous system, liver and kidney diseases. In the current research serum levels of adiponectin and its adiponectin receptor protein (AdipoR1) in obese individuals were measured in comparison with healthy controls. Both determined by using a highly sensitive ELISA method. It was determined that obese subjects having raised BMI values had increased mean values of adiponectin in their serum with a mean value of 545 ug/L as compared to healthy controls that showed mean value of 834 ug/L (p value < 0.05). While entirely opposite is true for adiponectin receptors which get raised in obese individuals ($p<0.05$).

Obesity associated problems represent disturbing and growing comorbidities. Discovering the marked changes in the serum levels of related adiponectin of obese and healthy individuals manifesting as imbalance in the adipokine state, is yet vital in predicting the severity of associated morbidity. The mainstay of current study is to determine the links between adiponectin and its receptor AdipoR1 in obese and healthy subjects for properly identifying individuals predisposed to risk state. Also, in current research, we determined the mean L/A ratio and LDL/HDL ratios and found their various associations with lipid profiles as marker of metabolic disturbance both in obese and healthy control groups.

In current research we focused on determining the significant role of Leptin/adiponectin ratio to find out its association with obesity parameters. Mean Serum adiponectin levels found to be 545 ug/L in obese while 834 ug/L in healthy ($p<0.05$). We estimated serum leptin concentrations and calculated mean L/A ratio in obese subjects. It was found to be 0.85 in obese and 0.44 in healthy subjects ($p<0.05$) (Table 1). The previous studies on L/A ratio demonstrated that the ratio persistently go on the upper side which depicts underlying metabolic disturbance. The ratio is proportional to adiposity of the growing lipid environment. Thus the higher the ratio the greater will be derangement in glucose as well as triglyceride metabolism[14].

In this study we also evaluated a correlation between L/A ratio both in obese and healthy subjects with individual adiponectin, its receptor and leptin along their respective lipid status. We found a very interesting finding that obese subjects showed positive and statistically significant correlation of L/A ratio with leptin levels in obese individuals ($p<0.05$) while in healthy controls L/A ratio correlated positive with both leptin and serum HDL levels and negatively with Adiponectin in healthy controls ($p<0.05$) (Table 3). According to a recent research explaining the fact that leptin Adiponectin ratio is found to have a better ability to discriminate the risk of underlying metabolic disturbance than adiponectin and leptin alone among apparently healthy subjects[15]. This proved to be consistent with our findings in this research.

Being insulin sensitive and providing protection in oxidative environment, a correlation was established between adiponectin its relative receptor $AdipoR_1$, leptin and lipid status in obese and healthy subjects (Table 2). The novelty of this

studies lies in the fact that due to imbalance in chemokines in obesity there occurs an increase in adiponectin receptors along circulating adiponectin in serum which positively correlated in this study showing significant results (Table 2). Thus it is observed from the findings that there exist a strong positive correlation between adiponectin and its receptor and serum leptin in obese individuals ($p<0.05$) as compared to healthy controls that demonstrated a positive statistically sound correlation of adiponectin with Triglyceride levels ($p<0.05$). These results make us consider that with increase in adiponectin there also occurs increase in its receptor and leptin concentrations only in obesity consistent with previous studies[16]. Previous studies described high LDL/HDL ratio is a significant predictor of the degree of carotid atherosclerosis. It also has been suggested that LDL-/HDL ratio showed a positive and linear relationship with the carotid plaques in diabetics[17]. A prospective cohort study also verified that LDL/HDL ratio is a better predictor of carotid intima media thickness progression than either HDL or LDL levels alone[18]. These studies show similar results with our study as Table 4 demonstrating a comparison done between LDL/HDL ratio both in obese and healthy subjects with individual lipid statuses. The ratio LDL/HDL shows significant correlations with Cholesterol, triglycerides, LDL and HDL obese as compared with healthy subjects ($p<0.05$), predicting the existence of an underlying metabolic derangement in obese subjects making them prone to obesity associated disease.

CONCLUSION

In obese subjects LA ratio is persistently high 0.85 and positively correlates with leptin however in healthy subjects the ratio is low 0.44 but strongly correlates with leptin, adiponectin and HDL. The ratio can be a better predictor of disease severity than individual concentrations of chemokine molecules. However the controls had high levels of adiponectin as compared to obese subjects showing the protective nature of adiponectin towards normal homeostatic state. The study also concluded the fact that LDL/HDL ratio positively correlates with Triacylglycerol, cholesterol and LDL contents and negatively with HDL in obese subjects.

However further studies are needed to categorize the values of LA ratio into low medium and high ranges as to further group the individuals on the basis of low medium and high risk class.

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