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ORIGINAL ARTICLE

Determining the Receptor Proteins AdipoR$_1$, Leptin and Lipid Status in Serum; Mitigating its impact on Obese and Healthy Individuals

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ABSTRACT

Aim: To investigate role of AdipoR1 concentrations and leptin levels in obese and healthy subjects along with their lipid status

Methods: This cross sectional study was conducted in residents of Sialkot city and included 132 subjects. Each group had 66 participants. Mean age was 39.6±0.97 years. Mean BMI for obese subjects was 31.55±0.6 while healthy group BMI was 20.5±0.2. Individuals with known history of diabetes, hypertension, chronic kidney disease, any malignancy and history of lipid lowering, antihypertensive, antipsychotics and antiepileptic were excluded. Fasting blood samples were taken after informed and written consent. Samples were centrifuged to extract serum and stored for analysis by ELISA method for AdiopR1 and leptin while lipid profile was determined by kits by Randox Diagnostics using micro lab. Data was analyzed by SPSS v. 26. Comparison between groups was carried out by Mann-Whitney U test.

Results: Our study revealed higher levels of serum AdipoR1 (28.9±22.86 ng/mL), leptin (320.7±59.38 pg/mL), cholesterol (216±5.31 mg/dl), triglyceride (177.8±4.93 mg/dl) and LDL (139.3±4.56 mg/dl) in obese when compared with healthy individuals, AdiopoR1 (17.8±1.96ng/mL), leptin (219±20.76 pg/mL), cholesterol (179.8±3.25 mg/dl), triglyceride (149.8±3.17mg/dl), LDL (108.5±25.7 mg/dl) respectively. No statistical difference was found for HDL between groups.

Conclusion: Our Study concludes that rising levels of AdipoR1 and leptin in addition to lipid profile are associated with obesity.

Keywords: Obesity, Receptor Proteins AdipoR$_1$, Leptin, lipid status, adipose tissue

INTRODUCTION

Obesity is a condition that may lead to more complicated array of disorders. The prevailing confusion and dilemma of the world towards the likely association of adipokines has made difficult to understand significance of obesity toward various disorders[1]. One third of the people in the world are categorized as obese and overweight. These two conditions have increased dramatically over the past 35 years. According to World Health Organization (WHO) it has been evaluated that over a billion adults are overweight and obese[2]. In America, the prevalence of overweight increased from around forty five percent to almost sixty five percent while prevalence of obesity increased from 12.9% to 28.3% from year 1980 to 2015[3]. In Pakistan it is estimated that there is a considerable increase in prevalence rate from 24% in 1980 to 35% in 2013[4]. Apart from other non-communicable diseases obesity has emerged as an epidemic which is still not recognized in many areas of Pakistan[5]. The growing incidence of obese individuals since the last decade has lead us to formulate new ideas towards understanding not only the evolving mechanisms linked to obesity but also simplify the doors towards understanding new research in our daily life[6]. Previously there is considerable data available on the receptor proteins of adiponectin (AdipoR1) supporting its anti-inflammatory, anti-platelet, anti-cancerous role, as well as its role in maintaining the membrane fluidity[7]. However, our understanding in visualizing this AdipoR1 is entirely diverse. As these receptor proteins are the transmembrane proteins that extend the entire surface of cellular membranes[8]. On activation, they produce an adenosine monophosphate kinase pathway in cells which produces a cellular cascade. It leads to activation of kinases that further initiate enhanced oxidation of fatty acids leading to decrease the triglyceride cellular pool[9]. Apoptosis, which might be the possible mechanism that leads to increase destruction of adipocytes, dramatically increase the adiponectin receptor protein level in serum of obese subjects. In this study our main goal was to study the concentration of these receptor proteins in healthy and obese individuals.

Previous studies on leptin provide elaboration of its specific function in regulating energy intake and expenditure by communication and integration of signals in the brain[10]. The levels of leptin show a linear relationship with fat content and energy consumed. Increase of high caloric diet will enhance its secretion while low caloric and low-fat diet will suppress its circulating levels[11]. The central nervous system influences energy balance through three mechanisms that include effect on feeding behavior, autonomic nervous system activity and finally its effects on

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the neuroendocrine system[12]. The integration between these three systems has been the subject of intense study. The regulation of feeding behavior may be divided into long- and short-term control systems. Short term involves the initiation and termination of meals with the onset of satiety, Long term signals that reflect the status of energy stores provide information to the CNS in the form of specific fat derived hormone like leptin, along with adiponectin and other adipokines thus further regulating feeding behavior to promote energy homeostasis[13].

Along with the receptor proteins, leptin is an important mediator performing explicit metabolically imperative functions. This study tries to answer the question, whether there is an impact of these specific adipokines on obesity. Clinical studies have shown that AdipoR1 receptors in obesity when down regulated can decrease response to the circulating adiponectin molecule that possess useful role in lowering lipotoxicity (cytosolic overload of lipids from excessive cytosolic acyl CoA)[14]. The question that needs to be addressed is that, do these changes influence the intracellular environment by way of the structural topology of AdipoR1 receptor proteins. If yes then what might be the indications that these changes have a direct impact on obese and healthy individuals. The main aim was to determine the levels of existing receptor protein (AdipoR1) and leptin along with lipid profile in serum to identify lipid rich environment manifesting itself as change in the structure of cellular contents of membrane in state of cellular stress that can be detected earlier in serum before any disease pathogenesis.

MATERIALS AND METHODS

Over a period of six months we identified 66 obese individuals through the obesity clinic outdoor department of Medical unit of Bethania Hospital along with 66 controls having healthy physique including all the paramedical staff. The basis of BMI according to Asian standards was set as criteria to recruit the individuals. Before taking the fasting blood samples, written consent was taken and subjects were counseled about proper fasting before one night of taking sample. The age range was between 20 to 60 years. Subjects having BMI range 25 to ≥29.9kg/m^2 were categorized as obese while BMI range 18.5 to 22.9 kg/m^2 as non-obese were included in this study[15]. Calculation of BMI was done according to formula;

Body mass index (BMI) = Weight in Kilograms divided by (Height in Meters) ^{2}

Serum Measurements: The blood samples were collected from each subject (control and patient) after an overnight fast (12 – 14 hours). Total 5 ml 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 3000 rpm. The serum was separated with the help of micropipettes and transferred to labeled sterilized Eppendorf tubes (1.5 ml). The serum stored at -70°C in laboratory refrigerator until analyzed. Before subsequent biochemical analysis, each serum tube was brought to room temperature to thaw the serum sample. After that the serum samples were analyzed by ELISA kit by Sinogen Clone (China). Results were obtained using micro plate reader 5.2. Fasting lipid profile was analyzed with micro lab using kits made by Randox Diagnostics.

Statistical analysis: Normality test revealed non normal distribution of data. Comparison between parameters was carried out by Mann-Whitey U test. Predictors for outcome were checked by multiple regression analysis. Level of statistical significance was set at $p \leq 0.05$. Statistical analysis was performed using SPSS for IBM, version 26.0. Data were expressed as mean ± S.E.

RESULTS

In this study 66 obese subjects and 66 non obese (healthy) subjects were included. Mean age was 39.6±0.97 years for obese and 38+1.42 in healthy controls. Anthropometric data in conjunction with mean BMI values for obese subjects was 31.55±0.6 while healthy group BMI was 20.5±0.2 as presented in Table 1. Obese individuals AdipoR$_1$ (28.9±22.86ng/mL), leptin (320.7±59.38pg/mL), cholesterol (216±5.31 mg/dl), triglyceride (177.8±4.93 mg/dl) and LDL (139.3±4.56 mg/dl) were found higher than healthy individuals AdiopoR1 (17.8±1.96ng/mL), leptin (219±20.76pg/mL), cholesterol (179.8±3.25mg/dl), triglyceride (149.8±3.17mg/dl), LDL (108.5±25.7mg/dl) displayed significant results ($p \leq 0.05$) respectively presented in Table 2).

Table 1 showing mean values along standard error of mean in study samples with distinct characteristics like age, weight , height and BMI values in obese and non-obese (healthy subjects). Mean BMI values for obese taken as 31.5 ± 0.6 and that of non-obese were taken as 20.5 ± 0.2 with respect to Asian BMI criteria.

Table 1: Anthropometric measurement

Parameters	Obese(n=66) $\bar{X}$ ± S.E	Healthy Controls (n=66) $\bar{X}$ ± S.E
Age (years)	39.6±0.97	38 ± 1.42
Weight(kg)	81.28±1.56	54.63±.92
Height(m)	1.60±0	1.62±0
BMI(kg/m^2)	31.55±0.6	20.50±0.2

Table 2: Comparison of biochemical parameters in Obese and Healthy Controls

Parameters	Obese (n=66) $\bar{X}$ ± S.E	Healthy Controls (n=66) $\bar{X}$ ± S.E	U Test p value
Serum AdipoR$_1$(ng/mL)	28.9±22.86	17.8±1.96	0.000*
Serum Leptin (pg/mL)	320.7±59.38	219.01±20.76	0.000*
Serum Cholesterol (mg/dl)	216 ± 5.31	179.8 ± 3.25	0.000*
Serum Triglycerides (mg/dl)	177.8 ± 4.93	149.8 ± 3.17	0.000*
Serum HDL (mg/dl)	41.3 ± 0.35	42.3 ± 0.42	0.082
Serum LDL (mg/dl)	139.3 ± 4.56	108.5 ± 2.93	0.000*

*Statically significant results p≤0.05

Table 3 explains the multiple linear regression analysis illustrating 32% of the variance in outcome variable (AdipoR1) with Cooks distance between -1 to 2 and standardized residuals ranging -1 to 3, suggesting

statistically significant finding ($p \leq 0.001$) interpreted from the model summary. Table interprets that among the predictor variables (leptin, cholesterol, triglyceride, LDL and HDL) only concentrations of leptin make a significant contribution in functioning of outcome variable $AdipoR_1$ ($p \leq 0.05$)

The above table 1.2 displays all the six parameters included in study along with their mean values and standard error of mean. Comparison between both the sample groups' obese vs non obese (healthy) was done using Mann-Whitney *U* test taking significance level with a $p \leq 0.05$. The serum concentrations Adipor1, leptin, cholesterol, triglycerides and LDL showed statistically significant results ($p \leq 0.05$).

Table 3: Multiple regression analysis of AdipoR1 with predictor variables in Obese subjects

Predictor Variables	$AdipoR_1$ in Obese Subjects, $R^2=0.32$			
	SE	β-Coefficient	t Value	P Value
Leptin	0.006	0.542	4.9	0.000*
Total Cholesterol	0.094	-0.177	-0.167	0.101
Triglycerides	0.907	0.035	0.211	0.834
HDL	0.847	-0.16	-1.5	0.139
LDL	0.103	-0.215	-1.28	0.205

Table 4: Multiple regression analysis of $AdipoR_1$ with predictor variables in Healthy Subjects

Predictor Variables	$AdipoR_1$ in Healthy Subjects, $R^2=0.13$			
	SE	β-Coefficient	t Value	P Value
Leptin	0.012	-0.124	-0.977	0.332
Total Cholesterol	0.07	0.035	0.293	0.771
Triglycerides	0.08	-0.047	-0.361	0.720
HDL	0.58	-0.297	-2.343	0.022*
LDL	0.07	-0.055	0.429	0.670

Table 4 explains the multiple linear regression analysis illustrating 13% of the variance in outcome variable ($AdipoR_1$) with Cooks distance 0.0 to 0.16 and standardized residuals ranging -1 to 3 ($p > 0.001$) interpreted from the model summary. Table interprets that among the predictor variables (leptin, cholesterol, triglyceride, LDL and HDL) only concentrations of serum HDL make a significant contribution in functioning of outcome variable AdipoR1 ($p \leq 0.05$).

DISCUSSION

In previous studies there were comprehensive factual studies on adiponectin receptors and leptin levels showing association of adiponectin, leptin and obesity[17]. However our study was rather different in characteristic for determining the receptor proteins in the obese people in a direction to identify the advancing lipotoxic environment that would propagate a dynamic response associated with receptors located on the membranous cellular architecture. The preceding studies on mRNA levels used to determine the expression of AdipoR1 and AdipoR2 in various tissues[18]. However, adiponectin receptor protein synthesis and release rely not only on mRNA but also other factors involved in translation[19], making these levels of adiponectin receptor protein determination essentially important in serum of individuals. The adiponectin levels have been well defined in obese people but to study the instigating role of receptors and their concentration in serum we conducted this study. Mean levels of adipoR1 were considerably on a higher range in obese as compared to healthy group. It was determined that obese subjects having raised BMI values had increased mean values of adiponectin receptor protein (AdipoR1) concentrations in their serum with a mean value of 28.9ng/mL as compared to healthy controls that showed mean value of 17.8ng/mL ($p \leq 0.05$). Actually, the concept to determine these receptor proteins was based on the fact that apoptosis mediated changes inside the obese individuals may lead to release of these receptor proteins in serum that is advanced progressing stage of developing obesity and may be responsible for damaging the health of these individuals. In a recent study circulating levels of soluble death receptors for apoptosis were higher in serum of patients with diabetes and correlated with markers of impaired glucose metabolism in non-diabetic subjects[20]. This predicts increased risk for development of diabetes and cardiovascular events in these individuals as mature adipocytes after their terminal differentiation undergo apoptosis[21]. However older cells soon undergo apoptosis and are removed by macrophages. This leads to stimulation of inflammatory mediated changes by macrophages in the process of differentiation. The endothelial cells become activated in state of hypoxia and inflammation, causing adipocytes to produce extracellular vesicles that further attract specific adhesion molecules. These include vascular cell adhesion molecules which facilitate further adhesion of leucocytes to endothelial membranes[22]. However limited studies during the process of expansion of adipose tissues are known about apoptosis mediating the mechanism. It has also been known that the dynamic changes proceeding in microenvironment of obese individuals creates a state of oxidative and mechanical stress[23] ultimately promoting adipocyte death directly linked with lipotoxicity which is aggregation of lipid intermediates leading to cellular dysfunction in non-adipose tissues as well. Similarly consistent with the previous studies advancing adiposity could also trigger an inflammatory response that can change the dynamics of serum and result in the increase in the concentration AdipoR1 receptor proteins in serum distinguished in our study.

Adipose tissue acts as a vigorously active organ, which may cause changes in adipokine levels in response to the volume and conditions in adipose tissue. However, in obese individuals the changes in secretion of adipokine levels lead to metabolic disturbance that plays a central role in succeeding towards disease[24]. Leptin is among the first studied adipokine secreted from adipocytes producing these changes. In obesity, a decreased sensitivity to leptin occurs, resulting in an inability to detect satiety despite high energy stores and high levels of leptin[25]. In the present study we also explored the role and function of leptin along with the $AdipoR_1$. We determined the serum leptin levels in 66 obese subjects and 66 controls according to WHO obese criteria for Asians. The average mean values for fasting leptin in obese subjects was found out to be 320.6 pg/mL and controls on the other hand had an average mean of 219.01 pg/mL. These values difference was statistically significant ($p \leq 0.05$). A study conducted in 2014

including over 11,000 subjects in which fasting leptin were determined and it was found that they had strong positive association with anthropometric measures that included height, weight, BMI and body circumferences[26]. Preceding studies investigated the relationship between leptin levels and body fat and it was found that leptin concentrations were positively correlated with adiposity in both men and women[27].

There is always a balance between synthesis and degradation pathways of cholesterol and fatty acid synthesis. Whenever this equilibrium gets disturbed it transpires itself as excess accumulation of lipids making progression towards atherosclerosis. An essential pathway that is protective against development of atherosclerosis is reverse cholesterol transport. The excess cholesterol gets transported to the liver from peripheral organs and is excreted in bile. High density lipoprotein is considered the main hub for performing this function. In assistance with HDL other proteins especially adiponectin and its receptor proteins are thought to play a vital part in this aspect as well[30]. In the light of above interesting findings levels of total cholesterol, HDL and LDL were also calculated in both groups. It was found that the levels again proved to have augmented in obese with cholesterol levels on the higher range than normal subjects. The baseline lipid profiles is elevated that supports previous findings in individual with higher lipid levels were more at risk to develop cardiovascular changes. The fact also proves that early determination could be pre-empted to reduce the progression with the help of determining the levels of such markers earlier to mitigate the impact of rising BMI on health.

CONCLUSION

Our study concludes that escalating levels of AdipoR1 and leptin and deranged lipid profiles are associated with obesity presenting a burden on health.

Significance/Recommendation: Increase in the value of AdipoR$_1$ may be responsive about underlying metabolic derangement and decreased values would specify truncated risk for development of obesity associated conditions.

This could also provide an insight regarding their respective concentrations which can become the target of various medicines and the raised levels could also serve as a diagnostic tool in identifying risk for particular obesity associated conditions.

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