Journal of
Obesity & Weight Loss Therapy

The Correlation between Overweight and Obesity with Plasma Levels of Leptin, Insulin and SdLDL in People Over 20 Years Old

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Abstract

The aim of this study was to co-analysis of sdLDL, leptin and insulin at a time and their association with overweight.

This experiment was performed as a case-control on normal people visited Bu-Ali Hospital of Zanjan. Two hundred and thirteen individuals over 20 years old were analyzed totally from which 122 individuals with BMI ≥ 25 and 91 individuals with BMI<25 were assigned as case and control groups respectively. Height, weight, blood pressure and waist circumference of all individuals were measured. The quantitative measurement of the plasma level of leptin and insulin were performed with the ELISA method and the measurement of sdLDL was accomplished with the column method (sdLDL-C SEIKEN Kit from DENKA SEIKEN CO., LTD., Tokyo, Japan). The plasma level of glucose, triglycerides, cholesterol and HDL were measured enzymatically.

Among all variables, only triglycerides and waist circumference were independently associated with overweight and the effect of other variables were not independently associated with overweight, although it showed direct association with Body Mass Index. It seems that the correlation of other factors such as sdLDL, leptin and insulin with overweight is dependent to sex and age.

Our findings showed that each of these factors were correlated with the Body Mass Index; however, the logistic regression analysis revealed that age, sex, triglycerides and waist circumference are independently associated with overweight.

Keywords: Leptin; Insulin; sdLDL; Overweight; Obesity

Introduction

Obesity makes individuals more susceptible to various diseases in the top of which are heart coronary diseases. Moreover, the risk of osteoarthritis, unconsciousness and fertility disorders increases significantly with the increment of Body Mass Index [1]. Several biological factors such as leptin and insulin are involved in regulation of appetite and energy metabolism [2,3]. Perhaps, the balance between leptin and insulin is effective in the incidence of obesity [4,5]. LDL is a low density lipoprotein involved in cholesterol and triglycerides transfer from liver to peripheral tissues. LDL consists of two parts: the bigger part with phenotypic pattern A is light and almost rich of cholesterol (LBLDL or Large buoyant LDL) and the smaller part with more special weight and phenotypic pattern B (sdLDL) composed of less cholesterol. These particles differ not only in weight and density, but also in the physicochemical composition as well as metabolic and atherogenic behaviors [6].

sdLDLs have an important role in the prevalence of heart coronary diseases. These particles are more easily adsorbed from vessel walls than larger particles and their transfer rate to endothelial coronaries are higher. The high prevalence of these atherogenic particles (sdLDL) mainly observed in individuals with familial hyperlipidemia, non-insulin dependent diabetes mellitus, and central obesity and insulin resistance syndromes [7]. In this study the relationship between sdLDL, insulin and leptin with obesity was inspected.

Materials and Methods

Subjects and sampling

The society under study was consisted of people visited Bu-Ali Hospital in Zanjan. Overall, 213 people with age of 20-85 were recruited to study. Individuals with endocrine diseases including diabetes mellitus, thyroid disorders, Cushing's syndrome, familial hyperlipidemia as well as individuals with heart coronary diseases who had the experience of MI or CVD or use heart drugs were excluded from the study. Fatty liver disorders, Gilbert's syndrome and acute diseases such as malignancies, arthritis, Anorexia nervosa, and crohn's disease were also excluded from the study. The ethical approval was obtained for the human studies and the study was in accordance with International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS, Geneva: 2002). All individuals were aware of the study and fill out the questionnaire and signing a written testimonial. Their height, weight, blood pressure and waist circumference were measured. One hundred and twenty two individuals with BMI ≥ 25 were categorized as case group and 91 remained individuals with BMI<25 were referred as control group. After 10 to 12 hours over night fasting, 10 ml of vein blood was collected from participants. A questionnaire including personal information and disease history was filled out by each person.

Insulin, leptin and sdLDL measurement

Plasma level of insulin was measured by ELISA method, using ELISA insulin kit of DRG Company, Germany, code EIA-2935. This kit has designed for the quantitative measurement of insulin in heparinated or citrated serum and plasma. Its accuracy was equal to

confidence coefficients of CV=19.2% and CV=57.2%, using the intra-assay and inter-assay, respectively. The normal range of insulin in this method was 2-25 µIU/ml. The serum level of leptin was also measured with ELISA method using the human leptin ELISA kit of Biovender Company, catalog number: RD191001100R. This kit has been designed for the quantitative measurement of leptin levels in serum or plasma and it was only for research usage and of no validation as a recognition method. The sensitivity of this method was 0.17 nanograms per milliliters and its accuracy is equal to confidence coefficients of CV=4.5% and CV=8.6% by intra-assay and inter-assay, respectively.

The sdLDL-C "SEIKEN" kit (DENA SEIKEN CO, LTD) with catalog number of 562524 was obtained from Randox Company, England. This kit was used for the separation and quantitative measurement of sdLDL and is for research only. Normal sdLDL ranges in men and women are 8-43 mg/dl and 6.9-39 mg/dl, respectively.

Statistical analysis

For all variables, average amounts, maximum, minimums and the standard deviation were calculated by SPSS software (v11.5). To compare averages and analyze the significance association between qualitative variables, T-test and chi-square test were applied, respectively. The Pearson coefficient and logistic regression were used to analyze the correlation between qualitative variables and their association with overweight, respectively, in the presence of each other. $P \leq 0.05$ was set as the confidence level for acceptance or rejection of hypothesis.

Results and Discussion

Results showed that the average of waist circumference, triglycerides, cholesterol, blood glucose, leptin, insulin and sdLDL of two groups had statistically significant difference (P<0.05), but there was no significant difference regarding HDL-c averages (P>0.05) (Table 1). The correlation between sdLDL and other variables in the study revealed that it was only associated with cholesterol (Table 2).

In order to realize the most important factor in prediction of overweight risk, logistic regression model were applied. All variables including age, sex, leptin, insulin, sdLDL, triglycerides, cholesterol, HDL, blood glucose and waist circumference put in logistic regression from which only four variables i.e. sex, age, triglycerides and waist circumference were considered independent predictors of overweight (Table 3).

There are several methods for measurement of small dense LDL (sdLDL) including ultracentrifugation, electrophoresis, HPLC and NMR. The measurement of sdLDL with NMR and HPLC methods is difficult, expensive and time consuming; since two other methods, ultracentrifugation and electrophoresis, are more common today

Variables	BMI<25	BMI ≥ 25	P Value
Age	40.8 ± 15.99	46.61 ± 13.57	0.005
Leptin (ng/ml)	7.13 ± 6.18	14.86 ± 16.52	<0.001
Insulin(µIU/ml)	7.07 ± 6.34	8.15 ± 8.87	0.019
sdLDL (mg/dl)	21.92 ± 45.79	29.07 ± 53.22	0.034
Cholesterol (mg/dl)	172.82 ± 42.21	193.66 ± 43.09	0.001
Triglyceride (mg/dl)	140.57 ± 79.45	190.82 ± 104.2	<0.001
HDL-c (mg/dl)	42.15 ± 4.02	42.57 ± 4.22	0.467
Fasting Blood Sugar (mg/dl)	84.65 ± 18.26	93.94 ± 25. 75	0.002
Waist circumference (cm)	84.03 ± 8.48	100.8 ± 9.17	<0.001

Table 1: Demographic and laboratory characteristics in subjects studied (normal group *vs* case group).

Variables	v BMI<25		BMI ≥ 25	
	P Value	r	P Value	r
Leptin (ng/ml)	0.236	-0.126	0.127	-0.139
Insulin (µIU/ml)	0.345	0.096	0.265	-0.096
Cholesterol (mg/dl)	0.001	0.347	<0.0001	0.675
Triglyceride (mg/dl)	0.197	0.136	0.097	0.151
HDL-c (mg/dl)	-0.052	0.625	0.597	-0.048
Fasting Blood Sugar (mg/dl)	0.095	0.176	0.497	0.062

Table 2: correlation of sdLDL with clinical characteristics in subjects studied (normal group *vs* case group).

Variables	Odd Ratio	Confidence Interval (95%)
Age (y)	0.94	(0.91-0.97)
Sex	14.6	(4.3-49.0)
Triglyceride (mg/dl)	1.01	(1.00-1.01)
Waist circumference (cm)	1.42	(1.28-1.58)

Table 3: logistic regression analysis of weight gain (obesity) with independent variables.

as they are faster and easier [8]. sdLDL-c SEIKEN method is an easy method for the quantitative measurement of sdLDL and consists of two steps. Although at this time it is considered as a research method only, it can be used as a routine laboratory method for the prediction of heart coronary diseases, obesity and metabolic syndromes in the near future. In this study a new method was used which is better than qualitative methods for sdLDL measurement regarding time, expenses and facilities. sdLDL was measured quantitatively after purification and its exact amount in the serum of individuals was calculated.

From all measured variables, only the triglycerides amount and waist circumference were independent factors associated with overweight which maintained their effect after applying influence of sex and age. Although other variables showed a direct association with body mass index, their effects were dependent to sex and age and did not show an independent association with overweight. In this study average amounts of sdLDL, leptin and insulin differed significantly in case and control groups, but sdLDL did not associate with the body mass index (p>0.05).

Halle et al. [9] (Germany) in their study showed that sdLDL particles are significantly more in individuals with BMI ≥ 25 than those with BMI<25 and the most amount of sdLDL was reported in individuals with BMI>27. This study showed that an important factor for expression of phenotype in obese individuals even in those with a normal insulin level is one of atherogenic subclasses of LDL. Miyashita et al. [10] showed that sdLDL amount in obese children is associated with high WHR, high amount of triglycerides and low amount of HDL-c. The regression analysis also revealed that body mass index is in association with fat accumulation in abdominal region and sdLDL can be considered as an important risk factor of metabolic syndromes. Gentile et al. [11] showed that sdLDL amount of an individual with metabolic syndrome and central obesity is more than that of normal individuals. According to studies performed in other countries, there is a statistically significant correlation between BMI, WHR and sdLDL but there was not such a relation between BMI and sdLDL in this study. A reason of this difference is possibly due to the qualitative measurement in other studies in comparison with the quantitative method used in this study. Moreover, since WHR has stronger association with overweight, obesity and sdLDL level in comparison with BMI in some races, it seems that WHR would show stronger association with sdLDL and metabolic syndrome than BMI.

In this study, there was also an increase in insulin and leptin

amount parallel to body mass index but this raise is more influenced by sex and age, which is in accordance with other studies. This similarity of results in different races can suggest that genetic influences of leptin and insulin in obesity and overweight differ from other environmental and cultural effects. Tong et al. [12] showed that in individuals with abdominal obesity, leptin and insulin amounts are more and there was significant difference in obese adults and non-obese ones and there was an inverse association between the age and leptin in obese individuals.

According to our study, it seems that the use of quantitative methods such as the sdLDL-c SEIKEN method, are more informative in assessment, comparison and measurement of the effective parameters in obesity.

Acknowledgments

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