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AGE AND GENDER-BASED ASSOCIATION OF SERUM LEPTIN AND CHOLESTEROL PROFILE IN PATIENTS WITH HYPERTENSION

Mohammed Ahmed M. Bafail, Abdulhalim S. Serafi * and Zahir Hussain

Department of Physiology, Faculty of Medicine, Umm Al-Qura University (UQU), Makkah, Saudi Arabia

ABSTRACT

Significance of leptin and cholesterol profile in health and disease especially related to hypertension and other related medical disorders has extensively been reviewed. Positive relationship of low-density lipoprotein cholesterol (LDL-C) and serum leptin was revealed in several studies. However, there are still controversies about the gender and age-based serum leptin and cholesterol profile in non-obese normal control male (NCm) and female (NCf) subjects, and non-obese hypertensive male (HPTm) and female (HPTf) subjects, as it was suggested that the change in leptin and cholesterol profile related to body mass index (BMI) might also be due to age, race, type of obesity and other factors. Hence, it was proposed in the present study to investigate the association of age with corresponding serum leptin and cholesterol profile levels. The ELISA-kit methods were employed, and standard statistical analysis were carried out. Significant increased serum leptin, LDL-C and high-density lipoprotein cholesterol (HDL-C) for HPTm and HPTf; significant association of age with serum leptin, total cholesterol (TC), LDL-C in all groups, and with HDL-C only for HPTm; and significant association of serum leptin with TC, LDL-C and HDL-C (in all groups except for HDL-C in HPTf) were obtained. The current study reveals quite interesting and new information for the association existing between age of subjects and their serum leptin and cholesterol profile levels in non-obese healthy and hypertensive subjects. This report, hence, seems promising for future studies.

Key Words: Serum leptin, hypertension, normotension, age and gender, serum cholesterol profile, non-obese hypertensive subjects

INTRODUCTION

It is well known that hypertension is one of the main multifactorial contributors in cardiovascular diseases (CVD) (Paula Bricarello *et al.,* 2018; Tanase *et al.,* 2019). There are a variety of factors involved in the pathogenesis of hypertension (HPT) (Coy, 2005; Luma and Spiotta, 2006; Tanase *et al*., 2019). Two of the important related factors are serum leptin (Dadarlat-Pop *et al.,* 2021) and cholesterol profile (Ding *et al*., 2021). The patients with HPT show rising trend in serum levels of low-density lipoprotein-cholesterol (LDL-C) (Nasri and Yazdani, 2006; Chiu *et al*., 2016). However, no change in LDL-C in patients with HPT was also revealed (Nguyen *et al.,* 2019) in patients with HPT without carotid plaques.

Association of total cholesterol (TC), LDL-C and high-density lipoprotein-cholesterol (HDL-C) in HPT was investigated (Hadaegh *et al.,* 2021). Non-obese male and female subjects with HPT showed variations in cholesterol profile (TC, LDL-C and HDL-C) (Chen *et al.,* 2019). Though studies mentioned in above explain the role of leptin and cholesterol profile in patients with HPT of various types, the precise involvement/ association of these factors is not clearly evident in view of controversial data. Hence, it was proposed to study and clarify the interactive role of leptin and cholesterol profile in non-obese subjects with/ without HPT.

Major research significance/ benefit of the current project is that it is an applied research study and is hoped to bring a series of research benefits at individual and public level. It will hopefully provide awareness to the scholars to conduct keen studies for the management of HPT by searching ways to identify and discover products, remedies, bioactive compounds and efficacious substances/ drugs that might become a huge research benefit.

METHODS AND MATERIALS

Collection of the data for subjects during whole duration of the research project till submission of the project report is as according to the instructions and regulations of the Biomedical Ethics, Faculty of Medicine, Umm Al-Qura University. Male and female patients with hypertension (HPT, n: 62) and normal controls (NC, n: 66) were studied as described below. A series of pilot studies were carried out for planning better studies to be performed by collecting the history of male (NCm n:33) and female (NCf n: 31) subjects and hypertension male (HPTm, n: 31) and female (HPTf, n: 31) patients and deciding which specific variables be studied. 128 subjects (age: 55 to 69 years) were studied quite comprehensively for the present project. The body mass index (BMI) was estimated by

***Corresponding Author:** Dr Abdulhalim S. Serafi, Professor and Chairman, Department of Physiology, Faculty of Medicine, Umm Al-Qura University (UQU), Makkah, Saudi Arabia. Email: asserafi@uqu.edu.sa, abdulhalims@hotmail.com

dividing the body weight in kg with squared body height (kg/m^2) (Shah and Braverman, 2012). The BMI was checked, and only those subjects were included in the present study who had BMI in the range of 18-22.9 (kg/m^2) as the non-obese range of population (Jafar *et al.,* 2004). Those having higher than the mentioned BMI levels were not included in the present study. The subjects were fully informed about the purpose of the present research and collection of required data. They filled specific forms as volunteers for participating in the present study with their own willingness. No subject was forced to provide data for the present study. The best option suggested to the subjects was to keep blood pressure not above 115/75 mmHg (Basile, 2008). The systolic and diastolic blood pressure ranges considered for the present study for hypertensive subjects were 140/ 90 mmHg or above these values (de Faria *et al.,* 2014).

Kit and chemical procedures were compared for initial pilot studies to know about the accuracy and inter assay and intra assay variations. Tests included for the research project study were age and sex matched serum levels of leptin, total cholesterol, LDL-C, and HDL-C, (in NCm, NCf, and HPTm, HPTf groups of subjects/ patients. Plasma levels of leptin were measured in ng/ml, whereas cholesterol profile (total cholesterol, LDL-C, and HDL-C) were in mg/dl. All aspects of the plan of investigation were to find the age and gender-based associations/ correlations of the serum levels of leptin and cholesterol profile.

Enzymatic colourimetric method was used for measuring total cholesterol. Enzyme's incorporation resulted dye formation of colored product. The manual method needed precipitation and measuring colorimetrically at 3000 rpm the concentration of LDL-C and HDL-C as well. Fully automated method showed direct proportionality for HDL-C with the intensity of dye and LDL-C was estimated at 585nm of absorbance. Several procedures were employed for comparative purposes in pilot studies. The HDL and LDL cholesterol were separated by using Abcam's (ab65390) kit for the final detection of cholesterol ester and free cholesterol.

For the determination of serum leptin, Leptin Human ELISA Kit (ab100581) was used. The Abcam's Leptin Human ELISA (Enzyme-Linked Immunosorbent Assay) worked nice for human serum samples for quantitative determination of leptin. It is colorimetric with 1x96 plates sandwich ELISA for the quantitative estimation of leptin. Its recovery was about 94%. Immobilized antibody (very specific for human leptin) gets bound with sample leptin in the wells that are used for standards and samples. By washing unbound biotinylated-antibody, the wells are again washed and TMB solution added. Color developed according to the amount of bound leptin and the reaction was stopped by stop solution showing yellow colour from blue that was measured (450nm) colorimetrically.

The t test (unpaired or paired) was used for comparison of two variables. For the comparison of more than two variables, one way ANOVA was employed. The values for F, p and F crit were obtained. The r^2 or R^2 was obtained for regression lines. The specifically organized spreadsheets were used for analyzing that data. However, comprehensive analysis was done using SPSS software version 23. The results were analysed/ compared statistically applying general statistical principles (Zahir *et al.,* 2014). Inter-assay and intra-assay coefficients for leptin estimation were less than 9%.

RESULTS

Age of hypertensive and control subjects

The mean ± SD values of the age (years) of the male hypertensives (HPTm; 62.23 ± 4.50) vs. male normal controls (NCm; 61.91 ± 4.35), female hypertensives (HPTf; 62.23 ± 4.50) vs. female normal controls (NCf; 61.91 ± 4.45), NCm vs. NCf and HPTm vs. HPTf did not show any significant variations ($p > 0.05$), as analyzed by Tukey-Kramer test. Age range of subjects in each group was 55-69 years. The one-way analysis of variance (ANOVA) showed non-significant variation (F: 0.054; p: 0.983) of age among the various subject groups (male and female hypertensive and normal control groups).

Serum leptin levels in hypertensive and control subjects

The mean ± SD values for serum levels of leptin for the subjects in various groups as compared by Tukey-Kramer test showed highly significant variations for NCm vs. HPTm (6.70 ± 4.85 vs. 12.19 ± 9.02; p: 0.0032) and NCf vs. HPTf (7.42 ± 5.35 vs. 14.77 ± 11.03; p: 0.0011), and non-significant variations for NCm vs. NCf (p: 0.5692) and HPTm vs. HPTf (p: 0.3187). The one-way ANOVA showed highly significant variations (F: 7.617; $p > 0.0001$).

Serum cholesterol levels in hypertensive and control subjects

Mean ± SD values of serum cholesterol levels for the subjects in various groups of NCm (185.59 ± 12.34, NCf (188.11 ± 13.90), HPTm (184.31 ± 20.23 and HPTf (186.56 ± 19.39) did not show significant variations. The one-way ANOVA (F: 0.297; $p > 0.05$) also did not indicate significant variation.

Serum LDL cholesterol levels in hypertensive and control subjects

Serum LDL cholesterol (mean ± SD) values for the subjects in various groups showed significant variations for NCm (92.16 ± 11.52) vs. HPTm (104.01 ± 19.63) giving p: 0.0043, and NCf (95.70) vs. HPTf (105.08 giving p: 0.0335. Other comparisons were found non-significantly different. The one-way ANOVA showed highly significant variation (F: 4.616; $p < 0.005$) of serum LDL cholesterol among various subject groups.

Serum HDL cholesterol levels in hypertensive and control subjects

The serum HDL cholesterol (mean ± SD) values for the subjects in various groups showed significant variations for NCm vs. HPTm (64.86 ± 6.88; $p < 0.0001$), and NCf vs. HPTf (64.18 ± 6.82; $p< 0.0001$). Other comparisons were found non-significantly different. The one-way ANOVA showed highly significant variation (F: 37.044; $p < 0.0001$) of serum HDL cholesterol among various subject groups.

Association of age with serum leptin, total cholesterol, LDL-C and HDL-C

Plot of the age of subjects against serum leptin as well as against TC and LDL-C in all subject groups presented significant positive linear correlation; whereas age plotted against HDL-C showed significant negative linear correlation only for HPTm (Table 1).

Table 1. Association of age and serum leptin in subjects with hypertension.

Subject groups	Coefficient of determination R^2 and value of p, R^2 (p)						
	Age and Leptin	Age and Cholesterol	Age and LDL-C	Age and HDL-C	Leptin and Cholesterol	Leptin and LDL-C	Leptin and HDL-C
NCm	0.2369 (0.0041)	0.2214 (0.0057)	0.1885 0.0120	0.0089 0.6010	0.3620 0.0002	0.6047 < 0.0001	0.2082 0.0076
NCf	0.2647 (0.0022)	0.3388 (0.0004)	0.3982 0.00008	0.0824 0.1050	0.6456 < 0.0001	0.7788 < 0.0001	0.1312 0.0380
HPTm	0.4491 (0.00004)	0.1280 (0.0480)	0.5592 <0.0001	0.5496 <0.00001	0.3279 0.0008	0.6804 < 0.0001	0.3252 0.0008
HPTf	0.4951 (0.00001)	0.3197 (0.0009)	0.4207 < 0.00008	0.0769 0.1310	0.7668 < 0.0001	0.8218 < 0.0001	0.0847 0.1120

NC and HPT, respectively represent normal control and hypertensive subjects, m and f denote male and female subjects

Association of serum leptin with total cholesterol, LDL-C and HDL-C

Association of serum leptin with TC, LDL-C and HDL-C presented significant positive linear correlation (Table 1) for all subject groups, except HPTf for HDL-C having negative linear correlation without significant variation.

DISCUSSION

The present study demonstrated that leptin levels are significantly higher in HPTf participants than HPTm participants independent of potentially relevant characteristics like BMI, fat mass (FM), and menopausal state; and due to gender variations in circulating serum leptin concentrations are mediated by at least two separate mechanisms: females have more adipose tissue mass and a greater leptin synthesis rate per unit than in males (Dadarlat-Pop *et al.,* 2021; Hadaegh *et al*., 2021).

The present work carried out in non-obese subjects revealed significantly higher levels of leptin in HPTm vs. NCm participants, and HPTf vs. NCf, whereas the non-significant variations were found in NCm vs. NCf, and HPTm vs. HPTf, that can be interpreted by several other reports (Nguyen *et al.,* 2019; Hadaegh *et al.,* 2021).

The present investigation of the significant correlation between age and serum leptin levels in non-obese NC subjects and HPT patients, relates to a previous study showing significant age-based relationship of high leptin levels and cardiometabolic risk profiles (Galletti *et al.,* 2012). Association between leptin and cardiovascular organ damage, such as arterial stiffness (AS), was found linked with increased risk of CVD (Williams *et al.,* 2018). Leptin has been shown in recent studies to affect blood vessels function through stimulating the renin–angiotensin aldosterone system (RAAS) (Faulkner *et al.,* 2018). A link has been discovered between leptin and AS in cardiovascular risk patients (with e.g., chronic renal disease, CHD, diabetes, and HPT) (D'Elia *et al.,* 2020).

Present work provides the findings that TC levels in HPT individuals are consistent with same results of prior work (Pooja *et al*., 2013). It was found that there is no direct relationship of TC vs. age in NC and HPT participants. These results can be explained by the help of previously conducted work that prove that it is commonly established that HPT is associated with increased TC (Patel *et al.,* 2016).

Dyslipidemia, described with changed ratio of increased TC beside other alterations is commonly related with elevated BP and the average serum TC levels were considerably higher and statistically significant in HPT individuals as compared to those in NC subjects (Patel *et al.,* 2016). The findings in the present study can further be explained by a prospective study showing elevation in blood TC in HPT patients than healthy NC subjects and by the most common abnormality among Mexican people as the increased LDL-C beside other lipid profile changes (Patel *et al.,* 2016).

The results obtained in the present report that non-significant change of LDL-C for NCm vs. NCf, HPTm vs. HPTf but significantly increased levels of LDL-C for HPTm vs. NCm, and HPTf vs. NCf, can be interpreted by a report wherein it was suggested that it is commonly established that CVD is associated to HPT and high LDL-C beside other changes in lipid profile (Mora *et al.,* 2013). Another report also verifies that serum lipid profile values vary widely around the globe across different demographic groups, and LDL-C in particular, has association with increased CVD risk factors (Chiu *et al.,* 2016).

The present report indicating that LDL-C increases significantly in HPT subjects has been observed in a report that reveals LDL-C considerably higher and statistically significant in HPT individuals compared to NC subjects (Chiu *et al*., 2016) and in another study wherein increased LDL-C in HPT patients was more than healthy NC subjects (Chiu *et al.,* 2016). No changes in LDL-C have also been found in patients with HPT without carotid plaques (Nguyen *et al.,* 2019). However, there is another study having similar results as obtained in the current study (Patel *et al.,* 2016).

The current project work shows significant variations for NCm vs. HPTm and NCf vs. HPTf that is in accordance with previous reports carried out to compare the results of male and female subjects (Pimenta, 2012). The correlation of age of all subject groups with LDL-C in the present work indicates gradual increase in LDL-C with the age as a general aging process, though more increase in LDL-C in HPT vs NC clarified as it is well known that the prevalence of HPT in both male and female subjects increases with age, and males have an increased incidence of hypertension than women until the age of 40s; but a variation occurs until the age of 50s, or menopause (Pimenta, 2012). Furthermore, other CVD risk factors, grow with age, particularly in women over the age of 50. It was revealed that older male and female, having neither large waist circumference nor high BMI substantially related with HPT (Sun *et al*., 2018; Al-Shoaibi *et al.,* 2022).

It was noted in the present work that though NCm vs. NCf, and HPTm vs. HPTf did not show significant change in HDL-C, but HPTm vs. NCm, HPTf vs. NCf showed significantly decreased levels of HDL-C showing as a metabolic syndrome component, that is verified previously (Patel *et al*., 2016). It is commonly established that cardiovascular disease is linked to hypertension and low HDL-C is one major manifestation and the non-obese male and female subjects with HPT reveal variations in cholesterol profile (TC, LDL-C and HDL-C) (Chen *et al.,* 2019).

Low levels of HDL levels are rapidly becoming known as a risk factor for unfavorable hypertensive and other CVD outcomes, regardless of low-density lipoprotein (LDL) levels (Chen *et al.,* 2019). The average HDL level in HPT individuals was lower than that in NC subjects, and this difference was found statistically significant. According to another report, the most common abnormality among Mexican people was reduced HDL-C, and changes in other lipid profile components (Patel *et al.,* 2016).

It is well known that the prevalence of hypertension in both male and female rises with age. Males have a higher incidence of hypertension than women until the age of 40s; however, inequalities between the genders reduce until the age of 50s, or menopause (Pimenta, 2012). Other cardiovascular risk factors, are well known to grow with age, particularly in women over the age of 50. Women have greater HDL-C concentrations than men (Williams, 2004) but there are distinct lipoprotein changes with aging in both sexes in terms of HDL-C protective function.

Most of the information about leptin-sympathetic and leptin-resistance actions has been predicted on the bases of in vitro and animal studies. Hence, it is essential to have data from human studies to clarify the role of leptin and related physiological changes in NC and HPT conditions, and to understand the diagnostic and pathophysiological involvement in patients with HPT. The present work hence, provides interesting and applied information about the role of leptin and cholesterol profile in patients with hypertension, and will hopefully help further in understanding the diagnostic and pathophysiological aspects of hypertension.

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