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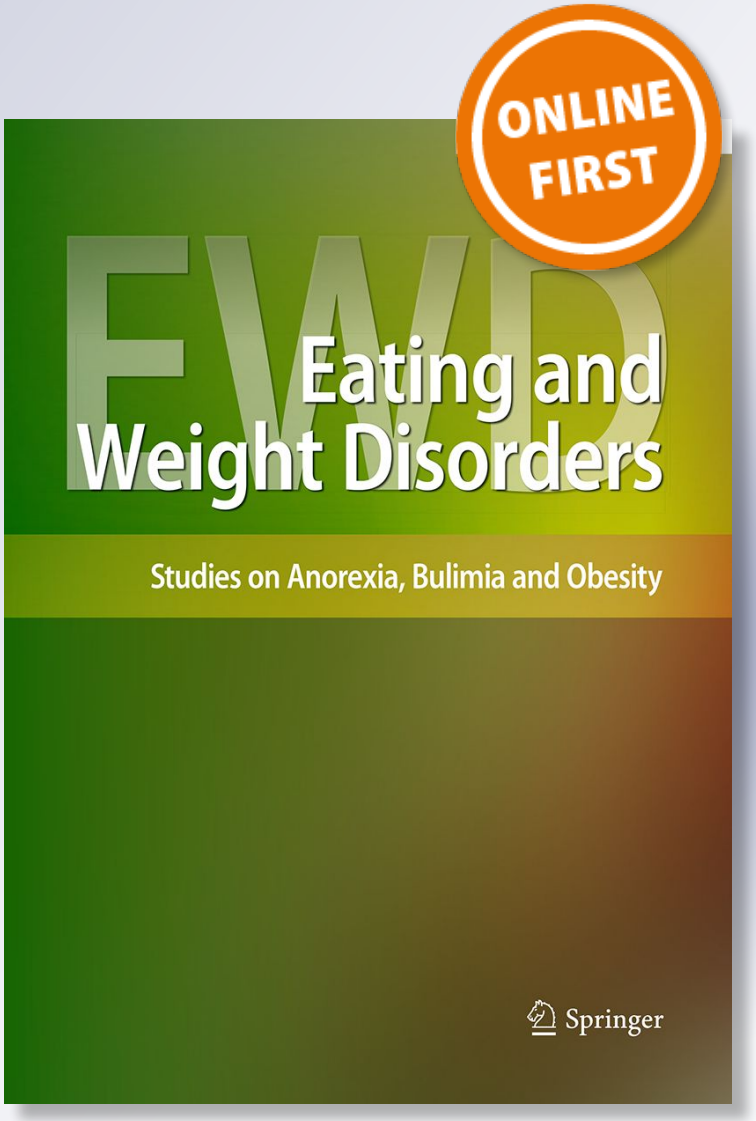

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ORIGINAL ARTICLE

Inter-relationship of serum leptin levels with selected anthropometric parameters among a non-diabetic population: a cross-sectional study

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Abstract
Purpose Association between serum leptin levels and anthropometric parameter is well established in western countries according to the specific WHO cut-off values assigned for those populations, whereas it is not clearly defined for Asians especially with respect to gender. Thus, the objectives of this study were to determine the relationship of serum leptin levels with body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR) to identify the variations of serum leptin levels with gender and to evaluate the serum leptin levels in risk and non-risk groups based on their anthropometric values.
Subjects/methods A cross-sectional study was carried out among 226 apparently healthy subjects (non-diabetics, age 20–70 years). Height, weight, WC, hip circumference (HC) and mid arm circumference (MAC) were measured. BMI, WHR, waist to height ratio (WHtR) were calculated. Fasting blood samples were collected. Serum leptin levels were measured using human leptin ELISA kits.
Results Majority of the participants were females (59.3%). Serum leptin levels were significantly higher in females (24.8 ± 17.1 ng/mL) compared to males (9.3 ± 7.9 ng/mL). Significant positive correlations ($P < 0.05$) were observed between serum leptin levels and all anthropometric parameters except height in both genders. The risk groups according to BMI, WC and WHR in females were hyperleptinaemic and had significantly ($P < 0.05$) higher serum leptin levels than the non-risk groups.
Conclusions Linear trend was observed for serum leptin levels with weight, BMI, WC, HC, WHR, MAC and WHtR in both genders. Though the serum leptin levels were higher among risk groups, according to WHR, WC and BMI, the hyperleptinaemia was observed only among females.
Level of evidence A descriptive cross-sectional study, Level V

Keywords Leptin · Obesity · Anthropometry · Gender differences

Introduction

Leptin is a peptide hormone produced predominantly by adipose tissues. It is a product of *ob* gene. The leptin controls energy expenditure and appetite [1]. Feedback signal to the Central Nervous System (CNS) by leptin regulates the energy homeostasis and feeding behaviors [2]. Leptin when bound to the receptors in the hypothalamus inhibits the synthesis and release of hypothalamic neuropeptide Y [3]. This neuropeptide Y enhances the secretion of insulin, corticosteroids, food intake and reduce thermo-genesis, thereby regulating the body weight of an individual [3]. Hence defects in the action of leptin and *ob* genes will lead to development of obesity [4]. Obesity is defined as 'a disorder of weight regulating system, characterized by an excess accumulation of fat in the body' [5].

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High prevalence of overweight and obesity, particularly abdominal obesity is reported globally with a higher incidence among South Asians. The data from around the world further indicate a gender-based prevalence of obesity with higher percentage of females in this risk category compared to males [6].

Studies have indicated that higher serum leptin levels are found in obese individuals in proportion to their body fat content. This condition is referred to as hyperleptinaemia [3, 7], where leptin levels in serum exceed the normal serum leptin range. Hyperleptinaemic condition induces resistance to leptin receptors, eventually promoting "leptin resistance" in obese individuals [7]. This in turn induces pancreatic β cells to secrete insulin continuously thus, promoting hyperinsulinaemia which gradually leads to the development of insulin resistance (IR) [9]. IR has strong association with development of metabolic syndromes and it's also a characteristic feature of type 2 diabetes [10]. Elevated serum leptin level is reported to have an association with IR suggesting that diabetics tend to have altered serum leptin levels [11, 12]. Thus, the present study was conducted in apparently healthy non-diabetic subjects.

Anthropometric measurements and indices such as waist circumference (WC), hip circumference (HC), mid arm circumference (MAC), body mass index (BMI), waist to hip ratio (WHR) and waist to height ratio (WHtR) are used as simple standard measures to assess obesity and body fat levels worldwide [13]. Both serum leptin and anthropometric parameters are used to assess obesity. Thus, it would be of value if both these parameters are compared as parameters to assess obesity and related disorders.

Most studies carried out globally to assess the association between serum leptin levels and anthropometric parameters were carried out according to specific World Health Organization (WHO) cut offs in western populations in order to study the applicability in the respective nations [14]. The South Asian population is reported to be more vulnerable in developing non communicable diseases as they have more centralized deposition of body fat and also have higher mean values for WHR and WC when compared to Europeans [15]. However, research conducted using the cut-off values of anthropometric parameters especially assigned for Asian populations were scarcely carried out along with serum leptin concentrations to determine the associations, if any. Also, no studies have been reported in apparently healthy subjects in a South Asian population according to WHO modified cut offs for Asians to study the relationship with serum leptin levels.

Thus, the aim of the present study was to compare the serum leptin levels among a selected Sri Lankan population of males and females with selected anthropometric parameters and to evaluate the relationship between serum leptin levels and the anthropometric parameters. Further, this study aims to categorize the subjects as overweight/obese and risk of development of non communicable diseases (NCD) based on BMI, WC and WHR [16] for Asians and to evaluate the serum leptin levels of these groups.

Methods

Study design and population

This was a descriptive cross-sectional study carried out in 226 non-diabetics (age 20–70 years) who attended a clinic attached to the Faculty of Medical Sciences, University of Sri Jayewardenepura which is situated in Colombo, Sri Lanka. All the participants for the study were selected using a non-probability (convenience) sampling technique. The study protocol was clearly explained to all the participants and informed written consent was obtained prior to the study. Non-diabetics [not diagnosed as having diabetes mellitus (DM)], adults aged between 20 and 70 years and no past history of myocardial infarction, cancer, heart failure and subjects with BMI values <40 kg m^{-2} were included in the study. Exclusion criteria of the study were individuals with diagnosed DM, pregnant mothers, cancer patients, individuals with other chronic diseases like tuberculosis, rheumatoid diseases, past history of myocardial infarction/stroke, subjects with BMI values >40 kg m^{-2} and patients with cognitive impairment. Pre-tested and standardized study instruments were used in the data collection process. Interviewer administrated questionnaire was used to collect information related to socio-demographic factors and lifestyle characteristics.

Anthropometric parameters

The anthropometric measurements such as height, weight, WC, HC and MAC were measured according to recommended guidelines [17, 18]. Body weight of all the selected participants was measured using an electronic weighing scale (Chyo, Mu-150 K, Japan) to the nearest 0.01 kg. Height of the participants was measured using a stadiometer to the nearest 0.1 cm. BMI, WHR and WHtR were calculated using recommended guidelines [14, 18]. In the present study, WHO modified anthropometric cut offs for Asian populations were considered when grouping the individuals [16] (Table 1).

Cut off values for MAC and WHtR were not specifically defined for Asian population. Thus, these two parameters were only used as continuous variable to assess the relationship with serum leptin status in the study population.

Table 1 Guidelines for the classification of obesity for Asian population Source [16]

Characteristics	South Asian cut-off values
BMI	
Underweight	<18.5 kg m^{-2}
Normal	18.5–22.9 kg m^{-2}
Over weight	23–24.9 kg m^{-2}
Obesity	≥25 kg m^{-2}
WC	
Obesity risk group	>90 cm (male)
	>80 cm (females)
WHR	
Obesity risk group	>0.89 (male)
	>0.81 (female)

Biochemical analysis

A fasting (12-h) venous blood sample was drawn from a peripheral vein from all the participants by a trained phlebotomist. Blood samples were collected into empty sterile centrifuge tubes and allowed to clot for a sufficient time (30–40 min). Serum was separated by centrifuging (KUBOTA, 6800, Japan) at 3600 rotations per minute (rpm) for 10 min at room temperature. An aliquot of 50 μL serum of individual samples was stored at −20 °C for batch analysis. The serum samples were tested for leptin levels using the human leptin ELISA kits (DRG, EIA-2395). ELISA reader (Immuno Mini NJ 2300, Japan) was used to read the absorbance values. ELISA procedures were followed as mentioned in the manuals of the kit and absorbance values were measured. The standard curve for the assay was constructed using the GraphPad Prism 6.02 software. The concentration of each sample was calculated from the standard curve. All collected date were entered and double checked for consistency.

Statistical analyses

Data were analyzed using the Statistical Package for Social Science (SPSS) software version 15.0. Values of leptin concentrations were log transformed to normalize the distribution. As leptin level varies with gender, serum leptin analysis was carried out separately for males and females. For comparative evaluations, the student's *t* test was performed. The Pearson correlation and non-linear regression analysis were used to determine the independent effect of anthropometric parameters on serum leptin concentrations. The data were presented as mean ± standard deviation (SD) and probability values less than or equal to 0.05 (two tailed) were considered to be significant.

Results

Among 226 subjects, majority of the population were females (59.3%) and within the age group of 18–38 years (48.2% in the whole population). gender differences in the serum leptin levels have been observed in previously reported studies, thus serum leptin levels and anthropometric parameters were analyzed separately for males and females. Fasting serum leptin concentration ranged from 1.86 to 50.8 ng/mL in males and 2.24 to 90.43 ng/mL in females. The ethnic variation of serum leptin concentration and anthropometric parameters were not analyzed as 93.4% of the study population was from one ethnic group (Sinhalese). Table 2 presents the serum leptin levels and anthropometric parameters of the selected population by gender. Significant differences were observed in serum leptin levels, weight, height, WC, MAC, WHR and WHtR between males and females.

All studied continuous variables (anthropometric parameters) were used in a standard regression analysis to assess the correlation with serum leptin levels. Table 3 indicates the correlations between log-transformed serum leptin concentrations and selected anthropometric parameters according to the gender. Among the male participants, all the parameters except age, height and WHtR correlated significantly with leptin levels. All the other parameters correlated significantly with serum leptin levels among females except for height. Among females WHtR, BMI and WC yield stronger correlation with the leptin levels (Table 3).

Serum leptin levels according to obesity grouping (WHO-modified definition for Asians) with regard to BMI, WC and WHR were further analyzed to observe the difference if any. These data were also analyzed separately for males and females. According to the current finding, serum leptin levels were significantly higher among risk groups compared to non-risk groups except WHR in males (Table 4).

Discussion

Leptin is secreted into the circulation in a pulsatile manner that follows a circadian rhythm. Secretion of leptin in the body is affected by sleep pattern of an individual. Highest levels of leptin secretion are found during midnight, early morning and lowest during the afternoons [19]. Thus, the sample collection was done during the morning hours.

In the present population, serum leptin levels were significantly higher among females (24.8 ± 17.1 ng/mL) when compared to males (9.3 ± 7.9 ng/mL). This was consistently observed in numerous other studies among western populations [3, 8, 20] as well as among other Asian populations from China and Middle East countries [21–23].

Table 2 Biochemical and anthropometric parameters of the population

Characteristics	Males ($n = 91$)	Females ($n = 135$)	P value
Biochemical parameter leptin (ng/mL)	9.3 ± 7.9	24.8 ± 17.1	<0.001*
Anthropometric parameters			
Height (cm)	167.1 ± 13.2	154.7 ± 7.5	<0.001*
Weight (kg)	68.3 ± 11.2	57.5 ± 11.7	<0.001*
WC (cm)	87.2 ± 10.5	81.0 ± 10.4	<0.001*
HC (cm)	98.2 ± 7.5	96.5 ± 8.9	0.245
MAC (cm)	30.2 ± 3.0	28.8 ± 4.1	0.006*
BMI (kg m^{-2})	24.0 ± 3.5	24.1 ± 4.3	0.693
WHR	0.89 ± 0.07	0.84 ± 0.08	<0.001*
WHtR	0.53 ± 0.11	0.52 ± 0.07	0.921

* The values are significantly different between males and females at 95% confidence interval. All the study parameters are presented as mean ± SD

Table 3 Correlation coefficients (r) of log-transformed serum leptin concentrations with anthropometric parameters

Characteristics	Males (r) ($n = 91$)	Females (r) ($n = 135$)
Age	−0.099	0.236**
Weight	0.402**	0.367**
Height	0.076	−0.095
BMI	0.512**	0.467**
WC	0.358**	0.460**
HC	0.330**	0.356**
WHR	0.245*	0.288**
MAC	0.291*	0.402**
WHtR	0.148	0.482**

* Correlations are significant at the 95% confidence level (2-tailed)

** Correlations are significant at the 99% confidence level (2-tailed)

Table 4 Differences in mean serum leptin levels according to the risk of development of central obesity

Characteristics	Concentration of serum leptin levels (ng/mL)	P value
Waist circumference		
Male	7.2 (≤90 cm)	0.002*
	12.4 (>90 cm)	
Female	17.1 (≤80 cm)	<0.001*
	32.9 (>80 cm)	
Waist to hip ratio		
Male	8.3 (≤0.89)	0.146
	10.7 (>0.89)	
Female	21.7 (≤0.81)	0.008*
	29.7 (>0.81)	
BMI		
Male	5.3 (<22.9 kg m^{-2})	<0.001*
	11.5 (≥23 kg m^{-2})	
Female	15.1 (<22.9 kg m^{-2})	<0.001*
	31.3 (≥23 kg m^{-2})	

* Mean differences in serum leptin levels are significant at 95% confidence level

Thus, the present study adds to the knowledge regarding the gender difference in serum leptin levels of a South Asian population. Reported studies have indicated that higher serum leptin levels are found in proportion to the body fat content. Thus, the differences of mean serum leptin levels among males and females might be due to the differences in fat metabolism and body fat distribution pattern among females and males. It is well defined that males generally have a lower percentage of body fat than females. A healthy range of body fat for male is 10–15% and for female is 20–25%. Fat storage among female is mainly around the hip and thigh region whereas males have the fat storage in and around the abdomen. Differences in the fat metabolism may be due to gender difference in hormones [24]. Furthermore according to other study findings, hormone receptors and enzymatic reactions play a major role for the gender difference observed in serum leptin levels; however, the mechanisms responsible for the above findings need to be further explored [24].

In addition, the data of the present study also adds some weight to the findings of Mente about leptin and ethnic variation (among South Asians and Western population) [11]. In the above-mentioned study, South Asians living in Canada had higher mean serum leptin levels when compared to western study populations. South Asians are reported to be more susceptible to develop central obesity and this could be a reason for the presence of higher serum leptin levels [16]. The serum leptin levels in this present study were much similar to the serum leptin values of South Asians living in Canada in the above-mentioned study [11].

When considering the anthropometric data, the mean BMI in both males and females, mean WC and WHR in

females indicate that this populations is at risk of developing obesity (according to the WHO modified cut-off for Asian) [16]. MAC and WHtR are considered as the novel indicators to assess obesity and malnutrition. However, data available to specify the cut-off values or values that can be used in risk assessments using WHtR and MAC in this population are very minimal. Thus, these two continuous variable parameters were only used in this study to assess the correlation with serum leptin levels.

In addition, different studies have stated different cut-off values for WHtR. In as study Cai et al. had stated optimal range/cut off for WHtR as 0.51–0.53 for males and 0.48–0.50 for females in a Chinese population [25] whereas another study carried out by Yaguang, in the same population had indicated 0.55–0.56 for males and 0.57–0.58 for females as WHtR cut off values [26]. Both studies conducted in Asian/Chinese population have recommended WHtR risk cut-off point for cardio vascular diseases as $\geq$0.5 [25, 26]. WHtR cut-off values for the African population (Haiti and Benin) was reported as 0.50 and 0.59 for males and females, respectively [27]. According to our study findings the mean WHtR for both genders is above 0.52. According to the available data, cut-off values for MAC are 27 and 29 cm for males and females, respectively [28]. In this present study, only the male population have a higher mean MAC compared with reported cut off values. Thus, in the present study population, both males and females are at risk of developing non communicable diseases when anthropometric parameters are considered as risk indicators.

Leptin levels are positively associated with weight, BMI, WC, HC, WHR, MAC and WHtR in both genders (Table 3). Previous studies have also established similar patterns of correlations between serum leptin levels and selected anthropometric parameters such as BMI, WC, HC and WHR [20, 21, 23, 29–31]. In addition, the present study has identified significant positive correlations for serum leptin with MAC and WHtR which has not been reported in other studies and adding a new knowledge. This needs to be further established with more data from different populations round the world.

Furthermore the study results showed a non-significant negative correlation between serum leptin levels and height in females and a non-significant positive correlation in males. However, height is not a reliable marker to determine the serum leptin levels since height is a constant factor after a period of age among both genders when compared to weight. Serum leptin levels positively correlated with weight irrespective of gender, and the relationship was significant ($P = 0.000$) in both males and females [8, 32].

Serum leptin levels were significantly higher in risk groups compared to non-risk groups in females. However, there was no significant difference in serum leptin levels between WHR risk and non-risk groups among males, whereas, the serum leptin levels of BMI and WC risk and non-risk groups were significantly different. There are no reported studies comparing serum leptin levels in risk and non-risk groups based on BMI, WC and WHR.

In the present study, leptin levels are associated with selected anthropometric parameters in accordance with other reported studies. According to reported data, anthropometric parameters were categorized to indicate the risk of developing obesity-related disorders among males and females separately. But in the present study serum leptin levels and anthropometric parameters were studied together to assess the relationship between the two indicators and to observe the variations in serum leptin levels in the risk and non-risk for obesity. Further, this study is unique where leptin levels were compared according to WHO modified cut-off values for Asians and evaluated the serum leptin levels in risk and non-risk groups.

Conclusion

Serum leptin levels of females were significantly higher than that of males. In addition serum leptin levels showed a positive correlation with weight, BMI, WC, HC, WHR, MAC and WHtR in both males and females. Though the serum leptin levels were higher among risk groups according to WHR, WC and BMI the hyperleptinaemia were observed only among females.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Ethics approval and consent to participate Ethical clearance for the study was obtained from the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. Written informed consent was obtained from all individual participants. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee.

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