

Leptin as a predictor of anthropometric cutoff points for obesity

Mozaffer Rahim Hingorjo^{1*}, Sitwat Zehra², Shazia Nadeem³ and Masood Anwar Qureshi⁴

¹Jinnah Medical & Dental College, Karachi, Pakistan

²Dr. AQ Khan Institute of Biotechnology & Genetic Engineering, Karachi, Pakistan

³Department of Physiology, Ziauddin University, Karachi, Pakistan

⁴Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan

Abstract: This study was conducted to find the association between leptin and adiposity indices. Secondly, to identify optimal threshold of various anthropometric indices for obesity, as assessed by 75th percentile of leptin levels, within a clinic sample of non-diabetic and diabetic Pakistani adults. Fasting serum leptin levels were compared with anthropometric markers of obesity in 164 diabetic and non-diabetic subjects (90 male, 74 female), aged 35 to 65 years. Obesity was defined by body mass index (BMI) of 25 kg/m² in either sex. The cutoff point of leptin was taken as the 75th percentile in non-obese subjects. Diagnostic accuracy for detecting excess fatness was evaluated through receiver operating characteristics (ROC) analyses with leptin taken as reference test against anthropometric indices as test variables. The 75th percentile of leptin in male and female was 7.0ng/mL and 17.9ng/mL, respectively. Leptin levels were significantly higher in females ($p < 0.001$) and had strong positive correlation ($p < 0.001$) with most anthropometric indices of obesity in both sexes; hip circumference (HC) being most prominent among these. Largest area under ROC curve (AUC) was between WC and leptin (AUC=0.844; CI=0.764, 0.925) in males and BMI and leptin (AUC=0.832; CI=0.740, 0.923) in females. The optimum thresholds for obesity indices in our study were: BMI, WC and HC as 25 kg/m², 96.25cm, 99.25cm for males; 27 kg/m², 95.50cm, 105.5cm for females, respectively. Leptin can be considered as a potential marker of obesity and may be used to identify obesity cutoffs in future demographic surveys. Longitudinal studies are required that include leptin in coronary artery disease risk assessment models.

Keywords: Leptin, obesity, diabetes mellitus, body mass index.

INTRODUCTION

Obesity has attained an alarming status worldwide with the general public becoming aware of their weight status, whether due to health or cosmetic reasons. In this regard, many techniques have emerged for its measurement. These include the use of sophisticated and expensive imaging equipment, having a high degree of accuracy for diagnosing excess body fat. However, these techniques are not widely available for everyone and one has to rely on anthropometric indices of obesity that are simple though less accurate. These include measurement like skin-fold thickness (Durnin and Womersley, 1974), body mass index (BMI) (Wang *et al.*, 1994), waist circumference (WC), waist-to-hip ratio (WHR) (WHO expert consultation, 2008) and many others.

The distribution of body fat also affects the health status of a person, major deposits being found at three places: subcutaneous, intramuscular and abdominal. Abdominal or central obesity is the accumulation of visceral fat inside the peritoneum in several locations and is particularly associated with dyslipidemia, cardiovascular disease, diabetes mellitus, metabolic syndrome etc, as opposed to generalized obesity (Sharma *et al.*, 2016). Anthropometric indices that measure central obesity are, therefore, of particular significance. In this regard, measurement of

WC either alone or in combination with some other variable becomes more important (Almeida *et al.*, 2009). Due to variations in body frames of Asians and Caucasians, the same cutoff points of anthropometric indices cannot be applied universally. For example, Asian BMI cutoff points suggested by WHO for obesity is ≥ 25 kg/m² which is different from Caucasian cutoff point (≥ 30 kg/m²) (Wang *et al.*, 1994; WHO expert consultation, 2004). Furthermore, regional, ethnic and racial differences call for separate cutoff values individualized to each nation (Hsu *et al.*, 2015).

Leptin is released mainly from the adipose tissue and is one of the hormones that control our feeding and satiety behavior. This is accomplished by suppression of 'orexigenic peptides' such as neuropeptide Y, melanin-concentrating hormone, orexins, and agouti-related protein as well as releasing 'anorexigenic peptides' such as pro-opiomelanocortin, cocaine and amphetamine-regulated transcript, and corticotrophin-releasing hormone from the hypothalamus (Jéquier, 2002; Flak and Myers Jr, 2016). A non-obese person has lower leptin levels compared to obese individual. However, the levels of leptin in obese do not match the degree of obesity and are higher. This may be due to an end organ resistance as in the case of insulin resistance, leading to persistently high leptin levels (Crujeiras *et al.*, 2015). Studies have shown association of high leptin levels with cardiovascular disease, diabetes mellitus, obesity and metabolic

*Corresponding author: e-mail: muzaffer_rahim@hotmail.com

syndrome, increasing the morbidity and mortality (Schmidt *et al.*, 2006). The purpose of this study was to find the association between leptin and anthropometric indices of obesity among diabetic and non-diabetic subjects. Based on the receiver operating characteristics (ROC) curve analysis, we also attempted to identify new cut-points for anthropometric indices that would better categorize Pakistani population as obese, using leptin as the reference test.

MATERIALS AND METHODS

Subjects

The study was carried out on 164 subjects: 90men (54.8%) and 74 women (45.1%), aged 35 to 65 years. The subjects were type 2 diabetics (73.7%) and healthy non-diabetics (26.2%). Subjects having T2DM were selected from Dr. Essa's Laboratory and Diagnostic Center. The laboratory is located at five places in Karachi and serves a vast population of subjects coming from five districts of Karachi. The controls came from the general population and were also analysed in the same laboratory. Hormone analysis was carried out in the main Dr. Essa's Laboratory and Diagnostic Center. Written informed consent was taken from each subject according to the principles of declaration of Helsinki and the study was approved by the research and ethical committee of Fatima Jinnah Dental College, Karachi. Exclusion criteria were: diabetic subjects on insulin, active infection, inflammatory disorder, cancer, thyroid dysfunction, pregnancy, lactation, end-stage hepatic/renal failure and cardiovascular events. Newly diagnosed diabetic, impaired diabetes (100 -125 mg/dl) on no hypoglycemic medication, hypertensive and those taking lipid lowering agents were, however, included in the study.

Anthropometric measurement

Weight was measured, using digital scale to the nearest 0.1 kg, while wearing light clothing. Height was measured without shoes using a wall-mounted stadiometer to the nearest 0.1cm. The BMI was calculated by dividing weight (kg) with height (m²). A BMI of ≥ 25.0 kg/m² was taken as obese (Asian cutoff value). We included body surface area (BSA) in the calculations as it is a better indicator of metabolic mass than body weight (Roy *et al.*, 2012) and is less affected by abnormal adipose tissue. The BMI is a modified form of BSA. It was calculated according to the Mosteller formula (Mosteller, 1987) as follows:

$$BSA = \sqrt{(Wt. \times Ht.)} \div 60$$

Where Wt. = weight in kg; Ht. = height in cm.

The WC was determined while erect, at the midpoint between the top of the iliac crest and lower margin of last palpable rib, to the nearest 0.1cm. Hip circumference (HC) was taken as the widest measure at the level of hips, while erect. Waist-hip ratio and waist-height ratio (WHtR)

were measured by dividing WC respectively with HC and Height. Neck circumference (NC) was taken at the level of larynx (just below the thyroid cartilage), perpendicular to the long axis of the neck, to the nearest 0.1cm. Conicity index (C index), a predictor of central obesity (Mantzoros, 1996; Mamtani & Kulkarni, 2005), uses WC for a given height and weight and was calculated as follows:

$$C\text{ Index} = WC(m) \div 0.109 \times \sqrt{(Wt.(kg) \div Ht.(m)}$$

Abdominal volume index (AVI) is a measure of central obesity as it measures the abdominal volume between WC and HC. The concept was introduced by Guerrero-Romero and Rodríguez-Morán (2003), who considered the abdominal cavity to resemble a truncated cone bordered by WC and HC. It is a geometric concept and has not been widely utilized in clinical practice. It was calculated as follows:

$$AVI = [2WC^2 + 0.7 (WC - HC)^2] \div 1000$$

Body fat percent (BF%) was estimated by first measuring skin fold thickness at four sites: triceps, biceps, suprailiac and sub scapular with Fat-O-Meter skinfold caliper. *Triceps*, a vertical fold over triceps muscles, midway between acromion and olecranon processes, while the arm is held freely by the body side; *Biceps*, a similar fold anteriorly over the biceps muscle; *suprailiac*, a diagonal fold, above the iliac crest in the anterior axillary line; *subscapular*, a diagonal fold 1 to 2cm below the inferior angle of the scapula.

Body density (D) was calculated according to the method of Durnin & Womersley (1974), using log of total skinfold measurements. Body fat percentage was then calculated as follows:

$$\text{Males} = (4.97 \div D) - 4.52$$

$$\text{Females} = (4.81 \div D) - 4.34$$

The normal limits for BF% in males and females were taken as 24% and 37%, respectively (Wang *et al.*, 1994).

Biochemical analysis

Fasting venous blood samples were taken to determine levels of glucose and leptin. Serum leptin was measured using highly sensitive sandwich enzyme-linked immune sorbent assay (EIA-2395; DRG Instruments GmbH, Germany), with sensitivity or minimum detection limit of 1.0 ng/ml. The intra-assay and inter-assay coefficients of variation of the kit were 6.91% and 8.66%, respectively.

The cutoff point for normal leptin level was taken as the 75th percentile in non-obese subjects with a BMI <25 kg/m² (currently accepted Asian cut point for obesity). This was 7.0ng/ml in males and 17.9 ng/ml in females. On the basis of this, the subjects were divided into two groups: those with normal leptin levels and those with higher than normal levels.

Table 1: Descriptive characteristics of the study population

Parameter	Male † (n = 90)	Min – Max	Female † (n = 74)	Min – Max	P
Age, yr	52.82 (10.48)	28 – 66	49.12 (9.90)	27 – 65	<0.001**
Weight, kg	72.18 (13.06)	41.0 – 112.0	68.09 (14.31)	46.0 – 116.0	0.008**
Height, m	1.69 (0.07)	1.50 – 1.83	1.57 (0.07)	1.43 – 1.87	<0.001**
BMI, kg/m ²	25.03 (3.95)	15.06 – 35.11	27.54 (5.07)	18.00 – 45.88	<0.001**
BSA, m ²	1.84 (0.18)	1.37 – 2.37	1.71 (0.19)	1.40 – 2.26	0.04*
WC, cm	96.24 (11.13)	67.0 – 126.0	96.30 (10.97)	72.0 – 125.0	0.98
HC, cm	99.67 (8.93)	80.0 – 141.0	106.97 (9.90)	89.5 – 133.0	<0.001**
WHR	0.96 (0.07)	0.79 – 1.15	0.90 (0.07)	0.72 – 1.11	<0.001**
WHtR	0.57 (0.06)	0.38 – 0.71	0.61 (0.06)	0.47 – 0.79	<0.001**
NC, cm	38.19 (2.80)	31.0 – 44.5	34.56 (2.94)	29.5 – 45.2	<0.001**
C Index	1.36 (0.08)	1.17 – 1.49	1.35 (0.09)	1.14 – 1.60	0.287
AVI, L	18.83 (4.30)	9.08 – 32.44	18.92 (4.23)	10.42 – 31.29	0.898
BF%, %	26.09 (5.09)	14.59 – 39.31	38.95 (6.06)	25.63 – 58.16	<0.001**
Leptin, ng/mL	9.08 (7.57)	0.50 – 47.30	29.65 (20.56)	6.9 – 107.6	<0.001**

Note: Paired-sample t test was used to compare means between male and female subjects.* $p < 0.05$, significant; ** $p < 0.001$, very significant; † Values expressed as mean (SD).

Abbreviations: BMI, Body Mass Index; WC, Waist Circumference; HC, Hip Circumference; WHR, Waist Hip Ratio; WHtR, Waist Height Ratio; NC, Neck Circumference; C Index, Conicity Index; AVI, Abdominal Volume Index; SD, Standard deviation;

Table 2: Correlation of Leptin with adiposity indices in male subjects

Parameter	Group A = Leptin $\leq$ 7.0 ng/mL (n = 47)		Group B = Leptin > 7.0 ng/mL (n = 43)	
	Mean (SD)	r	Mean (SD)	r
BMI, kg/m ²	22.94 (3.09)	(0.598**)	27.29 (3.52)	(0.450**)
BSA, m ²	1.75 (0.14)	(0.360*)	1.92 (0.19)	(0.221)
WC, cm	89.62 (8.84)	(0.484**)	103.46 (8.61)	(0.356*)
HC, cm	95.12 (6.06)	(0.357*)	104.65 (8.97)	(0.450**)
WHR	0.94 (0.07)	(0.358*)	0.99 (0.05)	(-0.090)
WHtR	0.53 (0.06)	(0.503**)	0.61 (0.04)	(0.471**)
NC, cm	37.13 (2.42)	(0.369*)	39.35 (2.76)	(0.055)
C Index	1.32 (0.08)	(0.194)	1.39 (0.06)	(0.083)
AVI, L	16.26 (3.04)	(0.470**)	21.58 (3.64)	(0.362*)
BF%, %	23.22 (3.99)	(0.384**)	29.24 (4.26)	(0.491**)
Leptin, ng/mL	3.91 (1.76)		14.71 (7.52)	

Note: Values expressed as Mean (SD), along with correlation coefficient (r) below the mean; * $P < 0.05$, ** $P < 0.001$.

Abbreviations: SD, Standard Deviation; BMI, body mass index; BSA, body surface area; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; NC, neck circumference; C Index, conicity index; AVI, abdominal volume index.

Table 3: Correlation of Leptin with adiposity indices in female subjects

Parameter	Group A = Leptin $\leq$ 17.9 ng/mL (n = 26)		Group B = Leptin > 17.9 ng/mL (n = 48)	
	Mean (SD)	r	Mean (SD)	r
BMI, kg/m ²	23.91 (3.86)	(-0.023)	29.51 (4.56)	(0.232)
BSA, m ²	1.58 (0.14)	(0.153)	1.78 (0.18)	(0.435**)
WC, cm	88.75 (8.32)	(-0.207)	100.39 (10.08)	(0.477**)
HC, cm	100.21 (7.08)	(0.220)	110.63 (9.30)	(0.503**)
WHR	0.89 (0.07)	(-0.411*)	0.91 (0.06)	(0.070)
WHtR	0.57 (0.06)	(-0.290)	0.64 (0.06)	(0.333*)
NC, cm	32.79 (1.95)	(0.130)	35.51 (2.96)	(0.491**)
C Index	1.34 (0.08)	(-0.331)	1.35 (0.09)	(0.314*)
AVI, L	16.01 (2.95)	(-0.217)	20.47 (4.06)	(0.486**)
BF%, %	34.77 (5.17)	(-0.059)	41.22 (5.29)	(0.160)
Leptin, ng/mL	12.34 (3.32)		39.02 (19.89)	

Note: Values expressed as Mean (SD), along with correlation coefficient (r) below the mean; * $P < 0.05$, ** $P < 0.001$.

Abbreviations: SD, Standard Deviation; BMI, body mass index; BSA, body surface area; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; NC, neck circumference; C Index, conicity index; AVI, abdominal volume index.

Table 4: Results of areas under ROC curves predicting optimal cut off points, sensitivity and specificity of anthropometric indexes of obesity as obtained by serum leptin levels.

Test Variable	Cutoff other studies	Cutoff this study	AUC $\pm$ SE (95 % CI)	Sen.	Spec.
Male Subjects					
BMI, kg/m ²	26.95 ¹⁸	25.23	0.811 $\pm$ 0.046 (0.720, 0.901)**	0.735	0.759
BSA, m ²	1.91 ²²	1.84	0.768 $\pm$ 0.039 (0.691, 0.844)**	0.691	0.688
WC, cm	94.5 ¹⁸ , 94.0 ⁵	96.75	0.847 $\pm$ 0.029 (0.791, 0.903)**	0.765	0.732
HC, cm	94.3 [†] , 94.0 ¹⁷	99.75	0.823 $\pm$ 0.034 (0.757, 0.890)**	0.765	0.750
WHR	0.95 ¹⁸ , 0.90 ³	0.97	0.672 $\pm$ 0.040 (0.593, 0.751)**	0.676	0.625
WHtR	0.55 ¹⁸ , 0.50 ²³	0.57	0.809 $\pm$ 0.032 (0.747, 0.872)**	0.765	0.732
NC, cm	37.0 ¹⁹	38.05	0.714 $\pm$ 0.038 (0.639, 0.789)**	0.676	0.571
C Index	1.40 ²⁴ , 1.25 ²⁵	1.37	0.713 $\pm$ 0.037 (0.639, 0.786)**	0.676	0.670
AVI, L	24.5 ⁹	18.81	0.846 $\pm$ 0.029 (0.790, 0.902)**	0.765	0.741
BF%, %	23.6 ²	26.52	0.811 $\pm$ 0.032 (0.748, 0.874)**	0.735	0.741
Female Subjects					
BMI, kg/m ²	29.19 ¹⁸	26.96	0.827 $\pm$ 0.034 (0.761, 0.893)**	0.744	0.771
BSA, m ²	1.71 ²²	1.65	0.806 $\pm$ 0.036 (0.736, 0.876)**	0.756	0.700
WC, cm	94.5 ¹⁸ , 80 ⁵	95.50	0.819 $\pm$ 0.034 (0.752, 0.886)**	0.692	0.686
HC, cm	95.6 [†] , 95.0 ¹⁷	105.5	0.779 $\pm$ 0.037 (0.606, 0.852)**	0.718	0.686
WHR	0.90 ¹⁸ , 0.85 ³	0.89	0.669 $\pm$ 0.045 (0.580, 0.758)**	0.641	0.657
WHtR	0.62 ¹⁸ , 0.50 ²³	0.61	0.814 $\pm$ 0.035 (0.745, 0.883)**	0.744	0.686
NC, cm	34.0 ¹⁹	33.95	0.805 $\pm$ 0.037 (0.733, 0.877)**	0.744	0.657
C Index	1.35 ²⁴ , 1.18 ²⁵	1.33	0.604 $\pm$ 0.047 (0.512, 0.696)*	0.641	0.600
AVI, L	24.5 ⁹	18.22	0.819 $\pm$ 0.034 (0.752, 0.886)**	0.718	0.686
BF%, %	36.8 ²	39.26	0.809 $\pm$ 0.035 (0.741, 0.878)*	0.718	0.714

Note: Figures in parenthesis are 95% confidence interval; *P<0.05, **P<0.001 for hypothesis test of whether area under the ROC curve is greater than 0.5; † mean value for HC in non-diabetic controls.

Abbreviations: Sen, sensitivity; Spec, specificity; AUC, area under concentration time curve; SE, standard error; CI, confidence interval; BMI, body mass index; BSA, body surface area; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; NC, neck circumference; C Index, conicity index; AVI, abdominal volume index.

STATISTICAL ANALYSIS

Statistical analyses were performed by using IBM SPSS Statistics (version 20.0). Descriptive statistics (means $\pm$ SD) were used to evaluate the characteristics of each participant. The data for continuous variables was examined for normality using Shapiro-Wilk W tests. Paired sample t-test was used to identify baseline differences in variables of either gender. Pearson's correlation coefficients were used to examine the relation between leptin and obesity indices. Gender specific, ROC curves were constructed in order to evaluate the accuracy of anthropometric indices as diagnostic tests to predict obesity, with serum leptin taken as the reference test. The ROC curve is a graph of sensitivity plotted against 1-specificity across a range of diagnostic test values, identifying the best threshold or cutoff for differentiating between positive and negative test results. Setting the cutoff value too low would give a highly sensitive result, without missing any disease but at the expense of including many normal tests in the diseased category. A high cutoff value would increase the specificity of the test but will be less sensitive. In this study, the optimal cutoff values were taken where the sensitivity and specificity

were maximum and not lower than 60%. The confidence interval was taken at 95%.

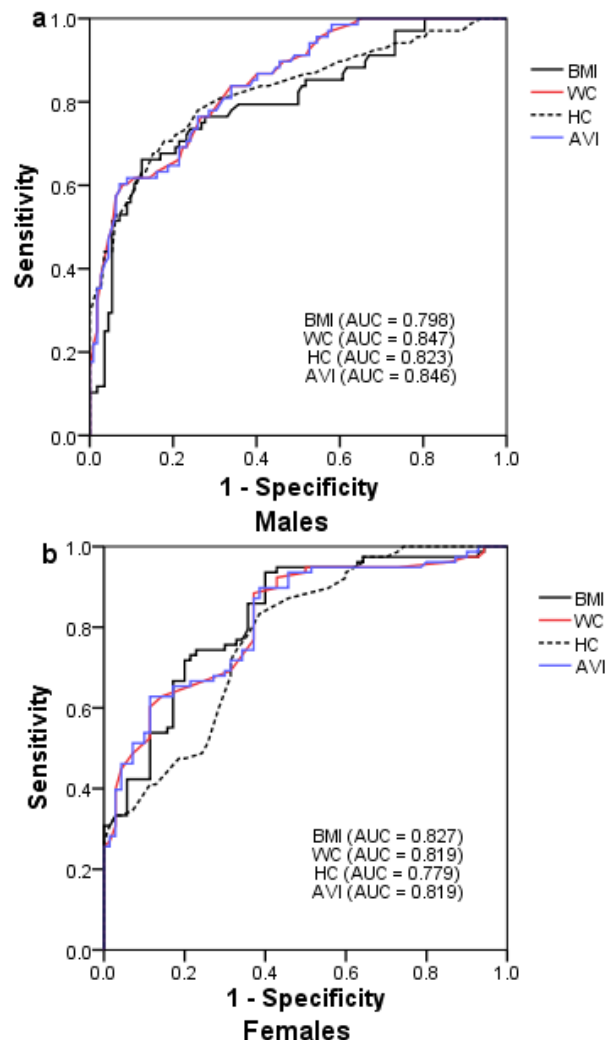
The area under the ROC curve (AUC) was used as a measure of the discriminative ability of anthropometric indices, to correctly classify those with and without obesity. It ranges from 1.0 to 0.0 with values closer to 1.0 indicating a good test able to discriminate between obese and non-obese. A value close to 0.5 indicates useless performance by the test as the result is not different from chance.

In the final stage of analysis, ROC curves were obtained again, however, this time using body fat percent instead of leptin as a reference variable against anthropometric indices (test variables). This was done to compare and validate the cutoff points obtained while using leptin.

RESULTS

Baseline descriptive statistics of data are presented in table 1. Shapiro-Wilk tests showed normal distribution of data ($p>0.05$) with W values ranging from 0.937 to 0.991. The total number of participants was 164, with 90

(54.9%) males and 74 (45.1%) females. Their ages ranged from 35 to 65 years with a median age of 51 years. The mean BMI (SD) was 25.03 (3.95) kg/m² in males and 27.54 (5.07) kg/m² in females. Fasting serum leptin concentration ranged between 0.6ng/mL and 107.6ng/mL. Mean serum leptin levels showed a significant difference ($p<0.001$) between males (9.08 ± 7.57 ng/mL) and females (29.65 ± 20.56 ng/mL), being more than three times higher in females. After stratification by BMI, leptin levels were substantially higher in obese than in non-obese individuals ($p<0.001$). The 75th percentile for leptin in non-obese subjects was 7.0ng/mL in males and 17.9 ng/mL in females.



(BMI, body mass index; WC, waist circumference; HC, hip circumference; AVI, abdominal volume index; ROC, receiver operating characteristics; AUC, area under the curve)

Fig. 1: Receiver Operating Characteristics (ROC) curves in (a) male and (b) female subjects for BMI, WC, HC and AVI for predicting the presence of obesity in Pakistani adults

Tables 2 and 3 show Pearson's correlation coefficient between leptin levels and anthropometric measures of

obesity in male and female subjects having normal or high serum leptins. For the male subjects, the correlation of leptin was strong in both low and high leptin groups ($p<0.001$) for most anthropometric measure. In females, however, leptin had significant positive correlation with anthropometric variables ($p<0.001$) in the high leptin group only. The low/normal leptin group in female did not demonstrate any significant correlation with leptin. Overall, BMI, HC, WHtR, WC and BF% displayed significant positive correlation with leptin in males ($p<0.001$) and BSA, HC, WC, NC and AVI in females ($p<0.001$). Especially prominent was HC which positively correlated with leptin in nearly all categories.

The relationship between studied indices and leptin was further evaluated and gender specific ROC curves were drawn to find out the optimal cutoff values of anthropometric indices for predicting obesity. Fig. 1 shows ROC curves for indices of general obesity (BMI, HC) and abdominal obesity (WC, AVI), for both sexes. In both male and female subjects, WC and AVI showed greatest AUC (>80%).

Table 4 shows the area under the ROC curve, sensitivity, specificity and optimal cutoff value for each anthropometric index of obesity. A comparison is also made with cutoff values obtained by other studies. Based on ROC curve analysis, lower cutoff levels were obtained for most anthropometric indices of obesity. The largest AUC in males was between WC and leptin (AUC =0.847; CI =0.791, 0.903) and in females, between BMI and leptin (AUC =0.827; CI =0.761, 0.893). For BMI the points of highest sensitivity and specificity were 25.23 kg/m² for males and 26.96 kg/m² for females. The cutoff observed for WC in males and females was 96.75cm and 95.50cm, respectively. The most accurate HC cutoffs for abnormal leptin were 99.75cm for males (sensitivity 0.765; specificity 0.750) and 105.5 cm for females (sensitivity 0.718; specificity 0.686). The cutoff values obtained by taking leptin as a reference test was compared with a second cutoff value taking BF% as reference test, while constructing ROC curves. We observed similar cutoffs with either method in both males and females.

DISCUSSION

Our findings suggest that serum leptin levels correlate strongly with anthropometric indices and can be used as an additional tool to identify and quantify obesity. The mean leptin levels were higher in females confirming many other studies. Saad *et al.* (1997), found women to have leptin levels 40% more than men at any level of obesity. Furthermore, the levels depended upon adiposity (42%), gender (28%) and insulinemia (2%). Throughout most of their lives, females have a higher percentage of body fat than males. The distribution of fat, however, differs with women having accumulation of fat around the

hip and thighs while men tending to accumulate fat around the abdomen increasing their waist. This was reflected by a higher WHR and NC seen in males than females ($p < 0.0001$). In contrast, females had significantly higher BMI, HC, WHtR and BF% ($p < 0.0001$).

Koester-Weber *et al.* (2014) computed the percentile distribution of leptin, cortisol, insulin and glucose in European adolescent population in order to define a reference range. The 75th percentile for males and females above 16 years was 8.99ng/mL and 39.56ng/mL, respectively. Paul *et al.* (2011), has reported a mean leptin level of 9.9ng/mL and 34.8ng/mL in overweight male and female Pakistani subjects, respectively. Mente *et al.* (2010), found serum leptin levels to be significantly higher in South Asian population (11.82ng/mL) compared to European (9.21ng/mL) and Chinese (8.25ng/mL). Gijón-Conde *et al.* (2015) carried out a study on 11540 Spanish subjects measuring leptin cutoff values in relation to cardio metabolic risk factors. The median levels observed were 7.2ng/mL in men and 24.5ng/mL in women, very similar to our study.

In our study, diabetic subjects had lower leptin levels, as seen in several other studies; however, in high leptin group, diabetic females had higher levels than non-diabetics. This may be due to the higher BF% in diabetic females of this group. Mohammadzadeh *et al.* (2012), found serum leptin levels in type 2 diabetes mellitus to be significantly lower than in non-diabetic subjects. In their study, the non-diabetics had greater body fat percentage that may have resulted in higher leptin values. Abdelqadir *et al.* (2002) demonstrated low leptin levels in Sudanese subjects with type 2 diabetes mellitus compared with controls in both males and females. They attributed low levels to differences in fat distribution. In a case-cohort study conducted on 570 incident diabetic cases and 530 non-cases, Schmidt *et al.* (2006) found high leptin levels to be predictors of increased risk of developing diabetes mellitus. However, after adjusting for obesity, hyperinsulinism, inflammation and metabolic syndrome, the high leptin levels seemed to protect against diabetes. Kumar *et al.* (2015) have, however, found increased leptin levels in type 2 diabetes mellitus patients as compared to controls without diabetes. This may have been largely due to the vast difference in BMI between cases and controls in his study (28.1 vs 22.8 kg/m²)

Body mass index is a common measure of obesity and has been used widely in epidemiological surveys. However, it does not truly represent the metabolic status and may misinterpret body fat mass (Shah and Braverman, 2012). Indices of central or abdominal obesity such as WC, WHR, WHtR, etc, relate more closely with the metabolic profile and cardiovascular diseases. Our results showed strong positive correlation of leptin with all anthropometric indices among diabetic and non-diabetic subjects. Indices for central obesity as measured by WC,

WHR, WHtR, NC, C index and AVI significantly predicted leptin levels in both male and female subjects. Hip circumference was the single most important variable that had significant correlation with leptin in all subjects especially females. Al-Daghri *et al.* (2007) has also found positive correlation between leptin and selective anthropometric measures of obesity, especially HC in non-diabetic Saudi subjects. However, this relationship was not maintained in diabetics, due probably to hyperinsulinemia or activation of sub clinical inflammation. The HC is a combination of muscles, fat and bones in the gluteal region and acts as a sink for free fatty acids in the blood, decreasing their concentration. Alone it has not received importance while measuring up for obesity and is a measure incorporated in WHR or WHtR. Hip circumference in our study was larger in non-diabetics reflecting a favorable metabolic profile as also observed by Seidell *et al.* (2001) and Conway *et al.* (2011).

Leptin is a reliable marker of adipose tissue status and correlates well with anthropometric indices. Different methods have been studied in the past to identify anthropometric cutoff points; however, to the best of our knowledge, this study is the first to use leptin as a reference test. Gender specific ROC curves were drawn to find out the optimal cutoff values of anthropometric indices for predicting obesity (table 4). Similar values were obtained when BF% was used as a reference test instead of leptin, to obtain ROC curves.

Suggested cutoffs for WC, WHR and WHtR for predicting cardiovascular disease in men and women by Hadaegh *et al.* in Iranian adults, were very similar to those observed by our study (Hadaegh *et al.*, 2009). The report of WHO Expert Consultation on Obesity (2000a), however, recommends 94cm for males and 80cm for females as increased risk for WC (WHO expert consultation, 2004). Almeida *et al.* evaluated the performance of WC, WHR, C-index and WHtR, all measures of abdominal obesity, for predicting coronary artery disease. Their cutoff points were lower than those of the present study, highlighting geographic, ethnic and racial differences (Almeida *et al.*, 2009).

Significant correlation has been found between NC and other conventional anthropometric markers of obesity like BMI and WC (Ben-Noun *et al.*, 2001; Ferretti *et al.*, 2015). We have previously shown NC to have potential value in indicating overweight and obesity in young Pakistani adults (Hingorjo *et al.*, 2012) and obtained similar cutoffs this time: Males 38.05cm, females 33.95 cm. The values in diabetics were higher than in non-diabetics, even after adjusting for BMI. Similar findings were observed by Aswathappa *et al.* (2013) who studied NC in relation to central obesity markers in both diabetics and non-diabetics.

Abdominal volume index measures the fat volume in the abdominal cavity between the waist and hip, and is strongly related to impaired glucose tolerance and diabetes mellitus. As yet it has not been used in clinical practice and its importance has recently been recognized as a model for abdominal obesity. We found strong positive correlation between leptin and AVI in both males and females. The cutoff values were lower, 18.0 liters as compared to some other studies, 24.5 liters (Guerrero-Romero and Rodríguez-Morán, 2003).

The BSA is measured with the help of various formulas and is utilized to calculate the cardiac index, a measure of cardiac output, as well as calculating the doses of pharmacologic agents (Verbraecken, 2006). Due to a smaller body frame seen in Pakistani individuals, the cutoff points obtained for BSA were lower than that seen in other studies (Sacco *et al.*, 2010). Conicity index did not correlate well with serum leptin levels and the cutoff values obtained were different from other studies (Garaulet *et al.*, 2000; Flora *et al.*, 2009).

Several points strengthen our study. First, use of a fractionalized portion of the ROC curve (for non-diabetics and diabetics) was a strength of the current analysis. All measurements were taken by a single person limiting variability of data. Some anthropometric indices studied in relation to leptin were either not studied before or very few studies were available. These include important measures of central obesity such as AVI, C index and WHtR. Furthermore, ROC curves obtained by using BF% as reference test gave cutoffs similar to those obtained by leptin verifying our results. However, we measured skinfold, a less accurate technique, to determine BF%.

Our study has some limitations. First, our sampling method was convenient and may not represent the whole population. Second, after stratification for BMI and diabetes, the sample size in some categories was small. Third, the whole study was focused upon obese and non-obese population, which represents an action level-II. Further studies need to be done to incorporate overweight in the measurement protocol so as to define action level-I.

CONCLUSION

Our findings suggest the significance of leptin when evaluating for anthropometric indices of obesity in both men and women. This relationship holds true for diabetic subjects as well. Furthermore, to accurately gauge obesity in terms of its metabolic consequence it is necessary to take into consideration the role of adipocytokines such as adiponectin, resistin, leptin and others. The cutoff points of anthropometric indices of obesity thus obtained for a given population may be utilized in future demographic surveys. With the addition of leptin in the family of factors modulating obesity, an ideal index can be suggested that best represents body fat mass, however, to

strengthen our findings, further studies may need to be done that include assessment of coronary artery disease risk factors as well.

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