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Identification of lipid accumulation product (LAP) as a marker of metabolic syndrome: a study in healthy population of MMU

Bhumija Sharma[1], Suvarna Prasad[2], Sunita Manhas[2], Bhawani Shankar Modi*[3], Priyanka Tangri[4]

[1]Department of Biochemistry, Farooq Hussain medical college and hospital, Agra 283201, Uttar Pradesh, India
[2]Department of Biochemistry, MM Institute of Medical Sciences, Ambala 133207, Haryana, India
[3]Department of Anatomy, Farooq Hussain medical college and hospital, Agra 283201, Uttar Pradesh, India
[4]Department of Biochemistry, Adesh institute of medical sciences, Shahbad 136135, Haryana, India

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ABSTRACT

Metabolic syndrome (MetS), is a collection of lipid and non-lipid cardiac related disease risk factors, is an important public health issue because of its higher prevalence with development of diabetes mellitus and cardiovascular diseases. Henry Kahn anticipated lipid accumulation product (LAP), as a novel marker of central lipid accumulation, to predict the risk of MetS. This study was conducted in Department of Biochemistry of MMIMSR, Ambala, Haryana. In the current study, we are researching over a new diagnostic tool for diagnosing MetS by LAP use. MetS is diagnosed by National Cholesterol Education Program Adults treatment panel III (NCEPATP III) and then compared with LAP to find its efficacy over other variables. 200 volunteers were enrolled in this study, their biochemical parameters were estimated and anthropometric measurements were taken. MetS was diagnosed in apparently healthy subjects. LAP was calculated by Henry Kahn formula and required statistical tools were applied to analyse results. The parameters of MetS positive cases were compared with LAP and its validity was tested statistically. 56 (28%) out of 200 subjects were positive for MetS. More females (30) than males (26) were diagnosed positive for MetS. LAP values were found to be higher in MetS cases, its efficacy was confirmed by ROC curves. MetS was diagnosed in volunteers who were considering themselves as healthy. Raised LAP and LAP2 were found, in MetS positive subjects than MetS negative. Relation of LAP and MetS came to be strong. Further, researches in this area are definitely recommended.

*Corresponding Author

Name: Bhawani Shankar Modi
Phone: 9896340163
Email: bhawanimodi.dr@gmail.com

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INTRODUCTION

The metabolic syndrome (MetS) is defined as a bundling of components that reflects the growing waist lines of the world (Cornier *et al.*, 2008). MetS, is a group of lipid and non-lipid factors revolving around risk of cardiovascular disease of metabolic origin. Mets is an important community health issue because of its high occurrence and relationship with development of diabetes mellitus (DM) and cardiovascular disease (CVD) (Sung *et al.*, 2009). MetS refers to a constellation of risk factors when occur-

ring together surges the risk for coronary artery disease (CAD), stroke, DM (Sharma *et al.*, 2013), metabolic abnormities including obesity, abnormal fat distribution (Tung *et al.*, 2011), dysglycemia, dyslipidemia and hypertension (Katulanda *et al.*, 2012).

Irrespective of the presence of any abnormalities of glucose metabolism, persons with MetS are at increased risk of DM. The presence of DM and MetS simultaneously potentiates the cardiovascular risk related with each of the two conditions. Characterizing MetS with the incidence of diabetes is therefore beneficial for the purpose of cardiovascular prevention (Kengne *et al.*, 2012). Clinically, exposure of MetS is also vital to catch a high-risk group of individuals who require intervention to prevent further complications. The most widely acceptable and used definitions are by the National Cholesterol Education Program (NCEP) on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults treatment panel III (ATP-III) proposed in 2001 and the International Diabetes Federation (IDF) proposed in 2005 (Mangat *et al.*, 2010).

Prevalence of MetS is in Pakistan and India was shown 34.8% and 25.3% respectively (Katulanda *et al.*, 2012). There is currently a debate surrounding the identity of MetS and its academic utility and diagnostic capacity. Along with the increase of visceral adipose cells, the serum concentrations of certain lipids would raise, during periods of lipid excess. This state is referred to as "lipid over accumulation" could tip to ectopic deposits of lipids in non-adipose tissues, where metabolic abnormalities and insulin resistance (IR) definitely would come into action. Henry Kahn projected the use of "lipid accumulation product" (LAP), as a novel guide of principal lipid accumulation, to calculate the risk of MetS. LAP is based on a blend of waist circumference (WC) and plasma TG levels. The LAP method was shown to predict DM and identify cardiovascular risk better than basal metabolic index (BMI) in previous studies (Kahn, 2006). Analysis done by areas under the curves (AUCs) for receiver-operating characteristic (ROC) curves showed that LAP was a better predictor of diabetes when compared to waist-to-hip ratio (WHR), WC and BMI, in both genders (Chiang and Koo, 2012).

LAP is based on a combination of WC and TG,

[LAP = {WC - 65) $\times$ TG (mmol/l)} for men and {(WC - 58) $\times$TG (mmol/l)} for women] (Kahn, 2006).

Recently, a strong relation between LAP and MetS was defined using the revised diagnostic standards of National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATP III), with area under ROC (0.91) in Argentinean males (apparently healthy), was reported (Taverna *et al.*, 2011). Cross-sectional analysis of the NHANES III cohort presented that higher LAP, was associated with LDL-C, apo B, uric acid levels, higher total cholesterol (TC)/HDL-C, apo B/apo A1 ratios and lower HDL-C levels when compared to BMI. In the present study, we are trying to stumble on a new diagnostic and economical tool for diagnosing MetS (NCEPATP III) by the help of LAP and moreover to find its efficacy over other variables.

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MATERIALS AND METHODS

Study design

Cross sectional and observational.

Target population and selection

The prevalence of MetS in India has been documented to be from 11% to 41% across vast country of India with numerous socio-cultural varieties (Kahn, 2006). Considering 15% prevalence 196 subjects were required so we took total 200 subjects who volunteered by random selection method.

Study area

This study was carried in Biochemistry department of Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala, Haryana. The study was conducted among the residents and staff members of MMU campus.

Study plan

200 total apparently healthy subjects were randomly selected, after taking consent. Out of them, 100 were females and 100 were males. A cross sectional study design was used for studying the predictive efficacy of LAP in diagnosing MetS (NCEPAT-PIII). Pregnant females, subjects on medicines or steroid therapy or any other hormonal therapy, any endocrine disorders (hypothyroid or hyperthyroid, Cushing syndrome) etc., were excluded.

All procedures completed in studies were in accordance with the ethical standards of the institutional research committee. Informed consent, both in English and vernacular language, was taken from the volunteers included in the study. Biochemical and other investigations were done to meet the criteria of NCEPATPIII for diagnosing MetS [fasting blood glucose, serum triglycerides, serum HDL]. LAP was calculated from data available. Gender wise data was collected and results were tabulated. Anthropometric measurements included WC, weight, height, hip circumference and BP. All measurements were taken with standard instruments and standard techniques. LAP was calculated by using formula (Kahn, 2006),

LAP = [WC (cm) – 65) x TG (m mol/l)] males

[(WC - 58) x TG] females

We additionally calculated LAP2 by modifying the original formula according to our findings. In the original formula, LAP= [WC-65] ×TG in males, [WC-58] ×TG in females. Where, 65 and 58 were lowest WC found in men and women respectively in that particular study. In our study, the minimum WC of 69 cm was found in men and 71 cm in women. This measurement was quiet similar in men but different in females, hence we calculated LAP2 and compared its values with original LAP and its usefulness in diagnosis of MetS.

Statistical analysis

Frequency distribution was presented in simple number and proportions (percentage). Descriptive statistics was calculated for the continuous data and to test the differences between means, independent t-test was used. ROC for various risk parameters was prepared. A 95% CI and p-value <0.05 have been reflected to be statistically significant. All the data was analyzed by using SPSS version 20 (IBM, Chicago, USA).

RESULTS AND OBSERVATIONS

In the present study overall BMI for 200 subjects was minimum was 16.15, maximum 51.6 with mean as 26.63±5.22 kg/m^2. Minimum, Maximum and mean of SBP was 110,130 and 117.050±6.93 mm Hg. Minimum, Maximum and mean of DBP was 60,90 and 78.10±7.11 mm Hg. Table 1 is depicting overall descriptive of total volunteers. All the parameters with minimum, maximum and mean with std deviation is shown.

Table 2 is depicting gender variation significant difference of values are found (0.013) in FSG, (0.000) in TG, (0.000) in TG/HDL, (0.000) in WHR, (0.000) in height, (0.000) in weight, and (0.002) in LAP2. These significant values are positive outcomes of our study. LAP was insignificant between males and females.

Table 3 is depicting values and significance between MetS present cases and MetS absent ones. Many significant values have come into this finding. First Age was found significant (p= 0.032). Except HDL and BMI all parameters are significantly different in his table. LAP and LAP 2 both came up to the expectations of this research with significant differences.

Table 4 is depicting AUC for parameters again LAP and LAP2 has achieved its position being better marker than rest with auc (0.867, 0.822) respectively.

Table 5 is depicting Diagnosis of MetS by NCEPATP III criteria.

DISCUSSION

The MetS is a multiplex risk factor for CVD. The syndrome develops through interplay of obesity and metabolic susceptibility (Grundy, 2007). Primary and timely identification of high risk persons for MetS could be important to forecast and prevent CVD and DM. NCEP and other organisations have proposed that MetS can be recognised clinically by a clustering of simple clinical measures including WC, BP, TG, HDL and glucose. According to NCEP ATPIII (2001) at least three of the following criteria should be met (Song and Hardisty, 2008).

In many initial studies, LAP method proved its efficacy to predict diabetes and recognize cardiovascular risk better than BMI (Chiang and Koo, 2012).

Mean of LAP and LAP2 in MetS positive subjects was found to be on higher side than MetS negative subjects. The large difference could be easily seen in Figure 1a, b this gives the evidence of LAP/LAP2 being an important marker for identifying MetS. Similar results were found in the study by Ejike, independent samples t-tests presented that mean LAP (p<0.001) and mean WHtR (p=0.022) dimensions were significant and higher in the MetS group, relative to the group without the syndrome (Ejike, 2011).

Descriptive statistics on total 200 subjects is given in Table 1. Observations are as follows,

1. Minimum age 18 and maximum age 73, with mean age as 37.675±14.14 years.
2. Minimum value of FSG was 52.43mg/dl; maximum was 147.81 mg/dl with mean 77.49±13.32 mg/dl.

Table 1: Total descriptive statistics

	N	Minimum	Maximum	Mean ± SD
Age (years)	200	18.00	73.00	37.675 ± 14.143
FBS (mg/dl)	200	52.43	147.81	77.498 ± 13.320
HDL (mg/dl)	200	23.51	67.62	38.746 ± 8.325
TG (mg/dl)	200	27.65	467.90	143.468 ± 77.832
TG/HDL	200	0.68	16.28	3.870 ± 2.347
WC (cm)	200	69.00	140.00	94.482 ± 12.858
WHR	200	.69	1.20	0.9171 ± 0.090
LAP (cm mmol/l)	200	3.50	396.50	54.618 ± 44.451
LAP2 (cm mmol/l)	200	0.00	375.40	41.763 ± 39.460
Height (m)	200	1.24	1.92	1.63 ± 0.096
Weight (kg)	200	40.00	120.00	70.765 ± 13.124
BMI (kg/m2)	200	16.15	51.60	26.633 ± 5.220
Valid N (listwise)	200	-	-	-

FBS= fasting blood sugar, TG= triglycerides, WC= waist circumference, WHR= waist to hip ratio, LAP= lipid accumulation product, LAP2= modified Lipid accumulation product, BMI= basal metabolic index, HDL= high density lipoprotein

Table 2: Gender wise descriptive statistics

	Gender	N	Minimum	Maximum	Mean ± SD	P-value
FBS (mg/dl)	m	100	55.70	147.81	79.71 ± 13.82	0.013*
	f	100	52.43	125	75.17± 12.44	
HDL (mg/dl)	m	100	24.06	67.62	37.89 ± 8.26	0.168
	f	100	23.51	27.7	39.55± 8.35	
TG (mg/dl)	m	100	35.5	467.9	163.49±74.53	0.000*
	f	100	27.7	388.5	123.29 ± 76.16	
TG/HDL	m	100	1.34	12.06	4.47 ± 2.23	0.000*
	f	100	0.68	16.28	3.26 ± 2.32	
WC (cm)	m	100	69	140	95.21±13.64	0.322
	f	100	71	122	93.58±12.02	
WHR	m	100	0.75	1.2	0.94 ±0.09	0.000*
	f	100	0.69	1.16	0.88 ±0.09	
Height (m)	m	100	1.50	1.92	1.69 ± 0.09	0.000*
	f	100	1.24	1.73	1.58 ± 0.07	
Weight (kg)	m	100	45	120	74.26 ±14.5	0.000*
	f	100	40	92	67.10±10.44	
BMI (Kg/m2)	m	100	18.25	51.60	26.16 ±5.56	0.309
	f	100	16.15	41.36	27.00 ±4.86	
LAP (cm mmol/l)	m	100	3.5	396.5	57.46±46.86	0.301
	f	100	6.8	214.33	51.36±41.88	
LAP2 (cm mmol/l)	m	100	0	375.47	50.08±44.36	0.002*
	f	100	0	157.47	33.04±31.76	

FBS = fasting blood sugar, TG=triglycerides, WC= waist circumference, WHR= waist to hip ratio, LAP= lipid accumulation product, LAP2= modified Lipid accumulation product, BMI= basalmetabolic index, HDL= high density lipoprotein, m= male, f=female, *Significantp< 0.005

Table 3: Statistics of Metabolic Syndrome present vs absent

	Met syndrome	N	Mean ± SD	p-value
Age (years)	Present	56	41.11 ± 13.40	0.032*
	Absent	144	36.34 ± 14.24	
FBS (mg/dl)	Present	56	83.08 ± 14.28	0.000*
	Absent	144	75.33 ± 12.31	
HDL (mg/dl)	Present	56	38.37 ± 8.43	0.690
	Absent	144	38.89 ± 8.31	
TG (mg/dl)	Present	56	212.04 ±78.29	0.000*
	Absent	144	116.80 ±59.29	
TG / HDL	Present	56	5.76 ± 2.58	0.000*
	Absent	144	3.14 ± 1.78	
WC (cm)	Present	56	99.91 ± 13.10	0.000*
	Absent	144	92.37 ± 12.17	
WHR	Present	56	0.95 ± 0.08	0.003*
	Absent	144	0.91 ± 0.09	
LAP (cm mmol/l)	Present	56	95.44 ± 59.78	0.000*
	Absent	144	38.74 ± 21.66	
LAP2 (cm mmol/l)	Present	56	74.28 ± 54.97	0.000*
	Absent	144	29.12 ± 20.72	
Height (m)	Present	56	1.64 ± 0.085	0.008*
	Absent	144	1.63 ± 0.099	
Weight (kg)	Present	56	74.45 ± 12.18	0.013*
	Absent	144	69.33 ± 13.24	
BMI (kg/m2)	Present	56	27.74 ± 4.34	0.060
	Absent	144	26.20±5.48	

FBS = fasting blood sugar, TG=triglycerides, WC= waist circumference, WHR= waist to hip ratio, LAP= lipid accumulation product, LAP2= modified Lipid accumulation product, BMI= basal metabolic index, HDL= high density lipoprotein, METS = metabolic syndrome,Independent t-test used, *p-value < 0.05 have been considered to be statistically significant

Table 4: Observed AUC under ROC for Mets parameters at Asymptotic 95% confidence interval (CI)

Parameter	AUC	Lower bound	Upper bound
FSG	0.668	0.582	0.754
HDL	0.483	0.396	0.570
TG/HDL	0.847	0.791	0.902
WC	0.680	0.595	0.764
WHR	0.650	0.5 6 8	0.7 3 1
BMI	0.632	0.549	0.715
LAP	0.867	0.813	0.922
LAP2	0.822	0.753	0.892

FBS = fasting blood sugar, TG=triglycerides, WC= waist circumference, WHR= waist to hip ratio, LAP= lipid accumulation product, LAP2= modified Lipid accumulation product, BMI= basal metabolic index, HDL= high density lipoprotein, AUC = area under curve , ROC =Receiver Operating Characteristic

Table 5: Diagnosis of MetS by NCEPATP III criteria (any 3 out of 5)

Parameter	Reference Values
Central obesity: waist circumference	≥102cm/40 inch ♂ ≥88cm/36 inch ♀
Dyslipidemia: Triglycerides	≥1.695m mol/L(150mg/dl)
Dyslipidemia: HDL-C	<40mg/dl♂<50 mg/dl♀
Blood pressure	≥130/85 mm Hg
Fasting serum glucose	≥6.1mmol/l (110mg/dl)

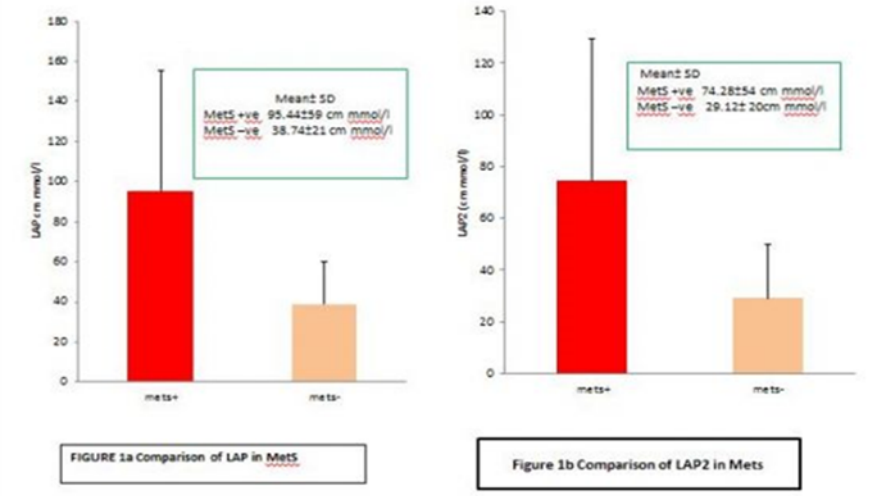

Figure 1: Comparison of Lap & Lap2 value in MetS positive and negative cases

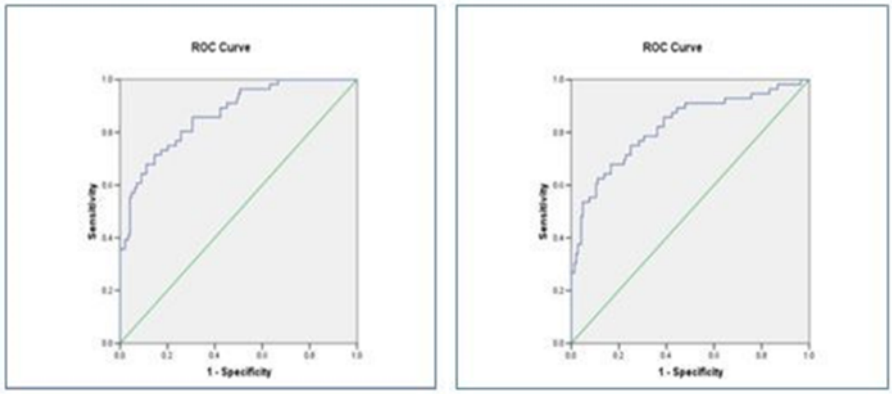

Figure 2a. ROC curve (LAP) Area: 0.867

Figure 2b. ROC curve (LAP2) Area: 0.822

Figure 2: Diagnostic efficacy of Lap & Lap2 in terms of area under curve (AUC)

3. Minimum, maximum and mean values of fasting TG are 27.65, 467.9 and 143.468±77.83 mg/dl respectively.
4. Minimum, maximum and mean of TG/HDL ratio was 0.68, 16.28 and 3.87±2.34.
5. Minimum, maximum and mean of WC was 69,140 and 94.48±12.85 cm.
6. Minimum, maximum and mean of WHR was 0.69, 1.2and 0.917±0.090.
7. Minimum, maximum and mean value of LAP came out to be as 3.5, 396.5 and 54.61± 44.45cm mmol/l.
8. Minimum, maximum and mean value of LAP 2 came out to be as 0, 375.4 and 41.76±39.46 cm mmol/l.
9. Minimum, maximum and mean of height was 1.24, 1.92 and 1.63± 0.096 m.
10. Minimum, maximum and mean of weight was 40,120 and 70.76±13.12 in kg.

LAP in our study was higher than found in the study of Taverna et al. (35.65±28.74) but LAP2 was closer to it. This could be probably due to the difference in WC of our collected data (Taverna *et al.*, 2011).

Data in Table 2 reveals gender statistics. Minimum, maximum, mean and p values of all the risk factors are specified between both the sexes. We found the significant values (0.013) in FSG, (0.000) in TG, (0.000) in TG/HDL, (0.000) in WHR, (0.000) in height, (0.000) in weight, and (0.002) in LAP2. All of the above p values are statistically very significant among males and females. Among the parameter of lipid metabolism, most of the mean values were higher in males. Contrary to our findings, Saxena et al. found higher values in female respondants. Liu *et al.* (2011) also did comparison between males and females, and they found significant p values for height (<0.001), weight (<0.001), WC (0.024), DBP (0.039) and FSG (0.040) (Liu *et al.*, 2011). In their study, most of the lipid parameters were more in males similar to our findings.

The data in Table 3 reveals that mean age in MetS present subjects is 41.11±13.4 years. Among 200 subjects, 56 (28%) subjects are found to be positive for MetS. Out of total Mets positive subjects, 26 (46.42%) are males and 30 (53.5%) are females. In our study, we found that females are more prone towards MetS. Similarly, Delavari et al. reported that by every standard definitions, the prevalence of the MetS was more in women. The prevalence of the MetS in one significant study of Iran was reported to be 33.7%, with a higher prevalence tending in female(42%) than in males (24%) (17). Contrary to this, Kawamoto et al. found 14.2% male and 8.3% females positive for MetS in their study Kawamoto *et al.* (2011). Table 4 is self-explanatory with higher achievements of LAP and LAP2.

Comparison of LAP, LAP2 as test variable

The area under ROC curve quantifies the total ability of the test to differentiate between various parameters to be used as diagnostic tool for MetS. By comparing the AUC of all test variables, we observe that AUC under ROC for FSG, HDL, WC, WHR, BMI are < than AUC under ROC for LAP and LAP2. AUC under ROC for TG/HDL lies in between the AUC under ROC of LAP and LAP2. Thus, LAP and LAP2 are more accurate than other variables. It concludes that LAP, LAP2 are better tools for identifying risk of MetS. ROC analysis was done to compare the skill to predict the occurrence of MetS. AUC ranges from 0 to 1, with 0.5 representing no predictive power and 1 representing perfect power. The superior the predictive ability, the more away from the diagonal line is the curve which is under examination. ROC curves of various risk factors are drawn to cover the possibility of detecting MetS as depicted in Table 4 and Figure 2a, b. The maximum possible value of AUC for an ideal test is 1.00 and true AUC cannot be less than 0.5.

1. HDL curve AUC is less than 0.5 hence it is not very efficient tool for diagnosing MetS.
2. TG/HDL can be used for identifying the person at risk of developing MetS because AUC for test =0.847 is close to unity.
3. LAP can be used for identifying the person at risk of developing MetS because AUC for test=0.867 is close to unity. It indicates the accuracy of the test.

The aim of this research paper is to evaluate LAP efficiency in diagnosing for MetS. From the construction of ROC curve for test variable LAP, we observe that value of AUC=0.867 . It is clear that LAP has maximum AUC followed by TG/HDL and LAP2, hence proving the aim of this study- "efficacy of diagnosing Mets". In the study by Kawamoto et al., various potential markers were compared by their AUC under ROC for IR. In that study, AUC for HDL=0.28 in men, TG/HDL=0.82 in men, HDL=0.25, TG/HDL=0.84 in women; these were similar to those in our study (Kawamoto *et al.*, 2011). In another study by Ejike (2011), the ROC analyses showed that LAP (AUC = 0.937) and WHtR (AUC = 0.905) predicted the MetS, better than the other indices. In a study by Chiang and Koo (2012), LAP showed the highest prediction accuracy among adiposity measures with an AUC of 0.901. LAP showed the highest diagnostic accuracy for MS-NCEP/ATP III (AUC 0.91 and 0.90 among males and females) in the study by Taverna *et al.* (2011). In a study by Yuan-Lung Cheng et al., males were strongly found associated with Mets, and the LAP and Fatty liver index (FLI) were better when compared to other variables to diagnose Mets among the 29,797 individuals. Both indexes were also found to be better than other variables to diagnose Mets in persons without fatty liver disease (AUROC: 0.871 and 0.879, resp.) and the predictive power was higher among women (Cheng *et al.*, 2017).

The diagnosis of MetS needs five factors –one anthropometric and four clinical. The measurement of blood pressure via auscultation method bring observer bias, and could possibly expose the individual and practitioner to mercury (if broken) and is moreover problematic to use in large epidemiological studies. Measurements of anthropometric indices could also include some observers prej-

udice. The measurement of circulating blood profiles for glucose, TG and HDL-C are all time consuming and costly implications. Therefore, the outcome that LAP delivers is sensitive and smart screening method for MetS. LAP requires only the measuring of circulating serum TG and the measurement of WC. Utilisation of LAP method (the superior marker of the MetS, based on the data presented in many researches) would be more economic to diagnose MetS considerably. According to a study of Ray. L, LAP index is a better predictor of MetS when compared to WC and BMI and should be merged in laboratory reports as timely, accurate, and budget friendly marker of metabolic syndrome (Ray *et al.*, 2018). The LAP method comes out as a superior predictor of the MetS, not just because of its specificity and analysis, but because it captures both indices of anthropometric and metabolic scopes of visceral fat over-accumulation. This suggests that no matter whether excess lipids are stored as visceral fat or as TG, it is arrested by LAP. Rationally speaking, LAP will tend to increase as more fats are deposited in non-adipose "ectopic" organs like liver, arteries, pancreas or kidneys, where they may undesirably affect tissue function and interfere with systems of cardiovascular regulation and/or when they are stored as TG with its attendant cardio-metabolic associations (Ejike, 2011).

CONCLUSIONS

To conclude, 56 Persons (28%) out of total subjects were positive for MetS, which is remarkable achievement both for researcher and subject as well. More females (30) than males (26) were positive for MetS. High values of LAP and LAP2 were found, in MetS positive subjects when compared with MetS negative which was statistically proven. The minimum and maximum value of LAP was (26.7, 396.5 cm mmol/l) and LAP2 was (17 and 375.4 cm mmol/l) in males MetS+ve. In females of MetS+ve the minimum and maximum value of LAP was (24.15, 214.33 cm mmol/l) and LAP2 was (2.52 and 157.4 cm mmol/l) respectively. LAP and LAP2 are predicted to be highly efficient markers of MetS.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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