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Leptin Level in Patients with Type 2 Diabetes as a Risk Factor for Cardiovascular Disease

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ABSTRACT

Leptin Level Background: Investigating adipocytokine leptin (Lep) levels in type 2 diabetes mellitus (T2DM) and their association with 6 anthropometry, lipid profile parameters could lead to understanding the role of Lep in T2DM and related risk of cardiovascular complications; Moreover, could further help prevention and management of these complications. **Aim:** The current study's objectives are to examine the association between blood levels of adipocytokine leptin (Lep) and the risk of metabolic syndrome and cardiovascular disease in Sudanese patients with T2DM. **Materials and method:** During the period of April 2012 and March 2013, a case-control study was conducted in Central Sudan. The study involved 300 participants who met the inclusion criteria and were divided equally into diabetes, diabetic hypertension (HTN), and non-diabetic non hypertensive (NDNH) groups to estimate FPG, HbA1C, lipid profile levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG) and, Lep. A questionnaire was completed, which included personal, clinical and anthropometric data beside biochemical measurements. Each respondent gave verbal consent and venous blood was drawn after an overnight fast. The statistical analysis was done with the help of statistical software for social sciences (SPSS version 16, Chicago, IL, USA). **Results:** Women represented 74% and men were 26%. Diabetic hypertensive group include the oldest participant who's their age ranged from 40-65 years and had the highest body weight 80.28kg, WC 104.14cm, BMI 31.65, SBP 128.10 mmHg and DBP 81.40mmHg. Diabetic and diabetic hypertensive had significant ($p<0.0001$) high mean level of FPG (215.33±9.93 mg/dl, and 164.63±6.65 mg/dl respectively). HbA1C mean concentration (8.32±0.29%) was significantly high in diabetic by ($p=0.012$) compared with diabetic hypertensive groups. Lep increased significantly between the three groups by ($p<0.0001$) and the diabetic hypertensive group had the highest mean level (1.79±0.11 ng/mL). Lep/BMI ratio increased significantly between the three groups by ($p<0.0001$); diabetics had the highest mean level (0.06±0.004). HDL-C decreased significantly between the three groups, by ($p=0.035$). SBP and DBP increasesd significantly between the three groups by ($p<0.0001$

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and p=0.008) respectively. Protinuria showed positive result in 70.1% of diabetic hypertensive. **Conclusion:** The study's main finding was that Sudanese patients with T2DM had lower mean Leptin adipocytokine concentrations than expected, despite considerable increases in waist circumference and body mass index. Women demonstrated greater mean concentration levels than men. The mean concentration of Lep in diabetes and diabetic hypertensive groups was significantly increased as compared to the NDNH group, and the mean levels of Lep were greater in the diabetic group than the diabetic hypertensive group. In addition to increased levels of LDL-C and TG, diabetic hypertensive patients demonstrated a significant reduction in HDL-C levels. Women's HDL-C showed significant decrease in lipid profile concentration, indicating a tendency for that group to develop dyslipidemia.

Keywords: Leptin, Obesity indexes, Lipid profile, Type 2 diabetes mellitus, Sudan

INTRODUCTION

Adipose tissue is a complex network of endocrine organs that has been divided into white adipose tissue WAT and brown adipose tissue BAT (Hahn and Novak, 1975) [1]. They release numerous regulatory molecules collectively known as adipocytokines such as Lep and adiponectin (Champe and Harvey, 2005) [2]. Their as function as endocrine cells and participate in autocrine and paracrine regulation within adipose tissue and can affect the functions of distant organs, such as muscle, the pancreas, the liver, and the central nervous system (Kronenberg et al, 2008) [3]. Moreover, adipocytokines modulate hemostasis, blood pressure, lipid and glucose metabolism, inflammation, and atherosclerosis (Fantuzzi, 2005; Rabe et al, 2008) [4,5].

Leptin means (leptos='thin') in Greek. It was discovered in mice in 1994 by Jeffrey M. Friedman (Williams, et al., 2009) [6]. It is a polypeptide hormone composed of 167 amino acids, of 16 kDa, encoded by the obese gene (Zhang, et al., 1994) [7] and expressed in white adipose tissue (Bluher and Mantzoros, 2009) [8]. Synthesis and secretion is by gastric chief cells in the stomach (Bado, et al., 1998) [9], but a higher amount is secreted by subcutaneous adipocytes than by the visceral adipocytes and it detected in many tissues including the placenta, mammary glands, breast milk, testes, ovaries, endometrium, stomach, hypothalamus, and pituitary gland (Van Harmelen, et al., 1998) [10], brain (Wiesner, et al., 1999) [11], bone (Morroni, et al., 2004) [12], macrophages (Lee, et al., 2013) [13], thyroid (Fan and Li, 2015) [14] and even the dental pulp (Martin-Gonzalez, et al., 2013) [15]. Functions of Lep include the regulation of energy balance (De Vos, et al., 1995) [16], reproduction, immunity (Caldefie-Chezet, et al., 2003) [17], and also as a pro-inflammatory factor (Lago, et al., 2007) [18]. Moreover, Lep is involved in glucose and lipid metabolism, angiogenesis, blood pressure regulation, bone mass formation (Housa, et al., 2006) [19]. The circulating Lep reflect the degree of adiposity and its release from adipocytes signals to the brain to trigger the suppression of food intake and to boost energy expenditure, thus Lep is serving as an "adipostat" (Rosen and Spiegelman, 2006) [20]. Circulating Lep levels are positively correlated with fat mass (Mantzoros, et al., 1997) [21] or BMI (Ruhl and Everhart, 2001) [22]. These levels range from 5 to 10 ng/ml in healthy individuals and from 40-100 ng/ml in obese individuals (Howard, et al., 2010) [23]. In pathological state which includes inflammation, malignant transformation, low birth weight and premature delivery have been linked to lower Lep levels (Mantzoros, et al., 1997) [21]. Likewise prolonged fasting decreases Lep levels, whereas over feeding greatly increases its levels (Kolaczynski, et al., 1996) [24]. A study has shown lower circulating Lep levels in Sudanese diabetics than in control subjects of similar age and BMI (Abdelgadir et al, 2002) [25].

Insulin can modulate adipocytokines production and interacts with Lep and Adiponectin. It is a positive regulator of Lep and increases its gene expression to suppress appetite whereas Adiponectin acts as an insulin sensitizing agent (Yamauchi, et al., 2001) [26]. On other hand, adipocytokines are thought to affect insulin action in other tissues and having a role on obesity-induced insulin resistance (Fantuzzi, 2005; Antuna-Puente, et al., 2008; Rabe, et al., 2008; Hansen, et al., 2010) [4,5,27,28]. Hansen, et al., 2010 suggested that obesity, HTN, dyslipidemia, and metabolic syndrome in T2DM were associated with increased plasma Lep levels.

The missing link between obesity and cardiovascular disease is adipocytokines profile. The aberrant production of adipocytokines, due to disruption of homeostasis of body weight that lead to inflammation and dysfunction of adipose tissue (Chrysant and Chrysant, 2013) [29].The prothrombotic effect of Lep appears in concentration of 50 ng/mL, this promotes adenosine diphosphate ADP-induced aggregation of human platelets via phosphoralation of tyrosine residue lead to thrombotic effect of Lep (Nakata, et al., 1999) [30] and change in BMI, blood pressure, total cholesterol, triglyceride, and inflammatory markers in addition to coronary heart disease (Sattar, et al., 2009) [31] and metabolic syndrome in

T2DM (Hansen, et al., 2010) [28], because Lep in the serum can influence the body fat distribution of patients with T2MD (Liu X, et al, 2019) [32]. Thus Lep is considered as independent risk factor for cardiovascular disease (Wallace, et al., 2001) [33] and hemorrhagic stroke (Soderberg, et al., 2007) [34]. High serum Lep concentrations was observed in patient with, coronary heart disease (Sattar, et al., 2009) [31] T2DM (Fruehwald-Schultes, et al., 1999) [35] and in patients of renal dysfunction (Heimburger, et al., 1997) [36], microalbuminuria or macroalbuminuria (Fruehwald-Schultes, et al., 1999), obesity (Widjaja, et al., 1997) [37] and were in risk for developing ESRD (Ritz, 1999) [38]. In addition to massively obese patient (Maffei, et al., 1995) [39] tend to develop glomerulosclerosis (Kasiske and Crosson, 1986) [40]. Recent study reveal that Lep resistance is a pathogenic factor generating obesity and related comorbidities, and leptin neural and cellular signaling are important in the control of metabolic balance (Liu, et al. 2022) [41].

Diabetes is one of the common chronic diseases in the Sudan with a prevalence of 447,000 in 2000, and this prevalence is projected to increase in 2030 to reach 1,227,000 (WHO, 2011) [42]. The prevalence of T2DM in the Sudanese population is 3.4%, and T2DM accounts for 75% of all diagnosed cases in northern parts of Sudan in 1996 (Elbagir, et al., 1998) [43]. In a survey carried out in Sudan in 2016, 954 participants with a mean age of 39.5 16.7 years and a range of 18-90 years reported having DM overall at a prevalence of 19.1% (182/954) and IGT at a prevalence of 9.5% (91/954). In the diabetic group, 125 (68.7%) were already aware of their condition, whereas 57 (31.3%) had been diagnosed only recently (Elmadhoun WM, et al. 2016) [44]. This brings with it the potential for a catastrophic increase in the prevalence of kidney and cardiovascular disease (Krum and Gilbert, 2003) [45]. No enough studies were performed in adipocytokines levels in diabetic Sudanease patients. This study was performed in 2012 in Central Sudan to measure the levels of serum adipocytokine Lep in diabetic T2DM participants and measure the relative risk between diabetes mellitus and incidence of diabetes complication particularly cardiovascular and renal disease.

SUBJECTS, MATERIALS AND METHODS

Study design, area and subjects: This study was a cross-sectional case-control study carried out at Abu A'gla health center for diabetic care- Central Sudan, from April 2012-March 2013. Three hundred participants of both sexes were included in the study. Among them 100 were diagnosed with T2DM (diabetic group), 100 were diagnosed with T2DM and later on become affected with HTN (diabetic hypertensive group), and the rest 100 participants were apparently healthy non-diabetic and non-hypertensive group NDNH (control group). The participants were from rural and urban areas around Wad Madani who get health services from Abu A'gla health care center. Participants who were included in this study were in the age range between 22 and 65 years with no current infection. The Participants included in the study if they have T2DM with or without HTN, and without diabetes complications. If a patient had hypertension prior to receiving a diabetes diagnosis or was having any of the complications of diabetes linked to micro- or macro vascular disease, they were excluded from the study.

Ethical approval: An ethical approval for the study was obtained from the Ethics Committee, Faculty of Medicine, University of Gezira and ministry of health. The study objectives and procedure were explained to each participant, with verbal consent.

Data collection: Data of this study was obtained by a structured questionnaire for each participant to obtain medical, personal, and family history information in addition to laboratory analysis of blood and urine sample.

Collection and preparation of Blood samples: Five ml of venous blood were drawn from each participant after an overnight fasting by standard aseptic procedure, and were divided into three parts: 1ml of blood was put in EDTA container for HbA1C measurement; 1ml was put in fluoride container for measurement of plasma glucose; and 3 ml were put in lithium heparin container, then plasma was separated after centrifugation and used for biochemical measurements lipid profile, and Lep. Plasma samples were analyzed for different biochemical parameters, using A15 (a random access analyzer) and ELIZA for Lep measurement using the method of human Lep ELISA kit. Urine samples were analyzed using urine strips (dipsticks).

Statistical analysis: Statistical analysis was carried-out using statistical package for social sciences (SPSS version 16, Chicago, IL, USA). All the numerical data were expressed as mean ± Standard Error of Mean. Differences in means of continuous variables between the study groups were compared using Analysis of variance (ANOVA). To compare differences between the study groups, multiple comparisons (post hoc tests such as Tukey HSD, Gabriel test, and Games Howell) were performed. Body mass index (BMI) was calculated applying to the formula: BMI = (weight in kg)/ (height in m)2 (Ng M, 2014) [46]. P-values were considered

significant at 0.05 or lower (p≤0.05).

Diagnosis of DM and hyperglycemia include measuring of plasma/blood glucose, from either fasting or random sample, using oral glucose tolerance test (OGTT), glucose in urine, and ketones in blood and urine (Alberti and Zimmet, 1998) [47] and also glycosylated haemoglobin HbA1C (WHO, 2011) [42]. The diagnostic criteria recommended by the National Diabetes Data Group NDDG or WHO include: Symptoms of diabetes plus random plasma glucose concentration >200 mg/dl (11.1 mmol/1); FPG ≥126 mg/dl (7.0 mmol/1) and 2HFBG ≥200 mg/dl during an OGTT (NDDG., 1979; Farooq, et al., 2008) [48,49].

Metabolic syndrome criteria by presence of three or more of the following metabolic abnormalities: abdominal obesity WC >102 cm in men and WC >88 cm in women, hypertriglyceridemia, TG ≥150 mg/dL, low HDL-C levels, HDL-C <40 mg/dL in men and <50 mg/dL in women, raised blood pressure (SBP ≥130 mmHg, DBP ≥85 mmHg), and raised FPG ≥110 mg/dL (Matthews, et al., 1985) [50].

Reference Ranges: BMI and WC: cut-off points and their association with disease risk to underweight <18.50, normal=18.50-24.99, overweight≥25.00, Obese class I 30.00-34.99, Obese class II 35.00-39.99, and Obese class III ≥40.00. WC: of men >102 cm and women >88 cm (NHLBI Obesity Education Initiative, 2000). FPG: <110mg/dl (Michael, 2002) [51]. HbA1C: excellent control less than 6.5%, good control 6.5%-7.5%, moderate control 7.5% - 8.9% and poor control greater than 9.0% (Grossman, 2011) [52]. TC < 200 mg/dl, LDL-C <130 mg/dl, HDL-C > 59 mg/dl and TG < 150 mg/dl (Expert Panel on Detection and Treatment of High Blood Cholesterol in, 2001) [53]. SBP >140 mmHg and diastolic DBP 90 mmHg (Anderssen, et al., 1995) [54]. Serum Lep: in adult: 0.3μg/L-8μg/L (Delbert, 2007) [55].

RESULTS

General Characteristic of the Study Population

This study included 300 participants divided equally into three groups (diabetic, diabetic hypertensive, and non-diabetic non-hypertensive NDNH).

Women were 74% and men 26% of the study group. The age range from 22-65 years. Diabetic hypertensive group include the oldest participant who's their age ranged from 40-65 years and had the highest body weight 80.28kg, WC 104.14cm, BMI 31.65, SBP 128.10 mmHg and DBP 81.40mmHg. Duration of DM was 7.18years and HTN was 5.78years. Diabetic group had an age range from 22-62 years and their mean body weight was 79.95kg, WC 98.69cm, BMI 30.36, SBP 118.60 mmHg and DBP 76.70 mmHg. Duration of DM was 4.75 years. The non-diabetic non hypertensive had age range from 24-65 years, WC 72.51 and BMI 27.54, Table 1.

Table 1: Anthropometric and biochemical measurements of the study groups.

Variable	Diabetic (n=100)	Diabetic-hypertensive(n=100)	NDNH(n=100)
Gender/men, women	26 /74	21/79	31/69
Age/years	49.67±0.71	56.17±0.72	46.74±0.78
Weight /kg	79.95±1.69	80.28±1.42	72.51±1.38
WC/cm	98.69±1.15	104.14±1.10	98.15±1.07
BMI Kg/m^2	30.36±0.58	31.65±0.59	27.54±0.55
SBP/mmHg	118.60±0.80	128.10±1.43	114.30±1.32
DBP/mmHg	76.70±0.71	81.40±0.93	82.00±1.95
Duration of DM/years	4.75±0.41	7.18±0.62	-
Duration of HTN/years	-	5.78±0.57	-
FPG/mg/dL	215.33±9.93	164.63±6.65	89.77±2.40
HA_{1C} %	8.32±0.29	7.42±0.21	-
TC/mg/dL	195.05±4.10	189.78±4.41	197.51±4.49
LDL-C/mg/dL	104.15±2.84	109.03±2.95	107.36±3.10
HDL-C/mg/dL	53.06±1.60	49.73±1.42	55.51±1.69
TG /mg/dL	172.60±8.58	160.48±7.36	146.76±7.75
Lep/ug/L	1.16±0.07	1.79±0.11	0.80±0.14

Data are expressed as mean±SEM, SEM= standard error of the mean, NDNH=non diabetic non hypertensive, DM=diabetes mellitus, HTN=hypertension, BMI=body mass index, WC=waist circumference, SBP=systolic blood pressure, DBP=diastolic blood pressure, FPG=fasting plasma glucose, HbA1C=glycated haemoglobin, TC=total cholesterol, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, TG=tri-glycerides, Lep=Leptin, Cm=centimeter, Kg=kilogram, mg=milligram, dL=deciliter, ug=microgram, mmHg=millimeter of mercury.

Comparison of mean of anthropometric and biochemical parameter in gender group

In women group:

In women group, comparison revealed statistically significant increase in the anthropometric measurements (weight, WC and BMI, SBP and DBP), and biochemical measurements (FPG, HbA1C and Lep). HDL-C was significantly decreased by. Statistically non-significant increase in TC, TG, and LDL-C. These results give marker for cardiovascular risk in women, Table 2.

Table 2: Comparison of mean of anthropometric and biochemical parameter in women groups.

Group	Women Subgroup (n=222)			p-value
Variable	Diabetic(n=74)	Diabetic-hypertensive (n=79)	NDNH (n=69)	
Age/years	48.92±0.79	55.44±0.81	46.48±0.86	<0.0001
WC/cm	99.03±1.33	103.99±1.19	100.65±1.26	0.017
BMI/Kg/m^2	30.78±0.62	32.22±0.65	29.09±0.68	0.004
SBP/mmHg	118.65±0.90	126.46±1.45	114.64±1.63	<0.0001
DBP/mmHg	77.03±0.83	81.14±1.10	82.90±2.48	0.029
Duration of DM/years	5.08±0.51	6.37±0.68	-	0.137
Duration of HTN/years	-	6.01±0.68	-	-
HbA_{1C} %	8.38±0.31	7.3506±0.22	-	0.007
TC/mg/dL	197.42±4.95	192.32±4.69	200.33±5.86	0.535
LDL-C/mg/dL	105.45±3.51	111.05±3.36	107.62±4.10	0.537
HDL-C/mg/dL	52.68±1.85	51.13±1.58	57.97±2.12	0.027
TG /mg/dL	173.62±10.35	162.96±8.58	149.19±9.44	0.203
Lep /ug/L	1.22±0.08	1.76±0.12	0.86±0.20	<0.0001

BMI=body mass index, WC=waist circumference, NDNH=non diabetic non hypertensive, DM=diabetes mellitus, HTN=hypertension, SBP=systolic blood pressure, DBP=diastolic blood pressure, FPG=fasting plasma glucose, HbA1C=glycated hemoglobin, TC=total cholesterol, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, TG=tri-glycerides, Lep=Leptin, Cm=centimeter, Kg=kilogram, mg=milligram, dL=deciliter, mmHg=millimeter of mercury , ug=microgram.

In men group:

In men group, comparison of means revealed statistically significant increase in the anthropometric measurements (weight, WC and BMI), and marginal significance increased in the biochemical measurements LDL-C. Men group showed well control for HbA1C and non-significant decrease in HDL-C level which indicate low risk of cardiovascular disease, Table 3.

Table 3: Comparison of mean of anthropometric and biochemical parameter in men groups.

Group	Men Subgroup (n=78)			p-value
Variable	Diabetic (n=26)	Diabetic-hypertensive (n=21)	NDNH (n=27)	
Age/years	51.81±1.51	58.90±1.46	47.32±1.64	<0.0001
WC/cm	97.73±2.33	104.71± 2.71	92.58 ±1.64	0.001
BMI/Kg/m^2	29.19±1.37	29.53±1.32	24.08±0.53	<0.0001
SBP/mmHg	118.46±1.73	134.29±3.88	113.55±2.25	<0.0001
DBP/mmHg	75.77±1.38	82.38±1.68	80.00±3.08	0.172
Duration of DM/years	3.82±0.57	10.24±1.38	-	<0.0001
Duration of HTN/years	-	±6.671.09	-	-
HbA_{1c}%	8.12±0.67	7.67±0.55	-	0.615
TC/mg/dL	188.31±7.09	180.24±11.38	191.23±6.25	0.626
LDL-C/mg/dL	100.46±4.50	101.43±5.93	106.77±4.17	0.575
HDL-C/mg/dL	54.15±3.25	44.48±3.00	50.03±2.48	0.089
TG/mg/dL	169.81±15.33	150.70±13.55	141.23±13.71	0.340
Lep/ug/L	0.99±0.12	1.91±0.24	0.68±0.12	<0.0001

BMI=body mass index, WC=waist circumference, NDNH=non diabetic non hypertensive,DM=diabetes mellitus, HTN=hypertension, SBP=systolic blood pressure, DBP=diastolic blood pressure, FPG=fasting plasma glucose, HbA1C=glycated hemoglobin, TC=total cholesterol, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, TG=tri-glycerides, Lep=Leptin, Cm=centimeter, Kg=kilogram, mg=milligram, dL=deciliter, mmHg=millimeter of mercury , ug=microgram.

Comparison of mean of biochemical parameter in the study groups using (ANOVA) test:

Levels of FPG, HA1C of study groups and their relation to hyperglycemia:

FPG increased significantly between the three groups by (p<0.0001); NDNH group had normal mean of FPG (89.77±2.40mg/dl), but diabetic and diabetic hypertensive had high mean level (215.33±9.93 mg/dl, and 164.63±6.65 mg/dl respectively). HbA1C increased significantly between the diabetic and diabetic hypertensive groups by (p=0.012); the diabetic group had a mean of 8.32±0.29%, whereas the diabetic hypertensive group had a mean level of 7.42±0.21%. These results indicated hyperglycemia and decrease control of FPG and HbA1C in diabetic and diabetic hypertensive group, Table 4.

Table 4: Levels of FPG, HA1C of study groups and their relation to hyperglycemia.

Variable	Diabetic (n=100)	Diabetic-hypertensive (n=100)	NDNH (n=100)	p-value
FPG (mg/dL)	215.33±9.93	164.63±6.65	89.77±2.40	<0.0001
HA_{1c} (%)	8.32±0.29	7.42±0.21	-	0.012

NDNH=non diabetic non hypertensive, FPG=fasting plasma glucose, HA1C=glycated haemoglobin, mg=milligram, dL=deciliter

Comparison of means of WC, BMI, and Lep/BMI ratio in the study groups:

WC increased significantly among the three groups by (p<0.0001); Diabetic and NDNH groups had less mean values (98.69±1.15cm and 98.15±1.07cm respectively) than the diabetic hypertensive group (104.14±1.10cm). BMI increased significantly between the three groups by (p<0.0001). NDNH group had slightly increased in BMI mean (27.54±0.55 Kg/m2), but diabetic and diabetic hypertensive participants were obviously obese with increased mean BMI (30.36±0.58 Kg/m2 and 31.65±0.59 Kg/m2 respectively). Lep differed significantly between the three groups by (p<0.0001); diabetic hypertensive had the highest mean level (1.79±0.11 ng/mL), the diabetic group had a mean level of 1.16±0.07 ng/mL, and the NDNH group showed the lowest mean level (0.80±0.14 ng/mL). Lep/BMI ratio increased significantly between the three groups by (p<0.0001); diabetics had the highest mean level (0.06±0.004); diabetic hypertensive showed the lowest mean level (0.03±0.004), and NDNH group had a mean value of 0.04±0.002. These results were point to direct correlation between WC and BMI with Lep concentration, Table 5.

Table 5: Comparison of means of WC, BMI, Lep and Lep/BMI ratio in the study groups.

Variable	Diabetic (n=100)	Diabetic-hypertensive (n=100)	NDNH (n=100)	p-value
WC/Cm	98.69±1.15	104.14±1.10	98.15±1.07	<0.0001
BMI/Kg/m²	30.36±0.58	31.65±0.59	27.54±0.55	<0.0001
Lep/BMI ratio	0.04±0.002	0.06±0.004	0.03±0.004	<0.0001
Lep/ug/L	1.16±0.07	1.79±0.11	0.80±0.14	<0.0001

NDNH=non diabetic non hypertensive Lep= Leptin; WC=waist circumference, BMI=body mass index Cm=centimeter, Kg=kilogram, m=meter

Levels of lipid profile, SBP and DBP and their relation to cardiovascular disease:

HDL-C decreased significantly between the three groups, by (p=0.035); NDNH group had the highest mean HDL-C (55.51±1.69 mg/dl), diabetic had a mean of 53.06±1.60 mg/dl and diabetic hypertensive had the lowest mean level (49.73±1.42 mg/dl). TG increased with a marginal significance between the three groups by (p=0.071); NDNH group had TG mean of 146.76±7.75 mg/dl, diabetic and diabetic hypertensive had higher mean levels (172.60±8.58 mg/dl, 160.48±7.36 mg/dl) respectively. SBP increased significantly between the three groups by (p<0.0001); diabetic hypertensive group had higher mean SBP (128.10±1.43 mmHg), but NDNH and diabetic group had the lower mean level (114.30±1.32mmHg and 118.60±0.80mmHg, respectively). DBP increased significantly between the three groups, by (p=0.008); NDNH group had higher mean DBP (82.00±1.95mmHg), but diabetic hypertensive and diabetic group had the lower mean level (81.40±0.93mmHg and 76.70±0.71mmHg, respectively). These finding designate increased tendency to improve cardiovascular disease, Table 6.

Table 6: Levels of lipid profile, SBP and DBP and their relation to cardiovascular risk.

Variable	Diabetic (n=100)	Diabetic-hypertensive (n=100)	NDNH (n=100)	p-value
TC/mg/dL	195.05±4.10	189.78±4.41	197.51±4.49	0.437
LDL-C/mg/dL	104.15±2.84	109.03±2.95	107.36±3.10	0.498
HDL-C/mg/dL	53.06±1.60	49.73±1.42	55.51±1.69	0.035
TG/mg/dL	172.60±8.58	160.48±7.36	146.76±7.75	0.071
SBP/mmHg	118.60±0.80	128.10±1.43	114.30±1.32	<0.0001
DBP/mmHg	76.70±0.71	81.40±0.93	82.00±1.95	0.008

NDNH=non diabetic non hypertensive, TC=total cholesterol, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, TG=tri-glycerides, mg=milligram, dL=decilite SBP=systolic blood pressure, DBP=diastolic blood pressure, mmHg=millimeter of mercury

Frequency of diabetic and diabetic hypertensive participants with proteinuria:

Protinuria showed positive result in 70.1% of diabetic hypertensive and 29.9% in diabetic participant where 62.0% of diabetic and 38.0% in diabetic hypertensive had negative result with strong statistically significant p<0.0001. These results are presented in, Table 7.

Table 7: Frequency of diabetic and diabetic hypertensive participants with proteinuria

Proteinuria	Diabetic	Diabetic Hypertensive	Total	p- value
Negative	75 (62.0%)	46 (38.0%)	121	<0.0001
Positive	23 (29.9%)	54 (70.1%)	77	

Post hoc analysis of the study group:

The results of ANOVA didn't indicate which of the three groups differ from the other groups, so multiple comparison analysis was performed using post hoc tests which included; Tukey HSD, Gabriel test, and Games Howell tests. The Tukey post hoc test indicated that mean WC increased significantly in the diabetic hypertensive group from diabetic group (p=0.002), and NDNH group (p<0.0001). For mean BMI, the NDNH group increased significantly from the diabetic group (p=0.002) and the diabetic hypertensive group (p<0.0001). Mean HDL-C decreased significantly between the NDNH and diabetic-hypertensive groups (p=0.027). Mean LDL-C level didn't differ between the three groups. Gabriel post hoc test revealed that mean TG increased between the diabetic and NDNH groups with a marginal significance (p=0.064). Games Howell post hoc test indicated that SBP increased significantly between the NDNH and diabetic group (p=0.017), and between diabetic hypertensive and each of NDNH and diabetic groups (all with p<0.0001). DBP increased significantly between the NDNH and diabetic group (p=0.023), and between diabetic hypertensive and diabetic groups (p<0.0001). Mean FPG increased significantly between the NDNH group and each of diabetic hypertensive and diabetic group (all with p<0.0001), Table 8.

Table 8: Post hoc analysis of the study group.

Group	Diabetic (n=100)			Diabetic hypertensive (n=100)			Diabetic hypertensive (n=100)		
Compared with	NDNH			NDNH			diabetic		
Variable	**Mean Diff**	**SE**	**p-value**	**Mean Diff**	**SE**	**p-value**	**Mean Diff**	**SE**	**p-value**
WC/cm†	0.54	1.57	0.936	5.60	1.57	<0.0001	5.45	1.57	0.002
BMI/Kg/m²†	2.83	0.812	0.002	4.11	0.81	<0.0001	1.29	0.81	0.254
SBP/mmHg§	4.300	1.546	0.017	13.800	1.949	<0.0001	9.500	1.644	<0.0001
DBP/mmHg§	-5.300	2.080	0.032	-0.600	2.165	0.959	4.700	1.173	<0.0001
FPG/mg/dl§	125.56	10.22	<0.0001	74.86	7.07	<0.0001	-50.70	11.96	<0.0001
TC/mg/dl†	-2.46	6.13	0.915	-7.73	6.13	0.418	-5.27	6.13	0.666
LDL-C/mg/dl†	-3.21	4.19	0.725	1.67	4.19	0.916	4.88	4.19	0.476
HDL-C/mg/dl†	-2.45	2.23	0.515	-5.78	2.22	0.027	-3.33	2.23	0.294
TG/mg/dl†	25.84	11.20	0.057	13.73	11.15	0.523	-12.1	11.18	0.625
Lep/ug/L§	0.36	0.15	0.053	0.99	0.18	<0.0001	0.62	0.13	<0.0001

†, Tukey HSD; ‡, Gabriel test; §, Games Howell. BMI=body mass index, WC=waist circumference, Diff=difference, NDNH=non diabetic non hypertensive, FPG=fasting plasma glucose, TC=total cholesterol, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, TG=tri-glycerides, Lep=Leptin, Cm=centimeter, Kg=kilogram, mg=milligram, mmHg= millimeter of mercury, dL=deciliter, meq=milliequivelent, ug=microgram.

Measurement of relative risk using odds ratio (OR)

The relative risks of diabetes in cardiovascular in case of increase Lep level:

In current study, researchers evaluate the relative risk of diabetes complication that will occurs among study participants in case of increase Lep level with reference to NDNH group. High Lep level in diabetes is a risk factor for high levels of LDL-C, TG, and BMI/WC ratio in addition to lower levels of TC, HDL-C and Lep/BMI ratio. Diabetes with HTN is a risk factor for high concentration of LDL-C, TG, and BMI/WC ratio; in addition to low level of TC, HDL-C, Lep/BMI ratio, Table 9.

Table 9: The relative risks of diabetes in cardiovascular in case of increase Lep level.

Variable median cut-off points	Group	Variable groups		OR/CI (95%)	p-value
		Low	High		
TC (mg/dl) **≤192** **>192**	NDNH Diabetic Diabetic-hypertensive	13(52.0%) 25(54.3%) 36(52.9%)	12(48.0%) 21(45.7%) 32(47.1%)	**Ref** 0.910(0.343-2.415) 0.963(0.385-2.4110)	 0.850 0.936
LDL-C (mg/dl) **≤104** **>104**	NDNH Diabetic Diabetic-hypertensive	15(60.0%) 23(50.0%) 37(54.4%)	10(40.0%) 23(50.0%) 31(45.6%)	**Ref** 1.500(0.559-4.025) 1.257(0.495-3.191)	 0.421 0.631
HDL-C (mg/dl) **≤52** **>52**	NDNH Diabetic Diabetic-hypertensive	12(48.0%) 25(54.3%) 36(52.9%)	13(52.0%) 21(45.7%) 32(47.1%)	Ref 0.775(0.292-2.057) 0.821(0.328-2.054)	 0.609 0.673
TG (mg/dl) **≤146** **>146**	NDNH Diabetic Diabetic-hypertensive	16(64.0%) 17(38.6%) 31(46.3%)	9(36.0%) 27(61.4%) 36(53.7%)	**Ref** 2.824(1.021-7.810) 2.065(0.801-5.324)	 0.046 0.134
Lep/BMI ratio ≤0.04 >0.04	NDNH Diabetic Diabetic-hypertensive	1(4.8%) 5(13.5%) 4(6.9%)	20(95.2%) 32(86.5%) 54(93.1%)	**Ref** 0.320(0.035-2.942) 0.675(0.071-6.408)	 0.314 0.732
BMI/WC ratio ≤0.29 >0.29	NDNH Diabetic Diabetic-hypertensive	17(73.9%) 18(43.9%) 27(46.6%)	6(26.1%) 23(56.1%) 31(53.4%)	**Ref** 3.620(1.185-11.058) 3.253(1.122-9.429)	 0.024 0.030

NDNH=non diabetic non hypertensive, HbA1C=glycated hemoglobin, TC=total cholesterol, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, TG=tri-glycerides, Lep=Leptin, mg=milligram, dL=deciliter, ug=microgram. Ref=reference; OR= odds ratio; CI= confident interval

DISCUSSION

Analysed data reveal associations between different parameters in the study groups. Family history of diabetes and HTN was observed in 71.67% and 69% of the study population and had large frequency in obese diabetic hypertensive participants with strong statistical significance indicates that DM and HTN were a genetic disorder (Lillioja, et al., 1993; Mannino, et al., 2001) [56,57].

The onset of diabetes and HTN are sex dependent and is more common in female than male, this find was in line with previously conducted studies of (Sowers, 1998; Aso, et al., 2000) [58,59], which indicates increased risk of developing T2DM in women more than men. Beside that in both sexes diabetic hypertensive participants had the highest WC, BMI, high concentrations of Lep, LDL-C, and low concentration of HDL-C. They showed increased mean of Lep/BMI ratio and also they characterized by presence of Protinuria. These findings were in agreement with that of (Danquah, et al., 2012) [60] which found that T2DM is socioeconomic disease affect mainly obese persons and is associated with increased risk of HTN and hyperlipidaemia (Ljungman, et al., 1996) [61]. Furthermore, diabetic hypertensive participants were found to be older compared with diabetic in agreement with that of (Harris, et al., 1995) [62] study which demonstrated

that the incidence of HTN in T2DM patients is increased from the age of 45-75 years by 40%-60% (Stein and Colditz, 2004) [63]. The increased concentration of Lep in diabetic hypertensive than in diabetic may by of long duration of DM and increase in age and the preasence of HTN may indicates tendency of those participants to improve diabetic complication in the presence of other independent factors which included family history of HTN and DM and those factors exacerbated by increases in BMI and WC since increased in Lep concentration is associated with obesity and insulin resistance (Antuna-Puente, et al., 2008) [64]. A case control study which was conducted in Sudan showed that circulating Lep levels were lower in diabetic subjects (men and women) than in controls of similar age and BMI and had higher mean concentration in females than in males and was significantly correlated to BMI. However, there was no difference in BMI mean between patients and control subject (Abdelgadir, et al., 2002) [25].

Data was analysed using median cut-off points to find the relative risk of diabetes in case of increased Lep level in study patient using the odds ratios. The results indicate a significant increase in Lep concentrations by 1.982 folds in diabetic and 4.760 in diabetic hypertensive compared to NDNH group and increased in BMI/WC ratio by 3.620 folds in diabetic and 3.253 times in diabetic hypertensive, indicating that the increased in WC, BMI and long duration of diabetes in diabetic hypertensive participants may be a cause of increased concentration of Lep in diabetic hypertensive and diabetic groups. These findings were in agreement with that of (Matsubara, et al, 2002) [65]. Proteinuria which is a good indicator for micro and macrovascular disease had a positive result in all BMI subgroups, which may increase as result of increase in FPG increase (Ljungman, et al., 1996) [62]. These findings confirm that BMI and age were related to the presence of obesity related disorders (WHO., 2007) [66]. The most important finding was that the incidence of diabetes and HTN in current study are coexist, because the time of onset of diabetes in diabetic hypertensive participants was from 1 month up to 38 years and HTN onset was from 2 months up to 40 years. These findings were in line with the study of (Sowers and Epstein, 1995) [67].

Lipid profile which includes TC, LDL-C, HDL-C and TG showed marginal increase in the mean concentration of TG and significant decrease in HDL-C, but TC and LDL-C showed slight increases in their means in diabetic and diabetic hypertensive groups. Diabetic hypertensive showed the lowest mean concentration of HDL-C concentration in the study and diabetic group showed the highest mean of TG. These results were in line with that of (Muna, 1993) [68] which indicated that T2DM patients have metabolic abnormalities of both quality and quantity of lipoprotein. The non-significant alteration in lipid profile concentrations may explain the effectiveness of the HTN medication in the treatment of HTN, heart failure, and other cardiovascular problems, our results were in line with that of (Otamere, et al., 2011) [69], which was conducted in Nigeria and revealed that subjects under management showed no change in lipid profile concentrations. The concentration of lipid profile in current study is altered as Lep concentration increased by decreasing in HDL-C and increasing in TC, TG and LDL-C concentrations; this may lead to changes in other metabolite concentration. These results explain that Lep level is increased in low insulin states, these finding were in agreements with that of (MacDougald, et al., 1995) [70].

As in the study group women showed significant decreased in HDL-C concentration between diabetic hypertensive and NDNH participants. The little change in other lipid profile concentration was not significant. Lipid profile of men showed no significant differences between groups except for HDL-C which has marginal significant decrease compared to women; this difference may be due to variation of study population in life style and social habits. These finding were in disagreement with that of (Shahid, et al., 2005) [71] which suggested that male patients are at higher risk of diabetic complications than female patients. Our study was in agreement with that of (Oyewole, et al., 2008) [72] and (Onmwuliri and Puppet, 2004) [73] in that sex plays no important role in the pattern of lipid profile in response to DM. In contrast to our current results, a case-control study which was conducted in Sudan for determinations of lipid profile disorder indicated that nearly half of 250 diabetic patients had some disorder in their lipid profile; male diabetic patients having higher levels than female patients. The study also conforms to our results in that there was lower mean of HDL-C concentration in men than women compared with NDNH (Elnasri and Ahmed, 2008) [74].

CONCLUSIONS

- The study's main finding was that Sudanese patients with T2DM had lower mean Leptin adipocytokine concentrations than expected, despite considerable increases in waist circumference and body mass index. Women demonstrated greater mean concentration levels than men.

- The mean concentration of Lep in diabetes and diabetic hypertensive groups was significantly greater than in the NDNH group, and the mean levels of Lep were higher in the diabetic group than the diabetic hypertensive group.
- Participants' lipid profiles revealed no statistically significant differences in TC and LDL-C between the diabetic, diabetic hypertensive, and non-diabetic non-hypertensive groups, but diabetic hypertensive participants did exhibit significantly lower HDL-C concentrations in addition to higher means of LDL-C and TG. Women's HDL-C showed significant lipid profile changes, pointing to those individuals' tendency to develop dyslipidemia.

RECOMMENDATIONS

To prevent aggressive consequences of DM, diabetic and diabetic hypertensive patients should routinely monitor their blood sugar, hemoglobin A1C, and lipid profile. They should also take their medications as prescribed.

LIMITATIONS

The unavailability of chemical leptin reagent in Sudan is one challenge for this work, because importing material doubles the cost, which is reflected in the sample size.

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