Assessment of Apelin and Risk Factors in Relation with Diabetic Mellitus Type 2

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Abstract— This study was conducted on randomly selected 68 type 2 diabetic patients (27 Males and 41 Females) attending the diabetes mellitus center in Al-Sadder Teaching City in Al-Najaf province, Iraq and a group of 20 apparently healthy subjects (10 Males and 10 Females) were included as a control group. The study was carried out from February 2013 to July 2013. The age of patients and control groups were range of 35-65y. The concentration of fasting blood glucose, cholesterol, triglyceride, LDL, VLDL, HDL, Apelin and BMI were estimated in patients and control groups. The results show significant increase (P<0.05) in fasting blood glucose, cholesterol, triglyceride, LDL, VLDL and Apelin levels in patients compared with control groups. The results revealed that Apelin not significant difference (p>0.05) in patients and control groups at different ages. The results also revealed that Apelin level increase significantly (P<0.05) in males than females in both patients and control groups. The results also revealed that significant increase (P<0.05) in BMI in patients compared with control groups. The results also show that Apelin concentration increase significantly (P<0.05) with increasing BMI in males than females compared with control groups. The results have been shown significant positive correlation (P<0.05) between Apelin, FBG, cholesterol, triglyceride, LDL and VLDL in patients (males and females), while the results have been shown significant negative correlation (P<0.05) between Apelin and HDL in patients (males and females). The present study concluded that Apelin level was a marker for detection and diagnosis of diabetic patients type 2.

Index Terms— Apelin, Diabetic Mellitus Type 2, Al-Najaf province and Lipid profile.

1 Introduction

Diabetes Mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (Gadsby, 2002). There are mainly three types of diabetes; Type 1 diabetes is immune-mediated and requires daily administration of insulin. The other common type is type 2 diabetes and characterized by insulin resistance or relative insulin deficiency [1,2]. The gestational diabetes occurs in pregnant women who have never suffered from diabetes, but gestational hyperglycemia, which might be develop after the pregnancy into type 2 diabetes mellitus. Other more rarely types of this disease such as neonatal diabetes, congenital diabetes, cystic fibrosis-related diabetes and steroid diabetes[3].

Type 2 diabetes is the most common form and comprises of 90% of people with diabetes around the world. The prevalence of type 2 diabetes rates continue to increase with increasing number of patients at risk of serious diabetes-related complications. Having type 2 diabetes increase the risk of a myocardial infarction two times and the risk of suffering a stroke two to four times. It is also a leading cause of blindness, limb amputation and kidney failure [4,5].

Apelin, an endogenous ligand for the G-protein-coupled APJ receptor, has been recently studied in obesity research. It is not only expressed in adipocyte tissue, but also widely expressed in various other organs such as the heart, lung, kidney, gastrointestinal tract, brain, adrenal glands, endothelium and human plasma. Studies have shown the association between apelin and obesity. Apelin has higher circulating levels in obesity. Insulin exerts a positive action on adipocyte apelin production [6].

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2 Materials and Methods

The study was conducted on randomly selected 68 type 2 diabetic patients (27 Males and 41Females) and a group of 20 apparently control subjects (10 Males and 10 Females) were included as a healthy group.

Diabetes Mellitus was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the name, sex, age, weight, height. Patients with renal dysfunction, heart diseases, who were on drugs affect oxidative stress, i.e: antioxidants, antihyperlipidemic agents were excluded from the current investigation.

Five milliliters of venous blood samples were drown using a disposable needle and plastic syringes from each patients and controls subject. Blood was left at room temperature for 10 minutes for clotting, centrifuged 6000 rpm for 10 minutes, and then serum was separated and transported into new disposable tubes.

Apelin (EIA-APC-1) ELISA Kit for quantitative determination of apelin in human serum was supplied by RayBiotech, Inc. Apelin C-Terminus ELISA Kit is an in vitro quantitative assay for detecting apelin C-Terminus peptide based on the principle of Competitive Enzyme Immunoassay.

3 RESULTS

3.1 Fasting blood glucose and serum Lipid profile level

The results of table (1) indicate a significant increase (P<0.05) in fasting blood glucose (FBG) level in diabetic patients (274.29 ± 59.20 mg/dI) in comparing with control group (102.05 ± 9.66 mg/dI). Also the results show that there is a significant increase (P<0.05) in serum Cholesterol, Triglycerides, LDL-C and VLDL-C level in diabetic patients (5.46 ± 0.12, 3.06 ± 0.06, 3.17 ± 0.04 and 1.37 ± 0.11 mmol/L) respectively comparing with control group (4.12 ± 0.06, 1.65 ± 0.11, 2.36 ± 0.06 and 0.79 ± 0.07 mmol/L) respectively, and a significant decrease (P<0.05) in HDL-C level in diabetic patients (0.86 ± 0.08 mmol/L) in comparing with control group (1.28 ± 0.14 mmol/L).

TABLE 1
SERUM LEVEL OF FBG AND LIPID PROFILE COMPONENTS IN PATIENTS AND CONTROL GROUPS

Groups / Parameters	Mean ± S.D	
	Control	Patients
	n = 20	n = 68
FBG (mg/dI)	102.05 ± 9.66	274.29 ± 59.20 *
Cholesterol (mmol/L)	4.12 ± 0.06	5.46 ± 0.12 *
Triglyceride (mmol/L)	1.65 ± 0.11	3.06 ± 0.06 *
LDL-C (mmol/L)	2.36 ± 0.06	3.17 ± 0.04 *
VLDL-C (mmol/L)	0.79 ± 0.07	1.37 ± 0.11 *
HDL-C (mmol/L)	1.28 ± 0.14	0.86 ± 0.08 *

* means significant difference at (P<0.05).

3.2 Aplin Levels

The results in figure (2) show a significant increase (P<0.05) in apelin level in diabetic patients (314.14 ± 50.73 ng/ml) in comparing with control group (149.74 ± 24.46 ng/ml).

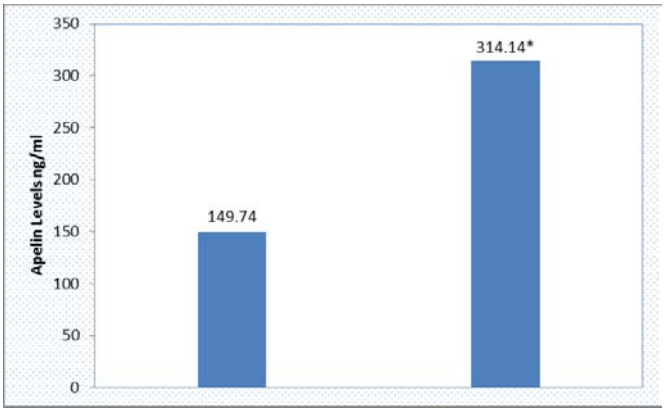

Fig. 1. Apelin level in diabetic patients and control groups
* means significant difference at (P<0.05)

3.3 Comparison Apelin level between diabetes and control groups according to age

The results of table (2) show a significant increase (p<0.05) in serum apelin level in patients at different ages, while serum apelin level is highly significant increase (p<0.05) in patients at different ages in comparing with control groups. Also the results show that there is no significant difference (p>0.05) in serum apelin in patients at different ages.

TABLE 2
APELIN LEVEL OF PATIENTS AND CONTROL GROUPS IN DIFFERENT AGE

Age	Mean ± S.D	
	Visfatin	
	Control	Patients
	n = 20	n = 68
35 -45	151.90 ± 32.12 A	314.58 ± 84.63 B
46 – 55	143.3 ±24.76 A	322.03 ± 77.04 B
56 – 63	140.32 ± 10.48 A	339.60 ± 68.70B

a,b means significant difference at (P<0.05), between patients and control groups

3.4 Comparison Apelin level between diabetes and control groups according to gender

The results of table (3) reveal a significant increase (p<0.05) in serum apelin level in males than females in both patients and control groups, while serum apelin level are highly significant increase (p<0.05) in both males and females in patients in comparing with control groups.

TABLE 3
BIOMARKERS LEVEL IN BOTH GENDER OF PATIENTS AND CONTROL GROUPS

Marker	Mean ± S.D			
	Control		Patients	
	n = 20		n = 68	
	Male	Female	Male	Female
	n =10	n =10	n =27	n =41
Visfatin ng/ml	192.30 ± 9.18*	157.17 ± 39.75	324.78 ± 27.64*#	307.12 ±73.82#

* means significant difference at (P<0.05), different gender at the same group
# means significant difference at (P<0.05), at the same gender in different group

3.5 Comparison Apelin level between diabetes and control groups according to BMI

The figures (3),(4),(5) show a significant increase (P<0.05) in apelin level in all groups normal weight, over weight and obese weight in comparing with control groups. Also the same figures show a significant increase (P<0.05) in apelin level in all groups normal weight, over weight and obese weight in

males (297.31, 389.79, 381.20 kg/m2) in comparing with females (251.53, 321.15, 320.21 kg/m2).

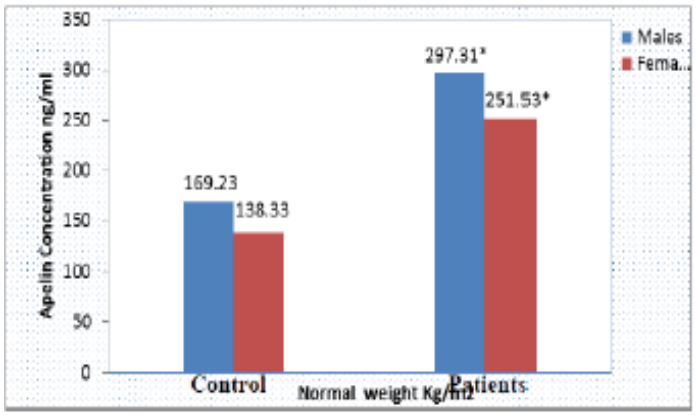

Fig. 2. Apelin concentrations of patients and control groups in normal weight
* means significant difference at (P<0.05)

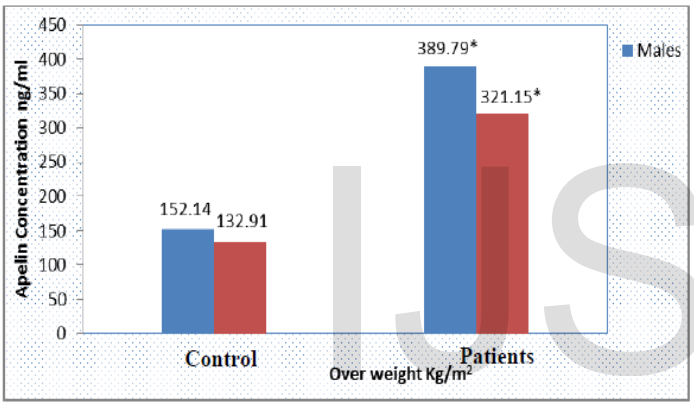

Fig. 3. Apelin concentrations of patients and control groups in over weight
* means significant difference at (P<0.05)

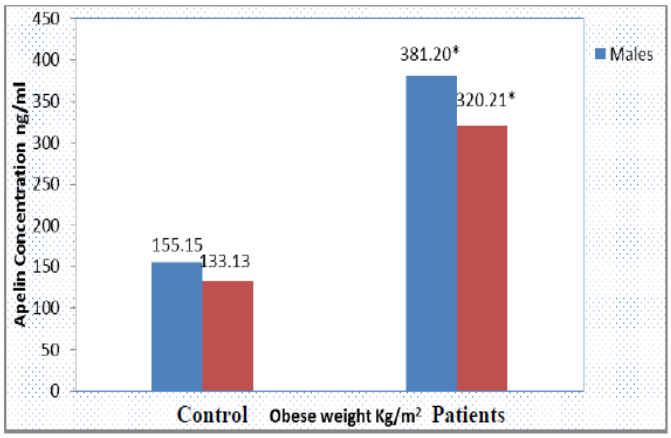

Fig. 4. Apelin concentrations of patients and control groups in obese weight
* means significant difference at (P<0.05).

3.6 Relationship between Apelin and fasting blood glucose levels

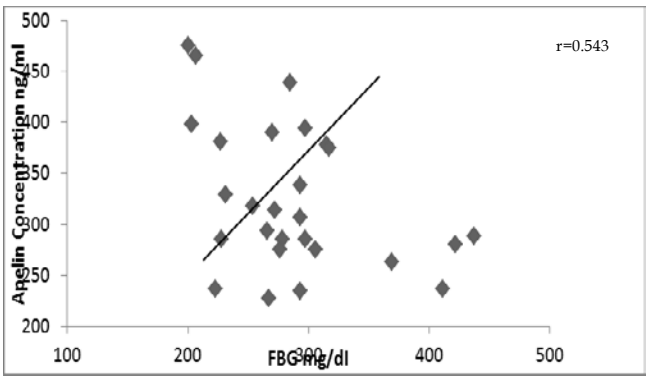

Fig. 5. Correlation between Apelin and FBG in males of Diabetic Mellitus patients

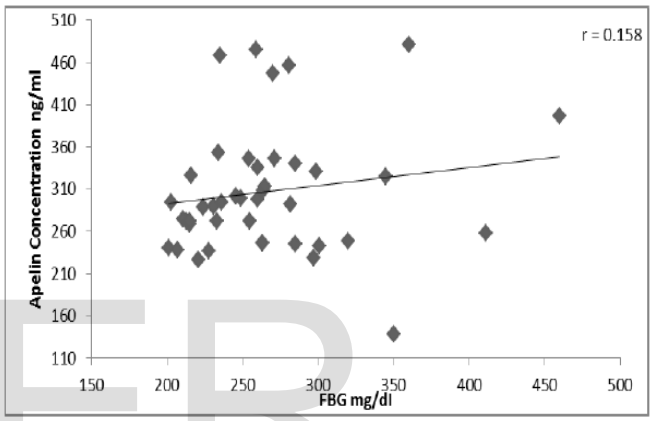

Fig. 6. Correlation between Apelin and FBG in females of Diabetic Mellitus patients

3.7 Relationship between Apelin and lipid profile levels

A. **Cholesterol**

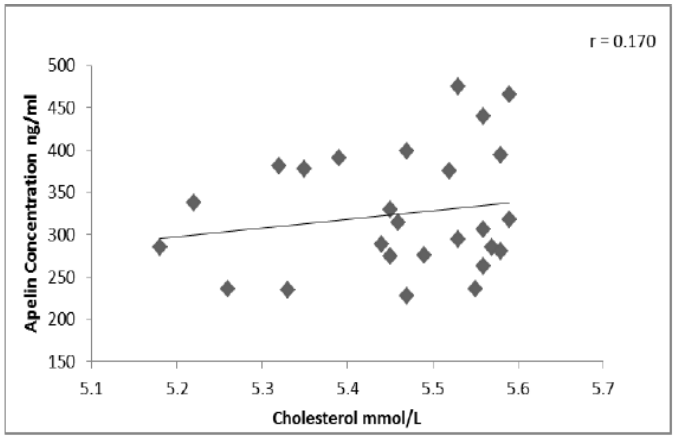

Fig. 7. Correlation between Apelin and cholesterol in males of Diabetic Mellitus patients

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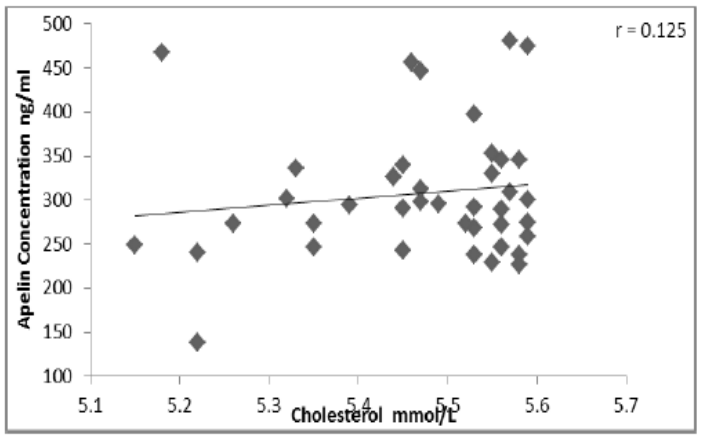

Fig. 8. Correlation between Apelin and cholesterol in females of Diabetic Mellitus patients

B. Triglycerides

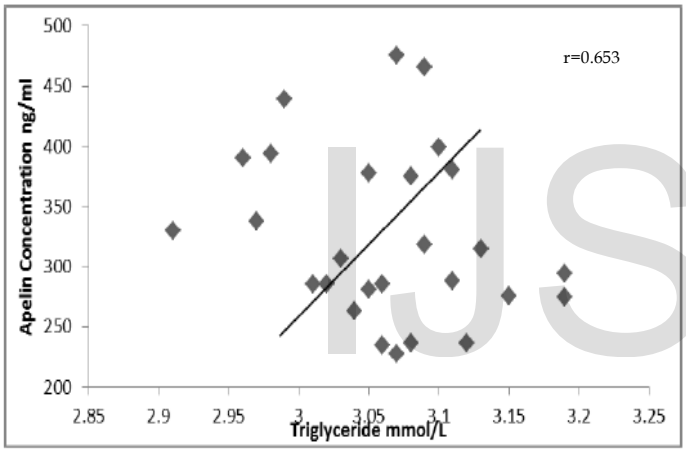

Fig. 9. Correlation between Apelin and triglycerides in males of Diabetic Mellitus patients

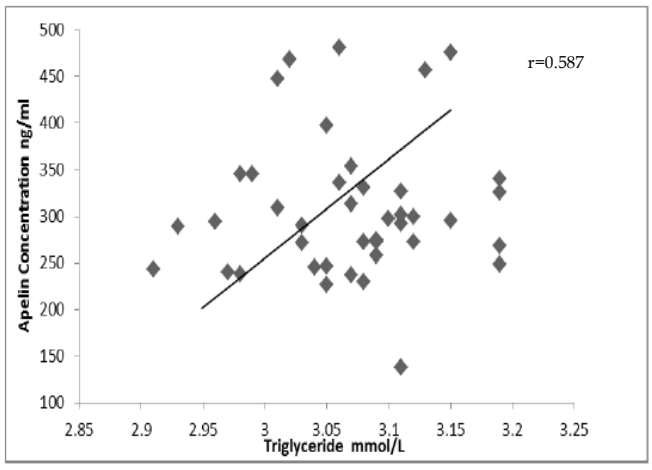

Fig. 10. Correlation between Apelin and triglycerides in females of Diabetic Mellitus patients

C. DL-C

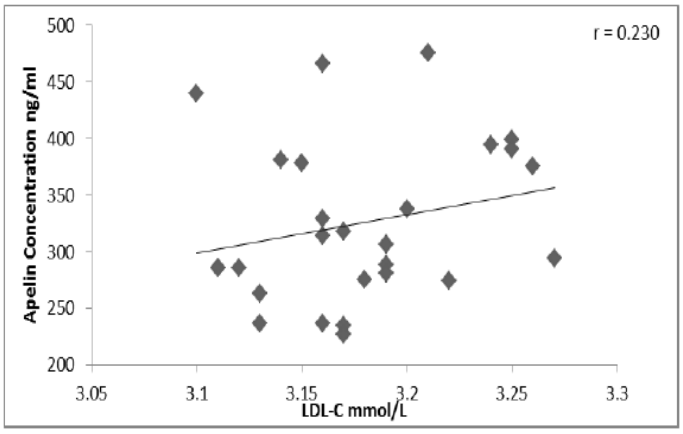

Fig.11. Correlation between Apelin and LDL in males of Diabetic Mellitus patients

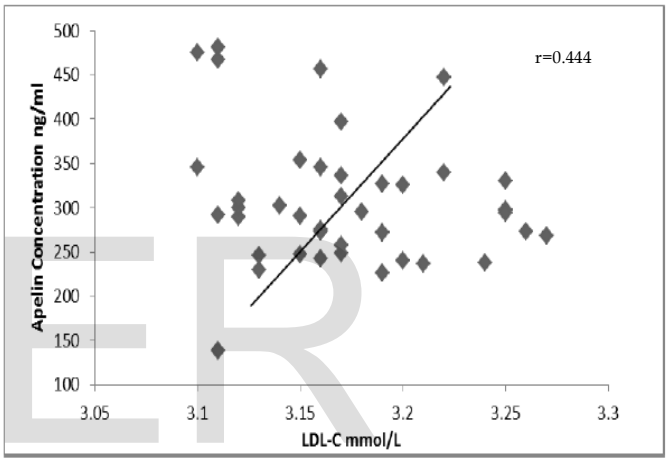

Fig. 12. Correlation between Apelin and LDL in females of Diabetic Mellitus patients

D. VLDL-C

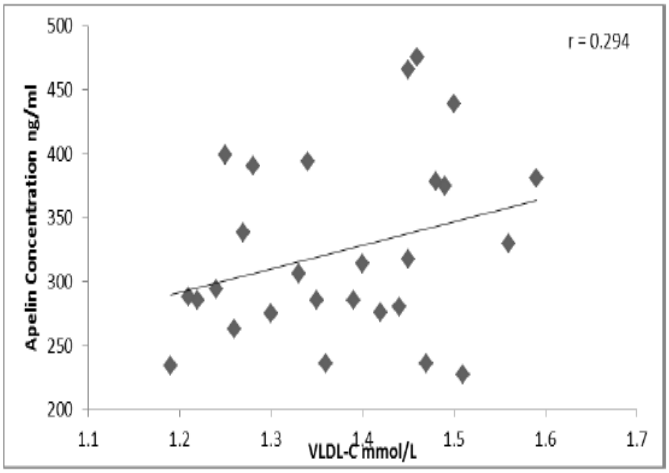

Fig. 13. Correlation between Apelin and VLDL in males of Diabetic Mellitus patients

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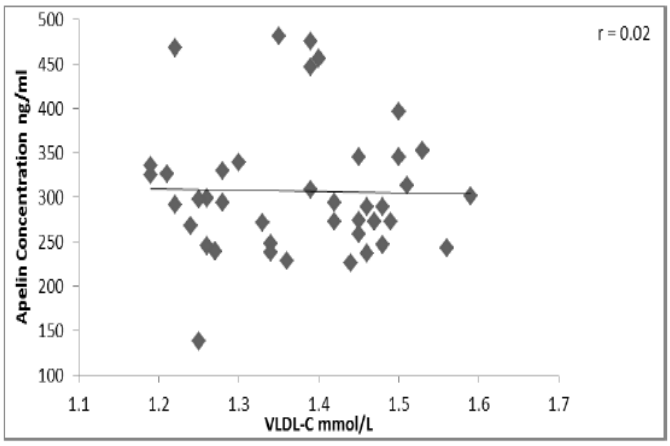

Fig. 14. Correlation between Apelin and VLDL in females of Diabetic Mellitus patients

E. HDL-C

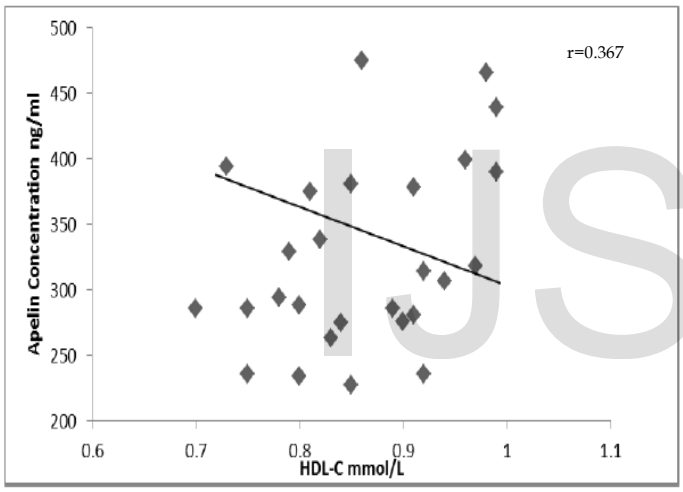

Fig. 15. Correlation between Apelin and HDL in males of Diabetic Mellitus patients

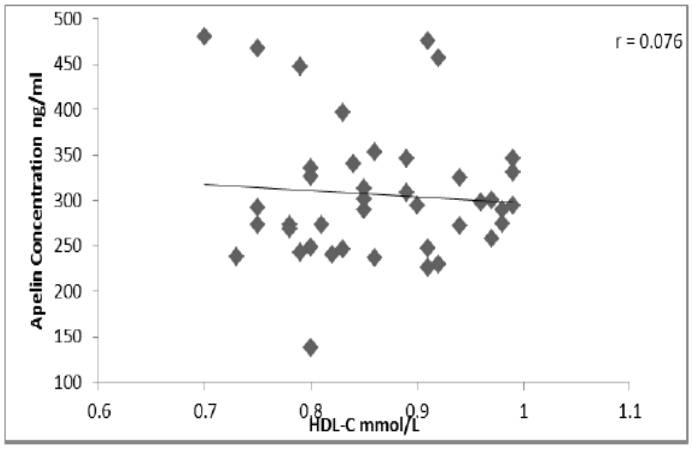

Fig. 16. Correlation between Apelin and HDL in females of Diabetic Mellitus patients

4 Discussion

The study revealed a significant elevation in fasting blood glucose in patients comparing with control group as presented in table (1). These results are expected due to the fact that the main characteristic feature of DM is hyperglycemia. Blood glucose is tightly controlled by two key processes: insulin secretion by pancreatic ß-cells in response to a nutrient and insulin action on major target organs, i.e., skeletal muscle, liver and adipose tissue. T2DM, is often associated with obesity and results from insufficient insulin production/secretion and IR [7].

The results show that there is a significant increase in serum cholesterol, triglycerides, LDL-C and VLDL-C and decrease HDL-C in patients comparing with control group as presented in table (1). The dyslipidemia noticed in the patients group are common in diabetic patients and has different explanations [8-12].

The results of figure (1) show a significant increae ($P<0.05$) in apelin level in diabetic patients in comparing with control groups. Recent studies indicated that significantly higher apelin level serum concentrations in patients with type 2 diabetes mellitus[13-15].

Previous studies finding that elevated apelin serum concentrations may be due to significantly increase in adipose tissue apelin mRNA expression, suggestion a fat depot-specific regulation of apelin expression found significantly higher apelin expression in omental compared to subcutaneous fat depots in T2D [16,17].

Some studies revealed of a significant correlation between adipose tissue apelin production and higher level of circulating apelin in serum [15,18]. Two cross sectional studies [19,20] in diabetic patients indicated a relationship between apelin and insulin resistance and plausible explanation is that insulin-sensitizers trigger apelin secretion through adenosine 5′ monophosphate activated protein kinase (AMPK) activation leading to insulin resistance in diabetic type 2 patients. A study of [21] concluded that circulating apelin increases as a compensatory mechanism to improve insulin sensitivity and this suggestion is supported by studies in apelin deficient mice in which supplementation of apelin causes improved in insulin sensitivity. Another study [22] indicated that apelin effects on insulin sensitivity may be direct by improved glucose uptake and intracellular signaling or indirect by improvement of energy metabolism by increased mitochondrial biogenesis and tighter matching between fatty acid oxidation and tricarboxylic acid cycle.

The higher level of apelin concentrations in serum may be associated with adipose tissue inflammation can be contribute to higher expression of apelin mRNA. Changes in apelin level may be related by a many parameters of inflammations such as TNF and IL-6 [16]. Apelin may be contribute to impaired glucose metabolism by inhibit insulin secretion.

The present study revealed in table (3) a significant increase in apelin level in male than female patients. The results of present study agreement with other studies that indicated increase in apelin level of male diabetic patients than female and this result may be discussed as male have a higher expression of apelin mRNA than female and also increase macrophage infiltration which consider as a source of apelin production in adipose tissue may be correlated with increase in

omental apelin expression in males than females [16,23]. Apelin mRNA expression and protein concentrations may be negatively correlated with estradiol level in women [19].

The results in Figures (2), (3), (4) show a significant increase in apelin level in all groups normal weight, over weight and obese weight in diabetic patients in comparing with control groups.

Study of [24] concluded that a higher apelin level in human associated with higher BMI and are primarily due to obesity. A study of [18] suggested that a strong association between weight loss and lower apelin concentration and also elevated in obese and over weight patients. Avariant in apelin gene has been associated with high BMI in diabetic patients [25]. A significant differences in the mRNA expression of apelin receptors in adipose tissue may be discussed and a high level of apelin in obese and over weight diabetic patients in comparing with control groups. A study of [18] improved that linear regression analysis of BMI changes significantly predict changes in circulating apelin.

The present study showed a significant positive correlation in apelin level in the fasting blood glucose in both males and females in type 2 diabetic patients.

Previous study demonstrated the relation between apelin level and plasma glucose concentrations [24]. Study of [25] proved that an association between apelin gene and blood glucose in larg chinese cohort. Recent report found that higher apelin serum concentration associated with higher glucose concentration in type 2 diabetic patients [15].

From the results of present study indicated a significant positive correlation between apelin level and cholesterol, triglycerides and LDL-C in both males and females and VLDL-C only in males and negative correlation with HDL-C in in both males and females.

Study of [26] reportred a significant up-regulation of apelin plasma level in patients with hypercholesterolemia. Further investigation clarified that changes in apelin level increasing to the LDL-C in diabetic patients because targeting on it [27,28].

Apelin has a modest impact on early atherosclerosis compared to advanced stage and lead to plausible greater changes in cholesterol and triglycerides in order to affect atherosclerosis progression [29,30].

Study of [24] confirmed that higher apelin serum concentration associated with hypercholesterolemia, hypertriglyceridemia and higher BMI in diabetic patients.

The pro-inflammatory functions of apelin may lead to accumulation of other sclerotic plaque destabilization [31,32]. Kadoglou in 2010 [29] found that apelin and Ghrelin among adipocytokines changes positively correlated with LDL-C and negatively with HDL-C.

More data proved that adipose tissue-derived apelin constitute a compensatory mechanism to insulin resistance which enhanced in cholesterol, LDL-C and macrophage of human carotid and coronary atherosclerosis lesion [31,33,34].

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