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Evaluation of TNF-α, Apelin and Visfatin in a sample of Iraqi patients with newly diagnosed Type2 Diabetes Mellitus

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ABSTRACT: Type 2 diabetes mellitus, previously referred to as "Noninsulin-dependent diabetes" or "adult-onset diabetes," accounts for 90–95 % of all diabetes. This type involves people who suffer from relative (rather than absolute) insulin deficiency and suffer from peripheral insulin resistances. The present study aimed to detect serum marker in newly diagnosed type two diabetic mellitus patients in Baghdad province through evaluation of Tumor necrosis factor alpha, Apelin and Visfatin levels. In the current study, (140) participants were recruited and grouped into: patients group (70 patients with newly diagnosed T2DM, and (70 individuals as apparently healthy control group). In the current study, age and sex matched healthy individuals were enrolled. Results showed the serum level of TNF-α, Apelin and Visfatin were estimated by the use of ELISA technique. Furthermore, the mean serum level of TNF-α, Apelin and Visfatin in T2DM patients was 366.08± 76.99 pg/ml, 96.61± 46.62 pg/ml and 9.59± 3.62ng/ml, respectively, which was higher than that of healthy subjects (175.86± 9.92pg/ml , 15.67± 1.74 pg/ml and 2.15± 0.33ng/ml, respectively) with highly significant differences (P<0.0001). Therefore, TNF-α, Visfatin and Apelin can be applies as a diagnostic biomarker and as useful tools for predict ion and prevention of pre DM complications.

KEYWORDS: Apelin; Tumors necrosis factor alpha (TNF-α); Type 2 diabetes mellitus (T2DM); Visfatin.

1. INTRODUCTION

The term (Diabetes mellitus) collectively refers to a metabolic disorder characterized mainly by chronic hyperglycemia. It is caused either by disturbed insulin secretions, different insulin resistance (IR) grades, or often by both. The number of patients with diabetes is expected to rise to 113 million by 2030 and 151 million by 2045, according to worldwide diabetes figures released in 2021 [1]. The three commonly detected of diabetes are Type-1 diabetes (T1D), Type-2 diabetes (T2D) and gestational diabetes (GD). T2DM has an abnormally low production or resistance to insulin [2]. Obese people are six times more likely to develop T2DM than those in good condition. However, all people who are obese are at risk of developing diabetes [3]. Changes in lifestyles such as urbanization, increased life pace, consumptions of high calorie diet and physical inactivity led to highly obesity burdens and accompanying diabetes [4]. Obesity is considered as a major risk factor for development of diabetes, and it is estimated that approximately 90% of diabetic individuals are overweight or obese [5].

The adipose tissue role developed from its being the major energy reservoir as triglyceride to being as the endocrine gland and the fundamental member within the endocrine systems, which is due to the fact of adipose tissue contribution to the secretion of hormone-like substance called (adipokine) or (adipocytokine) [6]. Adipokines involve the inflammatory mediators: interleukins IL-1β, IL-6, IL-8, IL-10, tumor necrosis factor (TNF), apelin, visfatin and others [7]. There may be a change or dysregulation in the concentration of such adipokines in certain metabolic diseases or conditions like type-2 diabetes and obesity [8].

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Tumors necrosis factor alpha (TNF-α) is an adipocytokine (protein) has a molecular weight of 17 KDa consisting of 157 amino acids, it is a homotrimer in solution that is mainly produced by activated macrophages, T lymphocytes, and natural killer (NK) cells [9]. There is a relationship between the cell signaling protein, TNF-α, and systemic inflammations, and TNF-α is a cytokine that establishes acute-phase reactions. The primary role of TNF-α is in regulation of immune cells [10]. The Monocyte/Macrophage cells mainly synthesize TNF-α, although it can be also synthesized by the intrinsic resident kidney cells.

The specific cell surface receptor mediates the actions of TNF-α. Several pathways are activated by TNF-α binding to its receptors 1 (TNFR1) or TNFR2 which lead to expressions of different cytokines, transcription factor, growth factor, receptor, cell adhesion molecule, inflammatory process mediator and acute phase proteins; in addition, it could mediate apoptotic and necrotic cell death [11]. The insulin transductions are inhibited by TNF-α and glucose metabolisms are disturbed by this cytokine [12]. A change in the metabolism of TNF-α plays a role in metabolic syndromes like insulin resistance and overweight; this demonstrates why TNF-α metabolism perturbation may influence the occurrence and development of T2DM [13].

The endogenous peptide, Apelin hormone, has been determined as the ligand for Orphan-G protein couple receptors APJ, thus, the term apelin refers to the APJ endogenous ligands. Bovine's stomach was the first part from which Apelin was isolated [14]. Apelin belonged to the adipokines' family, and adipose tissues are responsible for releasing this bioactive mediator. This peptide is involved in pathological conditions e.g., obesity, cardiac failures, Diabetes mellitus and cancers; it is a biomarker that predicts heart diseases and different cancer types [15]. Apelin is found in different subtypes such as Apelin-12, Apelin-13, Apelin-19, Apelin-28, Apelin-31 and Apelin-36 and so on., and Apelin-13 and Apelin-36 are the most common subtypes. The major form of endogenous Apelins is Apelin-36, whereas the form of short peptide is Apelin-13 [16]. Researches done on rodents showed that Apelin possesses insulin sensitizing's impacts exerting useful roles on the homeostasis of glucose. Depending on the information related to the physiological impacts, it may be anticipated that apelin can play a protective role against diabetes. Nevertheless, there is little data in large prospective studies that demonstrate the association between apelin and diabetes risk among the general population. Thus, assessment was carried out on the relation between serum apelin concentration at baselines and type2 diabetes incidence with related traits during 9 years follow up on 3,785 volunteers [17].

The visceral adipose tissues are the main sites of production of visfatin, which is a 52 KDa protein also called pre-B cell colony-enhancing factor (PBEF) or nicotinamide phosphoribosyl transferase (NAMPT). Many body tissues and organs express visfatin such as myometrium, fetal membrane, bone marrow, liver, muscles, heart, lungs, kidneys, macrophage and neutrophils [18]. The insulin-like effects are exerted by Nicotinamide Phosphoribosyl transferase through binding with insulin receptor-1. Therefore, hypoglycaemia is caused by visfatin via combined mechanisms including glycogenolysis reductions in hepatocyte, stimulations of glucose utilization in adipocytes and myocytes in downstream signaling [19].

2. RESULTS

2.1. Demographic characteristics of the studied groups

A total of 140 individuals have been recruited in the present study and grouped into: 70 patients who were newly diagnosed type 2 diabetes mellitus (T2DM) according to American diabetes association (ADA),2021 (Fasting plasma glucose (FPG) ≥126 mg/dL and Glycated hemoglobin (HbA1c) ≥6.5%), while 70 participants as apparently healthy controls.

The distribution of sex within the study population was examined and summarized in Table 1. The gender distribution was balanced between the case and control groups, and the chi-squared test did not reveal any significant association between gender and group allocation. The diabetic group consisted of 35 females and 35 males, accounting for a total of 70 individuals with an equal distribution of 50.0% for each gender. Similarly, the control group also comprised 35 females and 35 males, resulting in a total of 70 individuals and an equal gender distribution of 50.0% for each category. The significance level (p-value) was determined to be 1.0000, indicating no statistically significant relationship between sex and group allocation.

Considering age, the diabetic group (consisting of 70 subjects) exhibits a median age of 47.00, with a mean of 45.79±4.99 and a standard error of the mean (SEM) of 0.60. The observed no significant difference in age between the case and control groups, with a p-value more than 0.05. On the other hand, the control

group had a median age of 40.85 year, with a mean of 40.85±2.92 and an SEM of 0.35. Means & Confidence interval is presented in Figure 1.

Table 1. Frequencies and percentages of gender for each group in the study.

	sex		P.
Groups	**Female**	**Male**	
patients	35	35	70 (50.0%)
Control	35	35	70 (50.0%)
Total	70 (50.0%)	70 (50.0%)	140
Chi-square	0.000		
DF	1		
Significance level	P = 1.0000		

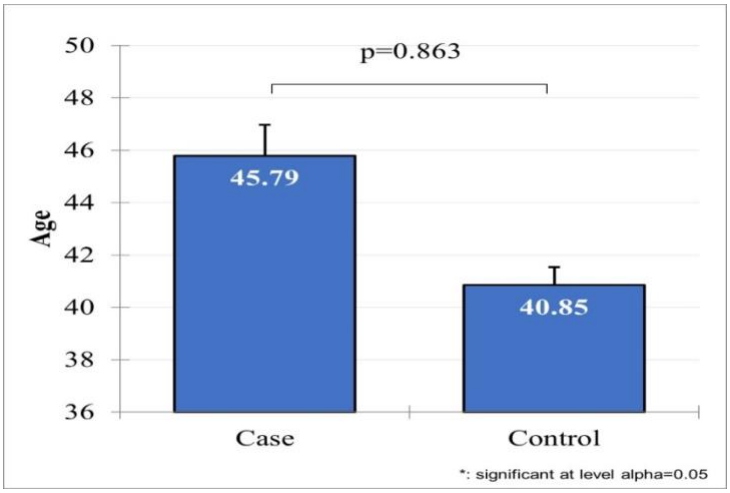

Figure 1. Means and Confidence interval of Age by group.

Table 2 displays comprehensive descriptive statistics and p-values for age on the characteristics of the study subjects.

Table 2. Descriptive statistics and p-values for clinical, demographic and glycemic control parameters in the case and control group.

Parameter	Subjects	No.	Median	Mean± SD	SEM	P-Values
Age (yr)	**Patients**	70	47.00	45.79± 4.99	0.60	P=0.863
	Control	70	40.85	40.85± 2.92	0.35	

The statistical analysis employed the two-tailed Mann-Whitney U test with a significance level set at $\alpha = 0.05$. The test yielded a significant result, with $U = 4887$, $z = -10.16$, and $p < 0.001$. The mean rank for the diabetic group was 105.31, while the mean rank for the control group was 35.69. These findings indicate a substantial distinction between the distributions of TNFα levels in the case and control categories. Moreover, the median value for the Diabetic group (354.53) was significantly higher than the median value for the control group (175.52).

The detailed outcome of the two-tailed Mann-Whitney U test is presented in Table 3, capturing the statistical parameter and test results. Furthermore, Figure 2 provides boxplot visualization, depicting the distribution of TNFα ranks stratified by group membership.

Table 3. Descriptive statistics and p values of TNF-α by groups.

Parameters	Subjects	No.	Median	Mean± SD	SEM	p-Values*
TNF-α pg/mL	Case	70	354.54	366.08± 76.99	9.20	<0.001
	Control	70	175.53	175.86± 9.92	1.19	

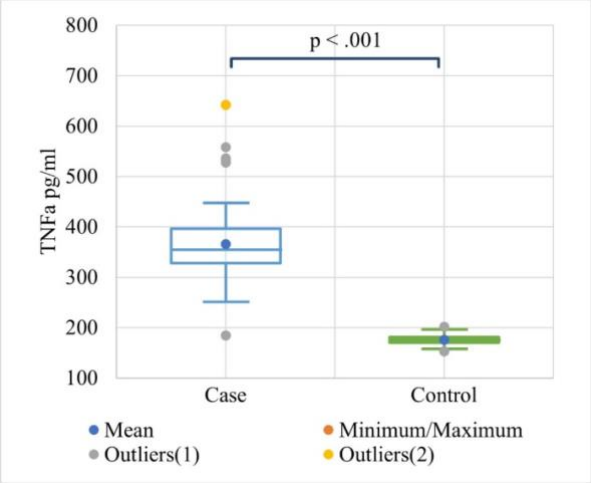

Figure 2. Mean of TNF-α in study groups.

2.2. Serum levels of Apelin among studied groups

The outcome of the two-tailed independent samples t-test yielded a statistically significant result, with a significance level set at 0.05. The test statistic (t-value) was calculated as t (69.19) = 14.52, and the associated p-value was less than 0.001. Consequently, the null hypothesis can be rejected, indicating a significant difference in the mean values of Apelin between the case and control categories within the groups. These results are provided in Table 4, while a graphical representation of the mean values is depicted in Figure 3.

Table 4. Descriptive statistics and p value for Apelin and Visfatin by groups.

Parameter	Subjects	No.	Median	Mean± SD	SEM	P-Values
Apelin pg/mL	**Case**	70	84.14	96.61± 46.62	5.57	**< 0.001**
	Control	70	15.76	15.67± 1.74	0.21	
Visfatin ng/mL	**Case**	70	9.16	9.59± 3.62	0.43	**< 0.001**
	Control	70	2.21	2.15± 0.33	0.04	

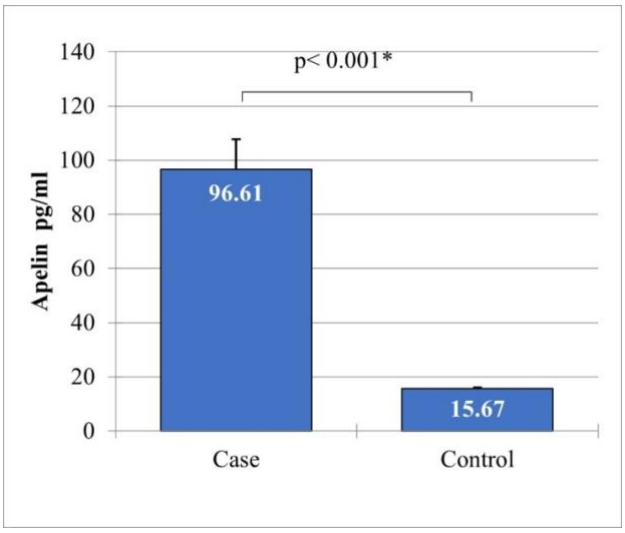

Figure 3. The mean of Apelin by levels of group with 95.00% CI.

2.3. Serum levels of Visfatin among studied groups

The outcome of the two-tailed independent samples t-test yielded a significant result, with a critical significance level (alpha) set at 0.05. The calculated test statistic t (70.12) was found to be 17.10, indicating a substantial difference between the case and control groups. The corresponding p-value was less than 0.001, demonstrating strong evidence to reject the null hypothesis. Specifically, this finding highlights a significant disparity in the mean values of Visfatin between the diabetic and control groups. Detailed statistical results are presented in Table 4, while a visual representation of the mean values is depicted in Figure 4.

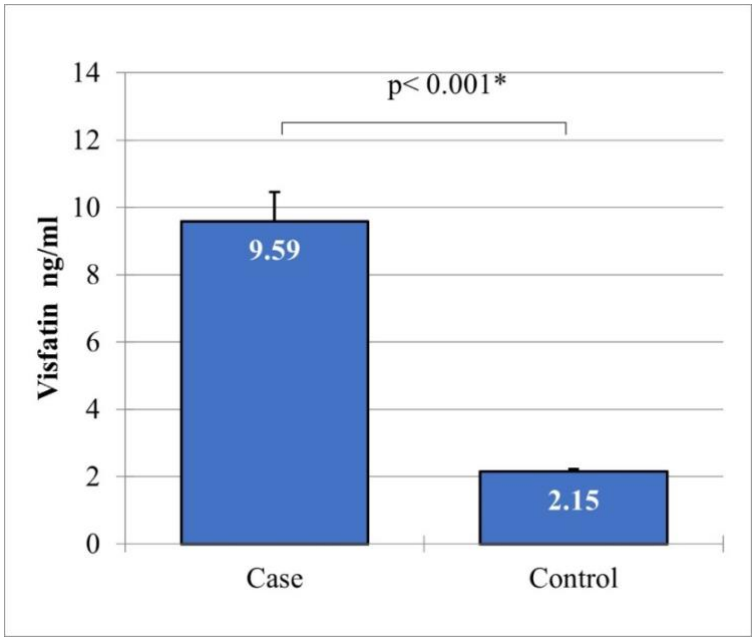

Figure 4. The mean of Visfatin by levels of group with 95.00% CI.

2.4. Receiver Operating Characteristic (ROC) analysis

The results presented in table 5 show the performance of parameters (Tumors necrosis factor alpha (TNF-α), Apelin and Visfatin) in terms of their areas within the (AUC) curve, p-value, cutoff value, specificity and sensitivity Evaluating the diagnostic accuracy of biomarkers.

2.4.1. Apelin

The results for Apelin suggest that it has excellent discriminatory power, with a perfect AUC of 1.000. The p-value indicates that the difference in Apelin levels between case and control groups is highly significant. The cutoff value of >18.31 suggests that values above this threshold may be indicative of the diabetes. The sensitivity and specificity values of 100.00% suggest that Apelin is highly accurate in identifying both true positives and true negatives. See Figure 5 and Table 5.

2.4.2. Visfatin

Similar to Apelin, Visfatin also demonstrates excellent discriminatory power with a perfect AUC and significant p-value. The cutoff value of >2.9 suggests that values above this threshold may be relevant. Both the sensitivity and specificity values of 100.00% indicate high accuracy in identifying true positives and true negatives. See Figure 6 and Table 5.

2.4.3. Tumors necrosis factor alpha

Tumors necrosis factor alpha demonstrates high AUC and a significant p-value, indicating good discriminatory power. The cutoff value of >201.43 suggests that values above this threshold may be relevant. The sensitivity value of 98.57% indicates the test's ability to correctly identify true positives, while the specificity value of 100.00% indicates high accuracy in identifying true negatives. See Table 5.

Table 5. Receiver-operating characteristics (ROC) analysis characteristics of the key markers in the study as they discriminate diabetic group from control.

Variable	AUC	SE	95% CI	P value	Criterion	Sensitivity	Specificity
Apelin pg/ml	1.000	0.000	0.974 to 1.000	<0.0001	>18.31	100.00	100.00
Visfatin ng/ml	1.000	0.000	0.974 to 1.000	<0.0001	>2.9	100.00	100.00
TNFα pg/ml	0.997	0.00274	0.969 to 1.000	<0.0001	>201.43	98.57	100.00

3. DISCUSSION

The current study result coincides with the results of Naif et al., which show a non-significant difference between the patient and control according to the gender distribution [20]. In contrast, Abusaib et al. show in their studies that this is highly significant [21]. Also, a study detected that women showed a higher T2D percentage when compared with men [22]. Additionally, because gender is matched between patients and control groups and also within groups.

These results were compatible with Al-Attaby and Lami et al., supporting that T2DM occur through the fifth decade of age [23]. In addition, our results agreed with those reported by Wong et al. in accordance with the study of, the prevalence of glucose intolerance [Prediabetes & T2D] elevated in patients ≥45 years [24]. Type 2 diabetes is thought to be caused by a decrease in insulin sensitivity and an inability of the beta cells' functional mass to compensate for the body's increased insulin resistance as people age [25]. The secretion of insulin typically decreased at about 0.7% rate yearly with ageing; and this decrease was accelerated in the functions of β-cell for about two folds in those with impaired glucose tolerances [26].

This study similar with other studies done by Bertrand et al., and Gao et al., [27, 28] who said that apelin has useful roles in energy metabolisms through glucose uptake increasing and insulin's sensitivity, also agreement with other study who conclude that among diabetics, apelin-12 concentration is elevated [15,29] extending susceptibility to diabetes [30. 31]. Therefore, serum apelin level estimation might be used as a prediction method for type-2 diabetes development [32,33]. Additionally, the increased apelin levels among rats indicated that it had a powerful anti-type-2 diabetes activities and acted as insulin-sensitization agents [34,35].

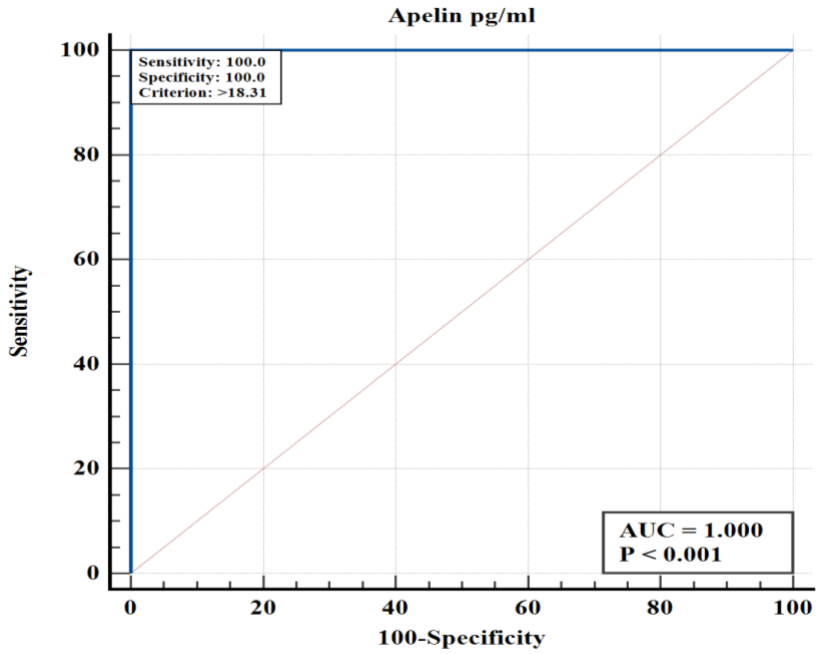

Figure 5. Receiver-operating characteristics (ROC) curves of the Apelin.

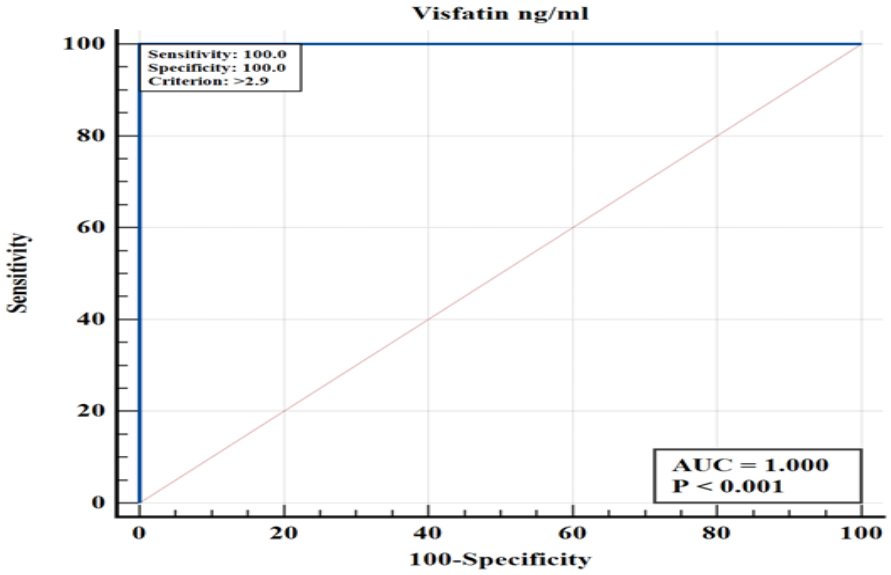

Figure 6. Receiver-operating characteristics (ROC) curve for the Visfatin.

The present study in agreement with result done by Hachim et al., who Stated Association of TNF-α 308 polymorphism with diabetes mellitus type 2 [36], another study also demonstrate by Liu et al., who said that Meta-analysis of 19 studies shows that elevated TNF- levels are strongly associated with an increased risk of developing Type 2 Diabetes Mellitus (T2DM) [37].

The current study results were consistent with Dakroub et al., who reported that serum visfatin was related to several hormones like signaling pathways and it activated certain cascades of intracellular signaling. It is important to mention that extracellular nicotinamide phosphoribosyl transferase (eNAMPT) is related to many metabolic diseases such as type 1, type 2 diabetes and obesity[8]. Our results are in agreement with those of earlier reports by Mir et al., who demonstrate that significant altered levels of 4 adipokine, adiponectin, leptin, visfatin and chemerin were detected in T2DM group in comparison with the control group, with more marked changes seen in obese & highly obese patients [38].

4. CONCLUSION

In this research highly significant differences were seen in mean of Tumors necrosis factor alpha (TNF-α), Apelin and Visfatin between T2DM compared with health control. With highly specificity and sensitivity in the cut off values in diagnosing type-2 diabetes, and measurement of serum levels of TNF-α, Visfatin and Apelin and can be applies in diagnosis as biomarkers and very important for prediction and prevention of pre-DM complications. And also increased serum TNF-α, Apelin and Visfatin are indicated to contribute to DM pathophysiology.

5. MATERIALS AND METHODS

This study was done on 140 serum samples ,70 samples collected from patients with newly diagnosed type 2 diabetes mellitus (T2DM) (35 males and 35 females), while 70 samples as apparently healthy controls (35 male & 35 female), their ages ranged from (25-70) yrs. Individuals who showed signs and symptoms were initially identified as type 2 diabetes mellitus (T2DM) by the physician and measurement the fasting blood glucose levels and HbA1c to confirm the subjects with T2DM, at Al-Imameen Al-Khdaimin Teaching Hospital, The Specialized Center For Endocrinology And Diabetes –Baghdad-Iraq during a period from December 2022 to April 2023. Samples from 70 patients were examined for Tumors necrosis factor alpha (TNF-α), Apelin and Visfatin levels and compared with 70 healthy people.

The study has approved by the Institutional Review Board (IRB) of the College of Health & Medical Techniques, Middle Technical University, Baghdad, Iraq (HMT23872)

About five milliliters (5mL) of venous blood were collected from each subject in the current study between 9 -11 AM after overnight fasting (8-12) hours. The samples were parted into two aliquots:

1. In the first tube 2mL of venous blood was put in ethylene diamine tetra acetic acid (EDTA) tubes for glycated hemoglobin level (HbA1c) measurement by using ROCHE COBAS e 411 Analyzer (Roche/Hetachi Diagnostics Ltd- Japan).
2. The second tube is gel tubes (3mL) of venous blood in a clean and dry gel tube and left to clot at room temperature (25°C) for 5 minutes then put gel tube in the centrifuge device at 3000 rpm for 5 min, From the separated serums were measure the level of fasting blood glucose (FBS) by using ROCHE COBAS e 411 Analyzer (Roche/Hetachi Diagnostics Ltd- Japan), and determine (TNF-α, Human Total Apelin and Visfatin).

TNF-α level was detected by using the ELISA kit (Sandwich-ELISA) (Cloud-Clone Crop-USA, Catalogue Number: SEA133Mu), Apelin and Visfatin levels was detected by using the ELISA kit (Competitive-ELISA) (Cloud-Clone Crop-USA, Catalogue Number: CED066 Hu and CEA638Hu).

5.1. Inclusion criteria (two of three)

All patients with T2DM were diagnosed by physicians, and measurement the fasting blood glucose levels and HbA1c. All patients with T2DM in early stage. To confirm the subjects with T2DM should include at least one of the following features according to the (Organization, 2006) and (Association, 2021) criteria:

1. Fasting plasma glucose (FPG) [≥126 mg/dL (7.0 mmol/L)].
2. Glycated haemoglobin (HbA1c) [≥6.5% (48 mmoL/moL)].

5.2. Exclusion criteria

1. Patients who had taken any drugs or under hormonal treatment.
2. Renal diseases.
3. Hepatic diseases.
4. Autoimmune diseases.
5. Type 1 Diabetes Mellitus.
6. Pregnancy.
7. Have any microbial infection.
8. CA and other malignant.
9. Familial hyperlipidemia.
10. Patients who had taken lipid lowering medication.
11. Obesity.
12. Hypertension.
13. Smoker and alcohol drinker**.**

5.3. Statistical analysis

Data were revised, coded and analyzed by using the SPSS version-26 program. The statistical analysis conducted in this thesis focused on examining the differences between two groups in terms of continuous variables. The study involved a comprehensive analysis that included the description of variables, comparisons between the groups, correlation analysis, and the use of Receiver Operating Characteristic (ROC) analysis. Various statistical tests were employed, such as independent samples t-test or Mann-Whitney U test depending on the nature of the data and assumptions of normality. Correlation analysis was conducted to explore the relationships between different scale variables within each group. Pearson's correlation coefficient or Spearman's rank correlation coefficient, were computed to measure the strength and direction of associations. The ($p<0.05$) value was regarded as significant.

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Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

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