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## **Serum retinol binding protein-4 and the risk of hypothyroidism in Egyptian diabetic patients with cardiovascular manifestations**

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**Abstract**---Retinol binding protein-4 (RBP4) levels have been associated with patients of type 2 diabetes (T2DM) including insulin resistance (IR), dyslipidemia, and cardiovascular disease (CVD). The present study examined the relationship between serum levels of RBP4 and the risk factors related to hypothyroidism (Hypo) and CVD in diabetic Egyptian patients. A total of 96 subjects were recruited and divided into 8 groups. Subjects were assessed for different biochemical parameters. In addition, serum levels of RBP4 were measured using specific immunoassays. There was a highly statistically significant difference in fasting blood glucose (FBG) and hemoglobin A1C (HbA1c) between the different patient groups and the control group. Thyroid stimulating hormone (TSH) concentration in the (T2DM+Hyp) group was considerably higher than in the other groups. There were statistically significant variations in TSH levels between all groups and controls. The mean RBP4 value in Group5 (T2DM+Hyp+CVD) was significantly higher than in the other groups. In (T2DM+Hypo+CVD) group, the receiver operating characteristic (ROC) curve revealed that RBP4 had a higher area under curve (AUC), making it a powerful discriminator of those diseases. However, when the AUC of RBP4 was

compared to the ROC curve of HbA1c, TSH, and the total cholesterol (TC) panel, the area under curve (AUC) of RBP4 was lower than the HbA1c panel. This study explored RBP4 as a potential biomarker for the design of diagnostically accurate biomarkers, especially in the case of T2DM, hypothyroidism, and CVD.

**Keywords**---T2DM, CVD, hypothyroidism, retinol binding protein 4.

## **Introduction**

Diabetes mellitus is a major public health issue that is also one of the world's fastest increasing diseases. It's a metabolic condition marked by excessive blood glucose levels caused by a lack of insulin secretion or function, or both. Hyperglycemia causes harm to several physiological systems, including the neurons and blood vessels [1]. Genetic and environmental factors impact inflammation, autoimmunity, and metabolic stress. They play an important role in insulin resistance and  $\beta$ -cell dysfunction leading to hyperglycemia which is associated with vascular complications [2, 3].

Retinol binding protein (RBP4) is an adipokine that has been linked to type 2 diabetes (T2DM) and cardiovascular disease (CVD) [4]. Several studies have found that T2DM patients with obesity, impaired glucose tolerance, and T2DM patients with hypothyroidism have higher plasma RBP4. RBP4 has also been linked to insulin resistance due to its influence on the insulin signaling pathway [5]. This adipokine has also been linked to CVD, a diabetes-related illness. While increased blood RBP4 levels have been associated to the development of systemic insulin resistance in rats [6], the evidence for RBP4's role in obesity and insulin resistance in people is conflicting. RBP4 levels have been found to be elevated or lowered in early stages of T2DM [7].

Hypothyroidism may increase the risk of cardiovascular disease in diabetic individuals, in addition to its impact on glycemic management [8]. Atrial fibrillation has been reported in as many as 10-15% of patients with overt hypothyroidism, and people with subclinical hypothyroidism are more susceptible than thyroid people to acquire it. In hypothyroidism, cardiovascular mortality is linked to advanced age, cardiac arrhythmias, or pre-existing organic heart disease [9].

The two most prevalent endocrinopathies observed in practice are thyroid problems and diabetes mellitus. Thyroid dysfunction is more common in diabetes patients than in the general population, and both illnesses frequently coexist. Thyroid dysfunction is still more common in T2DM patients than in the general population, and in certain studies, it is even more common than in T1DM patients, owing to the older age group of T2DM patients [10]. Previous studies were done on the role of RBP-4 in type 2 diabetic patients either with CVD or hypothyroidism, but not both [2, 4]. Up to the best of our knowledge, no previous studies were done on the role of RBP-4 in type 2 diabetic patients with both CVD and hypothyroidism. Consequently, the aim of our work was to study the

relationship between serum levels of RBP4 and the risk factors related to hypothyroidism and CVD in diabetic Egyptian patients.

## Materials and Methods

### Subjects

A total of 96 participants were enrolled in this study. Most participants were recruited from the National Institute of Diabetes and Endocrinology-Cairo, Egypt. Patients with CVD were recruited from National Heart Institute-Cairo, Egypt. The subjects were divided into 8 groups as follow: control group (group 1), T2DM patients (group 2), T2DM patients with hypothyroidism (group 3), T2DM patients with CVD (group 4), T2DM patients with hypothyroidism and CVD (group 5), hypothyroidism patients (group 6), patients with hypothyroidism and CVD (group 7), and patients with CVD (group 8). Samples were assessed for biochemical parameters related to diabetes, hypothyroidism, and CVD. All the individuals were matched in terms of age, gender, and ethnicity.

All participants in the study were subjected to thorough history taking and full clinical examination including measurement of height and weight. The mean body mass index (BMI), defined as weight in kilograms divided by the square of height in meters, was calculated. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also measured. A written consent form was obtained from each subject. Ethical approval for the study by the ethics committee of the General Organization for Teaching Hospitals and Institutes (GOTHI) with approved number (IDE00258).

### Blood sampling and laboratory testing

After an overnight fast, blood samples were taken from all participants. Sera were isolated after 1 hour of incubation and stored at  $-80^{\circ}\text{C}$ . Enzymatically, glucose concentration in plasma was estimated by GOD-POD methods of Trinder performed by the Dimension® Integrated Chemistry system [11]. HbA1c was estimated using HPLC fully automated system (Bio-Rad D-10 Hemoglobin testing system). It is intended for the percent determination of HbA1c in human whole blood using ion-exchange HPLC technique according to (Jeppsson, Jerntorp et al. ) [12]. The determination of insulin was done by DRG Insulin ELISA [13]. The homeostasis model assessment-estimated insulin resistance (HOMA-IR), developed by (Matthews et al.) [14] has been widely used for the estimation of insulin resistance.

$$\text{HOMA} - \text{IR} = \frac{\text{Glucose (mg/dl)} * \text{Insulin } (\mu\text{U/mL})}{405}$$

The estimation of serum cholesterol was done according to the endpoint method of Allain *et al.*, 1974 [15]. The estimation of triglycerides was done according to the endpoint method of McGowan *et al.*, 1983 [16]. Serum HDL was estimated by precipitation method of (Lopez-Virella *et al.*) [17]. The estimation of LDL-cholesterol was calculated according to the Friedewald formula [18]. Determination of TSH was done according to the method of The BioCheck.Inc; an ELISA

assay technique was used for the quantitative determination of serum TSH. (TSH) ELISA Kit was provided by BioCheck, Inc., USA. [19] The Cal biotech, Inc. (CBI) FT3 ELISA kit is used for the quantitative measurement of Free Triiodothyronine (FT3) in human serum [20] The Cal biotech, Inc. (CBI) FT4 ELISA kit is used for the quantitative measurement of Free thyroxin (FT4) in human serum [21].

#### **Measurement of serum retinol-binding protein 4**

Serum RBP4 was tested using a commercially available ELISA kit (Abnova, Abnova Corporation, Taipei City, Taiwan) according to the manufacturer's instructions [22].

#### **Statistical analysis**

We used mean  $\pm$  standard deviation (SD) for continuous variables and numbers (%) for categorical variables to assess the overall characteristics of the research population. The relationship between serum RBP4 and biochemical tests was evaluated using Pearson's correlation coefficient. The predictive accuracy of serum RBP4, the area under the curve (AUC), and the cutoff values for discriminating T2DM, hypothyroidism, and CVD among the study population were assessed using receiver operating characteristic (ROC) analysis. A 95 % confidence interval (CI), a *P*-value of less than 0.05 was declared significant. The GraphPad prism statistical tool was used for all studies.

### **Results**

#### **Baseline characteristics of the studied subjects**

Typically, the baseline characteristics (demographic data) of the subjects were as follows: There were no significant differences in age, duration of CVD, sex, and BMI among the groups ( $P>0.05$ ).

However, there were significant differences in duration of DM, duration of hypothyroidism, SBP, and DBP among all groups ( $P<0.05$ ) as shown in Table (1).

Table (1): Demographic characteristics of the studied groups

Group	Group1 (control)	Group2 (T2DM)	Group3 (T2DM+Hyp)	Group4 (T2DM+CVD)	Group5 (T2DM+Hyp+CVD)	Group 6 (Hyp)	Group 7 (Hyp+CVD)	Group8 (CVD)	P-Value	
Age (yrs.) (Mean±SD)	49.3±9.1	49.4±7.1	47±5.33	50.27±6.5	53.58±7.98	49.3±8.6	52.1±2.12	51.7±4.4	0.06	
Duration of DM (yrs.) (Mean±SD)		4.7±1.9 <sup>a**</sup>	6.8±2.7 <sup>a**</sup>	8.8±2.7 <sup>abcd**</sup>	11.3±3.9 <sup>abcd**</sup>	–	–	–	0.000	
Duration of Hypothyroidism (yrs.) (Mean±SD)	–	–	4.9±2.3 <sup>ab**</sup>	–	7.9±3.9 <sup>abcd**</sup>	3.6±2.3	6.3±6.6 <sup>d**</sup>	–	0.000	
Duration of CVD (yrs.) (Mean±SD)	–	–	–	3.7±2.5	3.0±2.2	–	2.3±1.8	2.6±2.2	0.50	
Sex	Male %	4(30.8%)	3(25%)	5(41.7%)	2(16.7%)	2(16.7%)	3(23%)	4(33.3%)	5(41.7%)	0.30
	Female %	9(69.2%)	9(75%)	7(58.3%)	10(83.3%)	10(83.3%)	10(77%)	8(66.7%)	7(58.3%)	
BMI (kg/m <sup>2</sup> ) (Mean±SD)	27.7±4.08	29.4±3.6	30.3±6.5	30.6±5.8	33.7±9.5	31.4±3.4	31.4±4.9	30.02±5.3	0.440	
SBP (mmHg) (Mean±SD)	115±12	115.4±10.3	124.2±21.9	155.5±17.5 <sup>ab**</sup>	143.8±20.8 <sup>abc**</sup>	118.4±4.2	135±10.7	139±7.4 <sup>a*</sup>	0.000	
DBP (mmHg) (Mean±SD)	76±8.4	76.7±7.8	77.5±12.9	90.9±7.01 <sup>ab*</sup>	86.7±9.8	79.3±3.1	87.5±10.4	88±7.9	0.000	

BMI; Body Mass Index, SBP; Systolic Blood Pressure, DBP; Diastolic Blood Pressure, T2DM; Type2 Diabetes Mellitus, Yrs.; Years

a. Sig Control Group 1 versus Group 2,3,4,5,6,7,8

b. Sig DM only Group 2 versus Group 3,4,5

c. Sig Hypothyroidism only Group 6 versus Group 3,5,7

d. Sig CVD only Group 8 versus Group 4,5,7; \*P-value <0.05; \*\*P-value <0.005

### Diabetic markers among the studied groups

Table (2) summarizes the biochemical results of diabetes markers and revealed that there was a highly statistically significant difference in FBG between the different patient groups and the control group ( $P<0.001$ ). Furthermore, there was a marginally significant difference in HbA1c levels across the patient groups ( $P<0.001$ ). In addition, all groups differed significantly from the control group in terms of insulin and HOMA-IR ( $P<0.001$ ).

Table (2): Diabetic markers in controls and different patient groups

Group	Group1 (control)	Group2 (T2DM)	Group3 (T2DM+Hyp)	Group4 (T2DM+ CVD)	Group5 (T2DM+Hyp +CVD)	Group 6 (Hyp)	Group 7 (Hyp+ CVD)	Group 8 (CVD)	P- Value
FBG (mg/dl) (Mean±SD)	96.4±8.7	184.7±48.5**	198.7±47.2ac**	210.5±37.3ad**	265.4±67.6acd**	88.5±8.6	95.4±6.1	88.2±9.3	0.00
HbA1c (%) (Mean±SD)	5.7±0.33	7.97±0.79a**	7.99±0.9ac**	8.2±1.4ad**	9.8±1.5 abcd**	4.9±0.46	5.5±0.32	5.6±0.48	0.00
Insulin (µIU/ml) (Mean±SD)	8.4±2.4	43.4±39.8a**	14.1±10.99b*	18.9±10.2b*	21.4±12.9	21.2±14.4	24.4±14.5	13.8±11.3	0.002
HOMA-IR (Mean±SD)	2.02±0.69	18.2±13.5a**	6.6±4.2b**	10.8±5.8a**	12.9±4.8a**d*	4.7±3.2	5.7±3.4	3.1±2.4	0.00

FBG; Fasting Blood Glucose HbA1c; Glycosylated Hemoglobin HOMA-IR; homeostasis model assessment-estimated insulin resistance.

- Sig Control Group 1 versus Group 2,3,4,5,6,7,8
- Sig DM only Group 2 versus Group 3,4,5
- Sig Hypothyroidism only Group 6 versus Group 3,5,7
- Sig CVD only Group 8 versus Group 4,5,7; \*P-value <0.05; \*\*P-value <0.005

### Lipid Profile among the studied groups

The levels and comparison of mean serum concentrations of lipid profile parameters between subjects are shown in Table (3). The results showed that the mean concentration of TC in (T2DM+Hyp+CVD) group was significantly higher than its concentration in other groups (312±53.8). There were statistically differences between TC concentrations in all groups and control groups (P<0.001). TG concentration bared higher mean concentration (340.3±93.3) in (T2DM+Hyp+CVD) group. The lowest concentration of TG was revealed in the group of hypothyroidism (Group 6) with mean concentration (170.6±23.9). There were statistically differences in all groups regarding TG concentration (P<0.001). Regarding HDL and LDL levels, there were statistical differences between all groups compared to the control group, hypothyroidism, CVD and/or DM group, with the lowest concentration of HDL and highest concentration of LDL in Group 5 (T2DM+Hyp+CVD) with mean concentrations of 29.4±3.7 and 187.6±50.5 respectively.

Table (3): Lipid profile parameters in controls and different patient groups

Group	Group1 (control)	Group2 (T2DM)	Group3 (T2DM+ Hyp)	Group4 (T2DM+CVD)	Group5 (T2DM+Hyp+ CVD)	Group 6 (Hyp)	Group 7 (Hyp+ CVD)	Group 8 (CVD)	P-Value
TC (mg/dl) (Mean±SD)	195±23.5	211.3±28.1	205.2±40.2	231.8±53.2	312±53.8 <sup>abc</sup> **	170.6±23.9	215.5±31.8	232±26.8	0.000
TG (mg/dl) (Mean±SD)	110.6±33.7	142.8±57.5	165.2±47.7	271.9±63.6**	340.3±93.3 <sup>abc</sup> **	157.7±37.1	228±82.7*	257.6±75.9**	0.000
HDL (mg/dl) (Mean±SD)	43.8±9.5	42.8±6.7	40.1±7.3	29.1±3.4**	29.4±3.7 <sup>abc</sup> **	46.2±6.04	34.1±7.6 <sup>abc</sup> **	31.7±4.9**	0.000
LDL (mg/dl) (Mean±SD)	110.8±14.2	134.1±20.8	127.8±27.6	144.8±18.3	187.6±50.5 <sup>abc</sup> **	105.5±22.8	129.1±45.7	156.5±27.7*	0.000

TC; Total Cholesterol, TG; Triglycerides, HDL; High Density Lipoprotein, LDL; Low Density Lipoprotein.

- Sig Control Group 1 versus Group 2,3,4,5,6,7,8
- Sig DM only Group 2 versus Group 3,4,5
- Sig Hypothyroidism only Group 6 versus Group 3,5,7
- Sig CVD only Group 8 versus Group 4,5,7; \*P-value <0.05; \*\*P-value <0.005

### Thyroid Profile among the studied groups

Table (4) shows the comparison of thyroid profile parameters mean serum values between participants. There were statistically significant variations in TSH levels between all groups and control group ( $P < 0.001$ ). The mean TSH concentration in the (T2DM+Hyp) group was considerably higher ( $7.5 \pm 6.3$ ) than in the other groups. In terms of FT3 level, all groups had statistically significant differences ( $P < 0.001$ ). There were significant variations in FT4 levels between all groups in terms of control group, hypothyroidism, CVD, and/or DM, with the lowest concentration in Group 7 (Hyp+CVD).

Table (4): Thyroid profile parameters in controls and different groups

Group	Group1 (control)	Group2 (T2DM)	Group3 (T2DM+ Hyp)	Group4 (T2DM+ CVD)	Group5 (T2DM+ Hyp+ CVD)	Group 6 (Hyp)	Group 7 (Hyp +CVD)	Group 8 (CVD)	P- value
TSH (uIU/ml) (Mean±SD)	1.8±0.86	2.1±1.1	7.5±6.3 <sup>abc</sup> **	2.8±0.68	4.2±1.05	5.3±2.1	3.7±0.82	2.2±1.1	0.000
FT3 (pmol/L) (Mean±SD)	1.5±0.4	1.4±0.4	2.6±0.65 <sup>ab</sup> *	3.1±1.03 <sup>abcd</sup> **	2.4±0.86	2.6±1.5**	1.7±0.7	1.4±0.34	0.000
FT4 (pmol/L) (Mean±SD)	3.4±0.93	3.2±1	1.1±0.18 <sup>abc</sup> **	1.4±0.43 <sup>abcd</sup> **	1.1±0.32 <sup>abcd</sup> **	1.4±0.75 <sup>abc</sup> **	0.7±0.43 <sup>abcd</sup> **	3.2±0.78	0.000

TSH; Thyroid Stimulating Hormone, FT3; Free Triiodothyronine, FT4; Free Thyroxine.

- Sig Control Group 1 versus Group 2,3,4,5,6,7,8
- Sig DM only Group 2 versus Group 3,4,5
- Sig Hypothyroidism only Group 6 versus Group 3,5,7
- Sig CVD only Group 8 versus Group 4,5,7\*P-value <0.05; \*\*P-value <0.005

### Retinol Binding Protein 4 (RBP-4) among the studied groups

Table (5) and Figure (1) shows the comparison of RBP-4 values between studied groups. There were statistically significant variations in RBP4 levels between all groups and control group ( $P < 0.001$ ). There was significant increase in levels of RBP4 in group 3; (T2DM+Hyp), 4; (T2DM+CVD), 5; (T2DM+Hypo+CVD), 7; (Hyp+CVD), and 8; (CVD) compared to controls. Moreover, there was significant increase in its level in group 4; (T2DM+CVD) and 5; (T2DM+Hypo+CVD) compared to group 2 (T2DM). In addition, there was significant increase in its level in group 5; (T2DM+Hypo+CVD), and 7; (Hyp+CVD) compared to group 6 (Hyp). Also, levels of RBP4 were significantly increased in group 5; (T2DM+Hypo+CVD) compared group 8 (CVD).

Table (5): RBP4 levels in controls and different patient groups

Group	Group1 (control)	Group2 (T2DM)	Group3 (T2DM+ Hyp)	Group 4 (T2DM+CVD)	Group5 (T2DM+Hy p+ CVD)	Group 6 (Hyp)	Group 7 (Hyp+CVD)	Group 8 CVD	P- value
RBP4 (pg/ml) (Mean±SD)	421 ±35.5	594.5 ±80.98	714.3 ±125.1**	940.05 ±83.8***b*	1163.11 ±398.8abcd**	512.6 ±53.99	871.9 ±88.4**c**	840.7 ±101.01a**	0.000

- Sig Control Group 1 versus Group 2,3,4,5,6,7,8
- Sig DM only Group 2 versus Group 3,4,5
- Sig Hypothyroidism only Group 6 versus Group 3,5,7
- Sig CVD only Group 8 versus Group 4,5,7; \*P-value <0.05; \*\*P-value <0.005

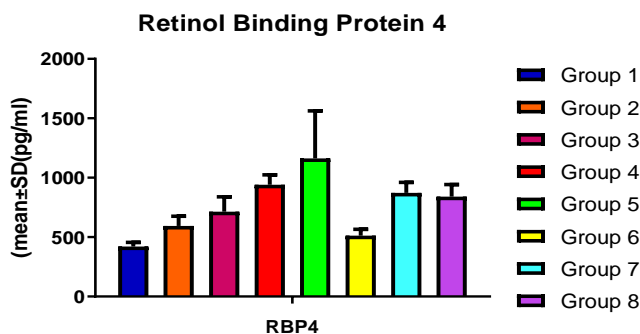


Figure (1): Retinol Binding Protein 4 levels in studied groups

### ROC curve analysis of the studied groups:

The receiver operating characteristic (ROC) curve was used to determine the cutoff point of RBP4 quantitation prior to diagnosis, which can detect the

diagnosis of the disease as it resembles a diagnostic value for concomitant disease of T2DM, hypothyroidism and/or CVD disease as shown in table (6).

### ROC curve pairwise comparison

The commonly approved diagnostic markers for the concomitant diseases of T2DM with/or hypothyroidism and CVD are the ordinary known biochemical test that were presented in the mentioned result section, however, by adding these ordinary marker to the RBP4, the ROC curve revealed that the RBP4 had an AUC of just 0.850 ( $P = 0.003$ ) in the combined diseased group (T2DM+Hypo+CVD), making it a strong diagnostic power of the concomitant diseases. However, when the AUC of RBP4 was compared to the ROC curve of HbA1c, TSH, and TC panel, the area under curve (AUC) of RBP4 was lower than the HbA1c panel. as shown in Table (7) and Figure (2). RBP4 was improved if combining it with the suggested HbA1c, TSH, and TC panel.

Table (6): The area under ROC curves and the cut-off points corresponding to the highest % sensitivity and % specificity of for all studied groups.

Parameters	Group2 (T2DM)	Group3 (T2DM+Hyp)	Group4 (T2DM+CVD)	Group5 (T2DM+Hyp +CVD)	Group 6 (Hyp)	Group 7 (Hyp+CVD)	Group 8 (CVD)	P- Value
Optimal cut-off value of RBP4 (pg/ml)	>610	>737	>940	>872.5	>504	>745	>998	0.006
AUC	0.600	0.650	0.750	0.850	0.900	0.60	0.600	0.230
95% Confidence interval	0.250-0.825	0.334-0.891	0.380-0.920	0.291- 0.859	0.594-0.996	0.29- 0.859	0.291- 0.859	0.04
Significance level P (Area=0.5)	0.752	0.450	0.181	<0.003	<0.001	0.752	0.805	0.01
Youden index (Optimal cutoff method)	0.40	0.50	0.50	0.40	0.8	0.40	0.50	0.321
Sensitivity	40	50	50	40	80	90	100	0.032
Specificity	100	100	100	100	100	50	50	0.121

Table (7): Comparison of patient groups regarding the RBP4 and HbA1c panel to differentiate between the diagnosis of T2DM, hypothyroidism and CVD

Variable	AUC	SE	95% CI
HbA1c Panel	0.725	0.140	0.404 to 0.933
RBP4	0.600	0.192	0.291 to 0.859
Pairwise comparison of ROC curves			
<i>HbA1c, TSH, TC Panel of DM+Hypo+CVD vs. RBP4</i>			
Difference between areas		0.125	

Standard Error <sup>a</sup>	0.153
95% Confidence Interval (CI)	-0.174 to 0.424
z statistic	0.818
Significance level	P = 0.4132

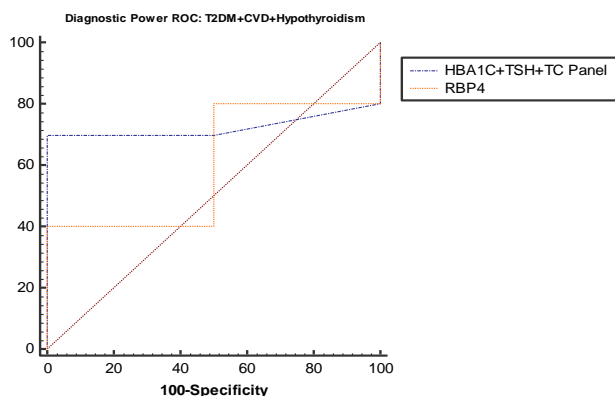


Figure (2): The ability of HbA1c panel and RBP4 candidates to discriminate the diagnosis of DM, hypothyroidism and CVD using a ROC curve analysis.

## Discussion

Type 2 diabetes mellitus patients are more prone to develop thyroid disorders. Many diabetic people develop symptoms of thyroid dysfunction over time. Insulin resistance is a major contributor to hypothyroidism in people with T2DM. In diabetics, hypothyroidism exacerbates dyslipidemia, hypertension, and cardiovascular disease [23]. Diagnosing and treating hypothyroidism in diabetic individuals is critical to avert further problems. A simple blood test that is frequently accessible can identify hypothyroidism. Thyroid dysfunction in diabetics should be addressed as soon as possible to help them achieve normal glucose and lipid profiles [24].

Chronic hyperglycemia, a symptom of diabetes mellitus, has long-term consequences when combined with other risk factors, including microvascular and macrovascular issues that can lead to organ damage, these Microvascular and macrovascular disorders are the main cause of morbidity and mortality in diabetic patients [25]. Diabetic nephropathy, neuropathy, and retinopathy are microvascular complications of diabetes, whereas CVD and stroke are macrovascular complications [26].

In vitro investigations have connected short-term insulin increases to increased TG synthesis. Metabolic syndrome, which is described as a clustering of several metabolic risk factors in one individual, is a significant component of atherogenic dyslipidemia, elevated blood pressure, high glucose, and a prothrombotic state [27]. With over 4 million diabetes cases registered in 2015, T2DM is a severe public health concern. Cardiovascular disease has been the leading cause of death for more than a decade, and diabetes is predicted to have a part in the

vascular disease burden [28]. Therefore, this study was done to evaluate the RBP4 in diabetic patients with hypothyroidism and CVD.

### **Baseline characteristics of the studied subjects**

In this study, the baseline characteristics of included patients revealed that there were no significant differences in age, duration of CVD, sex, and BMI among the groups ( $P>0.05$ ). However, there were significant differences in duration of DM and duration of hypothyroidism, among all groups ( $P<0.05$ ). For individuals with T2DM, biochemical screening recommendations are less obvious and are dependent on characteristics including sex, ethnicity, and age [29]. Gopinath et al found no difference in the 5-year incidence of thyroid dysfunction in elderly individuals with and without diabetes [30], and another study by Joffe and Distiller also reported no development of hypothyroidism in female type 2 diabetes who manifested subclinical disease [29]. This contrasts with the data presented in this study, which highlights the increased prevalence of hypothyroidism in patients with T2DM.

According to modifiable risk factors such as hypertension (SBP and DBP), our study found statistically significant variations in SBP and DBP across all categories. The processes underlying the relationship between diabetes and hypertension have been the topic of much debate and are yet unknown. Hyperinsulinemia and peripheral insulin resistance are thought to hinder insulin-mediated renal salt resorption, contributing to hypertension [31]. Hyperinsulinemia causes vascular over activity as a result of sympathetic activation, as well as enhanced erythrocyte  $\text{Na}^+/\text{Li}^+$  counter transport, which is linked to hypertension [32].

### **Diabetic markers, lipid profile, and thyroid profile among studied groups**

In terms of diabetes indicators, our findings demonstrated a highly statistically significant difference in FBG between the patient groups and the control group ( $P<0.001$ ). Furthermore, HbA1c levels changed slightly across patient groups ( $P<0.001$ ), while Insulin and HOMA-IR values differ considerably from the control group ( $P<0.001$ ) which is supported by previous studies on T2DM patients [33, 34]

Regarding the lipid profile, our study revealed that the mean concentration of TC in (T2DM+Hyp+CVD) group was significantly higher than its concentration in other groups ( $312\pm 53.8$ ). There were statistically differences in all groups regarding TG concentration. The lowest concentration of TG was revealed in the group of hypothyroidism (Group 6). Similar to the results of the present study, Soma *et al.* showed that relative risk of MI correlates directly with TG and inversely with HDL-c levels [35]. Singh *et al.* had found inverse correlations between thrombus formation and HDL-c levels, with enhanced platelet-dependent thrombus at low HDL-c levels and vice versa [36].

As per thyroid profile, this study displayed that the mean TSH concentration in the (T2DM+Hyp) group was considerably higher ( $7.5\pm 6.3$ ) than in the other groups. In addition, in terms of FT3 level, all groups had statistically significant

differences ( $P < 0.001$ ). There were significant variations in FT4 levels between all groups, hypothyroidism, CVD, and/or DM, with the lowest concentration in Group 7 (Hyp+CVD).

To make sense of the above information, there is limited agreement on thyroid disease screening procedures in normal diabetes management [37]. The key differences are thyroid function tests, testing intervals, whether routine screening is recommended for all diabetes patients, and whether a special screening policy is required in diabetic patients [38].

In one study, the 5-year incidence of thyroid dysfunction in older T2DM patients was not substantially greater than in non-diabetic controls [30]. Kadiyala *et al* [2] findings of a 0% progression rate to overt hypothyroidism in women with T2DM and subclinical hypothyroidism raises even more doubt on the utility of routine yearly screening in T2DM patients.

### **Retinol binding protein-4 among studied groups**

Regarding the RBP4, our investigations revealed that the mean RBP4 value in the Group5 (T2DM+Hyp+CVD) group was considerably higher ( $1163.11 \pm 398.8$ ) than in the other groups. There were statistically significant variations between all groups and control groups ( $P < 0.001$ ). RBP4 is primarily produced by hepatocytes and adipocytes [37]. RBP4 may induce abnormal vascular smooth muscle cell proliferation and migration, which is critical for the development of coronary atherosclerosis [38]. Furthermore, elevated levels of RBP4 aided macrophage-derived foam cell production by stimulating cholesterol uptake, speeding up the progression of atherosclerosis [39]. Other research has found no link between RBP4 levels in the blood and cardiovascular risk [40]. Our findings revealed that serum RBP4 levels in hypothyroidism and CVD groups were considerably higher than in the control group. They were also favorably linked with various vascular and metabolic concerns, as well as TSH. Chaker *et al* [41] observed no link between subclinical hypothyroidism and overall risk of stroke episodes or fatal stroke in their study. Younger individuals, particularly those under the age of 50, exhibited an elevated stroke risk in stratified analyses, despite the modest number of incidents. In contrast to their euthyroid counterparts, Chaker *et al* [41] discovered that individuals with a TSH level of 7.0–9.9 mIU/l had a higher risk of fatal stroke. As a result, we sought to probe the independent association with serum RBP4 in this investigation.

### **ROC curve analysis**

Concerning the ROC curve of RBP4 in Group5 (T2DM+Hyp+CVD) the RBP4 has the diagnostic power to diagnose the diabetic patients with hypothyroidism and CVD where our study revealed that the RBP4 had an AUC of 0.850 ( $P = 0.003$ ) in this group, making it a strong diagnostic power of the concomitant diseases. When an RBP4 cut-off of  $>872.5$  pg/ml was applied to detect the disease, it yielded a sensitivity of 40%, specificity of 100%, and overall, 95% CI 0.291 to 0.859.

Insulin resistance and glucose intolerance have been linked to an increase in cardiovascular atherosclerosis in previous research [42, 43]. The prevalence of glucose intolerance in thyroid disease has been estimated at 10.8% with most cases occurring in hypothyroidism about (30%) and subclinical hypothyroidism (50%). Hyperinsulinemia is a good marker for insulin resistance which is frequently associated with other risk factors in hypothyroidism [44].

## Conclusion

In conclusion, this exploratory work highlighted RBP4 as a promising candidate for the generation of diagnostically accurate biomarker extracted from genuine samples obtained in a non-invasive way especially in the concomitant diseases of T2DM, hypothyroidism and CVD.

## Conflict of interest

None

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