Serum retinol-binding protein 4 and the risk of ischemic stroke in Egyptian patients with hypothyroidism

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Objective
Ischemic stroke (IS) is one of the major causes of disability and death worldwide. Effective prevention remains the best approach to reduce the burden of stroke. Adipokines can serve as a key messenger to central energy homeostasis and metabolic homeostasis. Retinol-binding protein 4 (RBP4), a retinol transporter, is elevated in insulin resistance. Controversy exists regarding the role of RBP4 in thyroid diseases. The objective of this study was to evaluate serum RBP4 in patients with hypothyroidism and to assess the association of serum RBP4 with susceptibility of IS.
Patients and methods
This case–control study included 50 healthy individuals as a control group and 90 patients with hypothyroidism, who were stratified into two subgroups: patients with IS and patients without IS. All participants were subjected to history taking and clinical, laboratory, and radiological evaluation.
Results
Serum RBP4 levels were significantly higher in hypothyroid patient, especially patients with IS, compared with the nonstroke group. Interestingly, serum RBP4 level was positively correlated with vascular and metabolic risk factors. Moreover, diastolic and systolic blood pressures, triglyceride, free T3, as well as thyroid-stimulating hormone, were independently correlated with serum RBP4 by linear regression analysis test. The diagnostic power of serum RBP4 level in differentiating hypothyroidism from controls was revealed at the cutoff value of 12.25, with area under the curve of 0.909 (95% confidence interval: 0.861–0.957). However, the diagnostic power of serum RBP4 level in differentiating hypothyroid patient with IS from those without stroke was revealed at the cutoff values of 11.4, with area under the curve of 0.822 (95% confidence interval: 0.737–0.906).
In conclusion, the higher levels of serum RBP4 in hypothyroidism, especially in patients with IS, were associated with metabolic and glucose abnormalities, and thus, it could be used as a promising predictive biomarker of IS in hypothyroidism.

Keywords:
hypothyroidism, retinol-binding protein 4, stroke

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Introduction

Emerging evidence demonstrated that stroke, a leading course of death or disability, shares many risk factors with coronary artery disease (CAD), such as age, smoking, hypertension, diabetes mellitus, inactivity, overweight or obesity, and dyslipidemia [1,2]. Compelling evidence explored that ischemic stroke (IS) is the most preventable disease. Although CAD and stroke share risk factors, published data on the association between hypothyroidism and stroke are insufficient and conflicting [3].

A growing body of evidence has corroborated that stroke is an important cause of adult mortality and morbidity; however, its pathogenesis is still unknown. Substantial pieces of evidence implicate proinflammatory cytokines as a critical mediator in the pathophysiology of IS [4].

Retinol-binding protein 4 (RBP4) is an ~21 kDa secreted protein that transports retinol (vitamin A) in circulation [5]. RBP4 has been well known as an important adipokine that contributes to insulin resistance in both rodent and human [6,7]. Recent clinical studies have also linked higher circulating RBP4 to cardiovascular diseases, including hypertension [8,9], heart failure [10,11], and atherosclerosis [12].

RBP4 binds to transthyretin (TTR) forming a protein complex that prevents glomerular filtration and reduces the renal clearance of RBP4 [13]. Lowering TTR could decrease circulating levels of RBP4 by promoting its

renal clearance [14]. However, it remains unclear whether increased RBP4 is a cause or a result of hypothyroidism. Thus, we carried out the present study to evaluate serum RBP4 in patients with hypothyroidism and to assess the association of serum RBP4 with susceptibility of noncardioembolic stroke. To the best of our knowledge, this is the first study that explored the correlation of serum RBP4 with susceptibility of IS in patients with hypothyroidism.

Patients and methods

This case–control study was conducted at Internal Medicine, Neurology and Clinical Pathology Departments, Faculty of Medicine, Zagazig University. After approval from the Medical Ethical Committee of the Faculty of Medicine, Zagazig University, all participants signed an informed written consent. Ninety patients with hypothyroidism [patients with elevated thyroid-stimulating hormone (TSH) and low thyroid hormones levels diagnosed as hypothyroid patients] were included in this study, who were divided into nonstroke group (n=60) and stroke group (n=30), in addition to 50 healthy controls; all participants were matched regarding age, sex, and ethnic origin.

The patients were chosen with the following inclusion and exclusion criteria. The inclusion criteria were focal or global neurological deficit lasting greater than 24 h on initial neurological evaluation. Computed tomography (CT) scan of the brain showed evidence of cerebral ischemia. However, the exclusion criteria were nonischemic etiology such as hemorrhagic stroke (patients with intracerebral hemorrhage, subarachnoid hemorrhage). Patients with a history of respiratory disease, cancer, severe hepatic and renal diseases, acute illness, hormonal therapy, any active inflammatory diseases, alcoholism, carotid artery surgery, and chronic heart failure were excluded from the study.

All patients in the study were subjected to the following: thorough history taking and full clinical assessment including a general and neurological examination. Stroke severity within 72 h of the onset of symptoms was assessed using the National Institute of Health Stroke Scale [15]. CT scan of the brain was performed for each patient to exclude intracranial hemorrhage and to diagnose cerebral infarction including its site and size. If the CT scan result was negative, it was repeated after 72 h. The size of the lesion was calculated according to the formula 0.5×A×B×C [where A and B are the largest perpendicular diameters measured on CT and C is the slice thickness (10 mm).

Blood sampling and laboratory testing

Blood samples were drawn from all participants after an overnight fast. Sera were separated after 1 h longstanding and stored at −80°C. We determined the fasting plasma glucose (FPG) using the glucose oxidase method (Roche Cobas 8000-e507; Roche Diagnostics, Basel, Switzerland) (Spinreact, Girona, Spain). Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured by routine enzymatic methods (Roche Cobas 8000-e507). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [16]. 2.2 Assay of thyroid function.

The serum concentrations of free T4 (FT4), free T3 (FT3), and serum thyrotrophin (TSH) were measured by Roche Cobas 8000-e602.

Measurement of serum retinol-binding protein 4

Serum RBP4 was measured with commercially available ELISA kits according to the manufacturer's instructions (Abnova, Abnova Corporation, Taipei City, Taiwan) [12].

Statistical analysis

We examined the general characteristics of the study population using mean (SD) for continuous variables and numbers (%) for categorical variables. Pearson's correlation coefficient was used to assess the association between serum RBP4 and clinical as well as biochemical tests. Receiver operating characteristic (ROC) analysis was performed to assess the potential accuracy of serum RBP4, the area under the curve (AUC), and the cutoff values for differentiating of hypothyroidism and stroke among the studied population. A linear regression analysis was done to detect the main predictors of serum RBP4 values in hypothyroidism group. We considered P to be significant at less than 0.05 with a 95% confidence interval (CI). All analyses were conducted using SPSS version 21 (Chicago, Illinois, USA).

Results

Clinical, anthropometric, and laboratory characteristics of all studied participants

Among the studied participants, 90 patients with hypothyroidism (11 males and 69 females; mean age, 48.7±12.3 years) were enrolled. The control group consisted of 50 healthy participants (28 males and 22 females; mean age, 49.5±14.14 years). Hypothyroidism cases had significantly higher values of systolic and diastolic blood pressures compared

Table 1 Clinical, anthropometric, and laboratory characteristics of all studied participants

	Control group (mean±SD) (*n*=50)	Hypothyroidism group (mean±SD) (*n*=90)	*P*
Systolic blood pressure (mmHg)	127.79±9.2	140.4±61.9	<0.05*
Diastolic blood pressure (mmHg)	73.65±7.5	85.48±8.5	<0.001*
BMI (kg/m^2)	26.47±3.35	35.51±8.89	<0.001*
Total cholesterol (mg/dl)	166.76±18.87	199.9±29.36	<0.001*
Triglycerides (mg/dl)	115.62±49.75	136.76±70.39	0.0570
LDL cholesterol (mg/dl)	122.22±26.79	147.1±53.76	<0.001*
HDL cholesterol (mg/dl)	54.92±4.26	33.9±16.8	<0.01*
Fasting plasma glucose (mg/dl)	91.62±6.276	118.9±41.19	<0.001*
FT3 (pg/ml)	2.11±0.34	1.12±0.35	<0.001*
FT4 (ng/dl)	3.32±0.34	0.72±0.811	<0.001*
TSH (μIU/ml)	2.5±0.97	18.48±10.82	<0.001*

FT3, free triidothyronine; FT4, free thyroxine; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein; TSH, thyroid-stimulating hormone. *P<0.05.

with the control group. Moreover, the values of BMI, FPG, TC, triglyceride (TG), LDL, and TSH were increased compared with controls. On the contrary, patients with hypothyroidism had significantly lower values of HDL, FT4, and FT3 (P<0.001*) (Table 1).

Clinical, anthropometric, and laboratory characteristics of hypothyroidism groups, as shown in Table 2.

Stroke group had significantly higher values of systolic blood pressure, diastolic blood pressure, BMI, FPG, TG, TSH, and TG than non-IS group (P<0.001*). On the contrary, there were significantly lower values of FT3, FT4, and HDL in stroke group compared with nonstroke group (P<0.001*).

Comparison of serum retinol-binding protein 4 (μg/ml) levels in studied groups

Hypothyroid patients with IS had significantly higher values of serum RBP4 (20.31±6.257) compared with hypothyroid patients without IS (12.3±5.6) and controls (7.8±2.37) (P<0.001*) (Fig. 1).

Pearson's correlation between serum retinol-binding protein 4 (μg/ml) with other parameters

There was a positive correlation between serum RBP4 and BMI, TC, TG, FPG, as well as TSH (Fig. 2). However, there were significantly negative correlations between serum RBP4 level and HDL and FT3 as well as FT4 (P<0.001*) (Table 3).

Table 2 Clinical and laboratory characteristics of case groups

	Nonstroke group (mean ±SD) (*n*=60)	Stroke group (mean±SD) (*n*=30)	P_2
Systolic blood pressure (mmHg)	137.5±19.3	147.3±14.5	<0.001*
Diastolic blood pressure (mmHg)	77.2±9.1	85.18±9.7	<0.05*
BMI (kg/m^2)	30.5±8.94	43.7±2.49	<0.001*
Total cholesterol (mg/dl)	168.3±54.9	222.7±59.46	0.521
Triglycerides (mg/dl)	121.8±±47.37	167.8±84.81	<0.01*
LDL cholesterol (mg/dl)	140.5±48.61	162.1±57.5	0.792
HDL cholesterol (mg/dl)	34.08±9.2	31.8±6.4	0.227
Fasting plasma glucose (mg/dl)	91.7±5.82	162.7±33.5	<0.001*
FT3 (pg/ml)	1.13±0.3	1.01±0.21	<0.001*
FT4 (ng/dl)	1.3±0.3	1.2±0.2	<0.001*
TSH (μIU/ml)	9.6±5.8	16.3±6.4	<0.001*

FT3, free triidothyronine; FT4, free thyroxine; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein; TSH, thyroid-stimulating hormone. *P<0.05.

Linear regression analyses in patients with hypothyroidism

Linear regression analysis test was done to assess the main independent parameters associated with serum RBP4. Our results showed that diastolic and systolic blood pressures, TG, FT3, as well as TSH were independently correlated with serum RBP4 (Table 4).

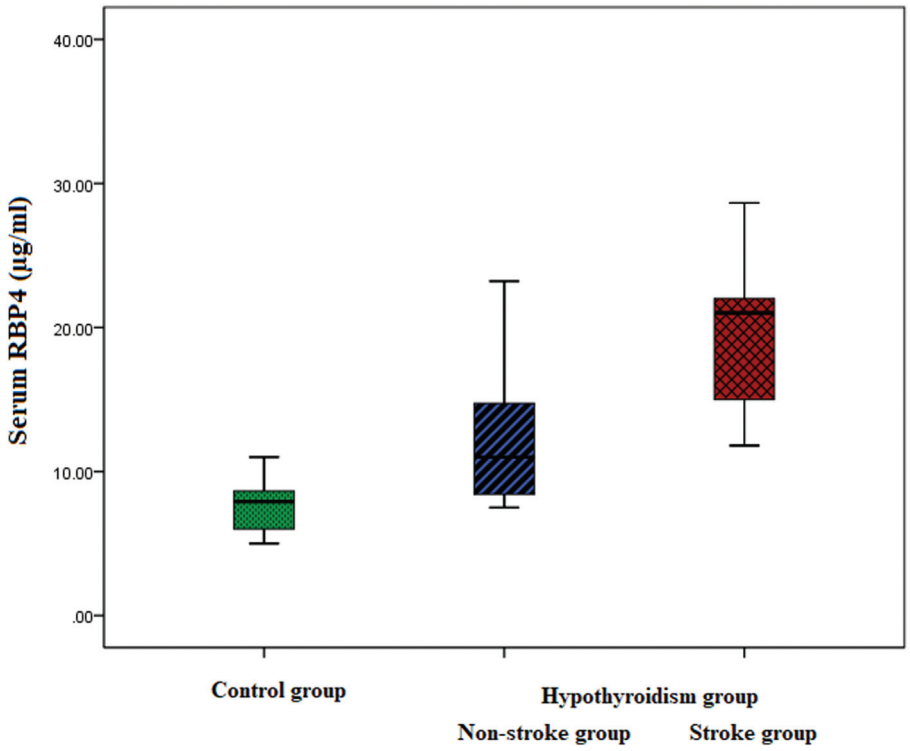
The accuracy of serum retinol-binding protein 4 (μg/ml) for discriminating hypothyroidism group from control group using receiver operating characteristic curve analysis

We further investigated the potential diagnostic value of serum RBP4 by ROC curves, as presented in Fig. 3. In the studied groups, when we discriminate hypothyroidism from controls, the cutoff values were 12.25 and the AUC was 0.909 (95% CI: 0.861–0.957). Additionally, the sensitivities and the specificities were 96.7 and 74.5%, respectively.

The accuracy of serum retinol-binding protein 4 level for discriminating ischemic stroke group from the nonstroke group by receiver operating characteristic curve analysis

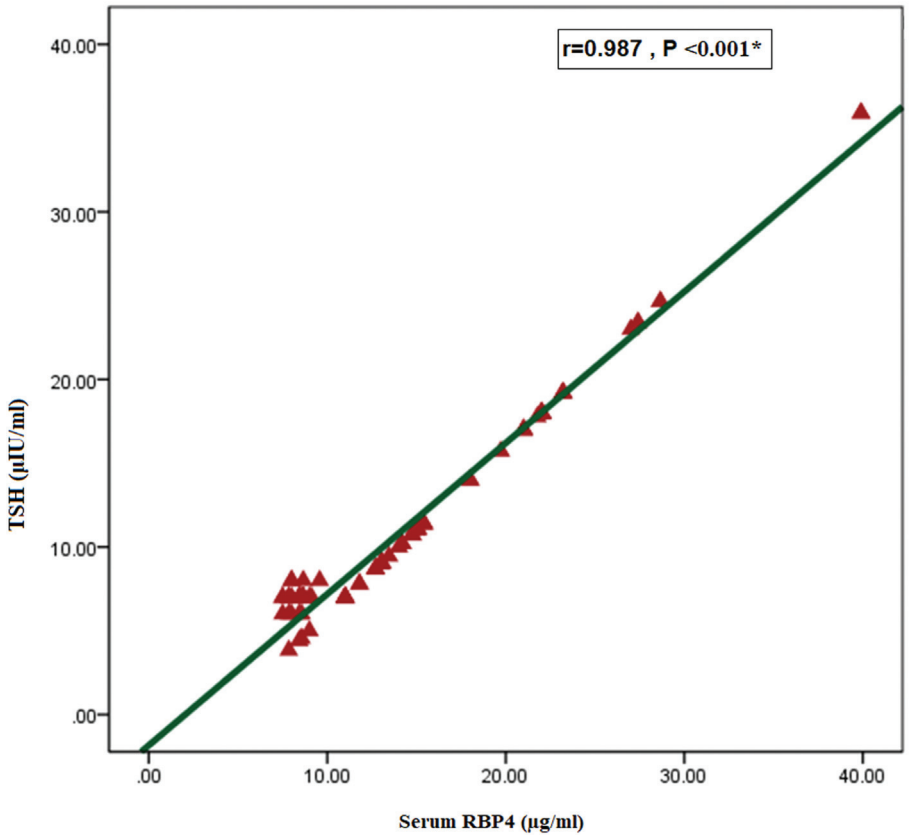
Regarding the diagnostic value of serum RBP4 level for discriminating IS group from the nonstroke group by ROC curves as presented in Fig. 4; among patients with hypothyroidism when we tried to discriminate IS group from nonstroke group, the cutoff value was 11.4, and the AUC was 0.822 (95% CI: 0.737–0.906). Additionally, the sensitivities and the specificities were 94.3 and 96%, respectively.

Figure 1



Serum retinol-binding protein 4 levels in the studied groups.

Figure 2



Correlation between serum retinol-binding protein 4 levels and thyroid-stimulating hormone in the hypothyroidism.

Discussion

Mounting evidence indicates that stroke is the third leading cause of death. The mortality rate after an ischemic incident is very high 30% and survivors almost always face disabilities that require costly long-term care [17].

RBP4 is a protein synthesized mainly by hepatocytes and adipocytes [18]. RBP4 could promote the abnormal proliferation and migration of vascular smooth muscle cells, which is important for the formation of coronary atherosclerosis [19]. Moreover, high level of RBP4 facilitated macrophage-derived foam cell formation through activating cholesterol uptake, and thus accelerated atherosclerosis progression [20].

Table 3 Pearson's correlations between serum retinol-binding protein 4 (μg/ml) and other parameters of patients with ischemic stroke

Variables	RBP4	
	r	*P*
Systolic blood pressure (mmHg)	0.134	0.115
Diastolic blood pressure (mmHg)	0.056	0.444
BMI (kg/m^2)	0.628	<0.001*
Total cholesterol (mg/dl)	0.546	<0.001*
Triglycerides (mg/dl)	0.881	<0.001*
LDL cholesterol (mg/dl)	0.086	0.226
HDL cholesterol (mg/dl)	0.289	<0.001*
Fasting plasma glucose (mg/dl)	0.487	<0.001*
FT3 (pg/ml)	–0.277	<0.001*
FT4 (ng/dl)	–0.292	<0.001*
TSH (μIU/ml)	0.987	<0.001*

FT3, free triidothyronine; FT4, free thyroxine; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein; RBP4, retinol-binding protein 4; TSH, thyroid-stimulating hormone. *$P<0.05$.

Even though omics studies observed increased RBP4 level in patient with CAD [21,22], some other studies demonstrated no significant relationship between serum RBP4 and cardiovascular risk [23,24]. Despite the high mortality and morbidity associated with IS, currently established therapies are limited. Thus, the current study aimed to evaluate serum RBP4 in patients with hypothyroidism and to assess the association of serum RBP4 with susceptibility of IS.

The results of the current study revealed that hypothyroid patient had significantly higher values of cardiometabolic risks compared with controls. Even more importantly, serum RBP4 levels were significantly higher in the hypothyroid group compared with controls.

Concordance with our finding, Choi *et al.* [25] observed that RBP4 level was elevated in hypothyroidism independently of obesity in elderly participants with normal glucose tolerance.

Similar to our findings, Kokkinos *et al.* [26] detected that serum RBP4 levels were significantly higher in clinical and subclinical hypothyroidism. Moreover, Güdücü *et al.* [27] detected higher levels of RBP4 in association with free testosterone and TSH in postmenopausal women.

RBP4 binds to TTR forming a protein complex that prevents glomerular filtration and reduces the renal clearance of RBP4. Thus, lowering TTR could decrease circulating levels of RBP4 by promoting its renal clearance [14].

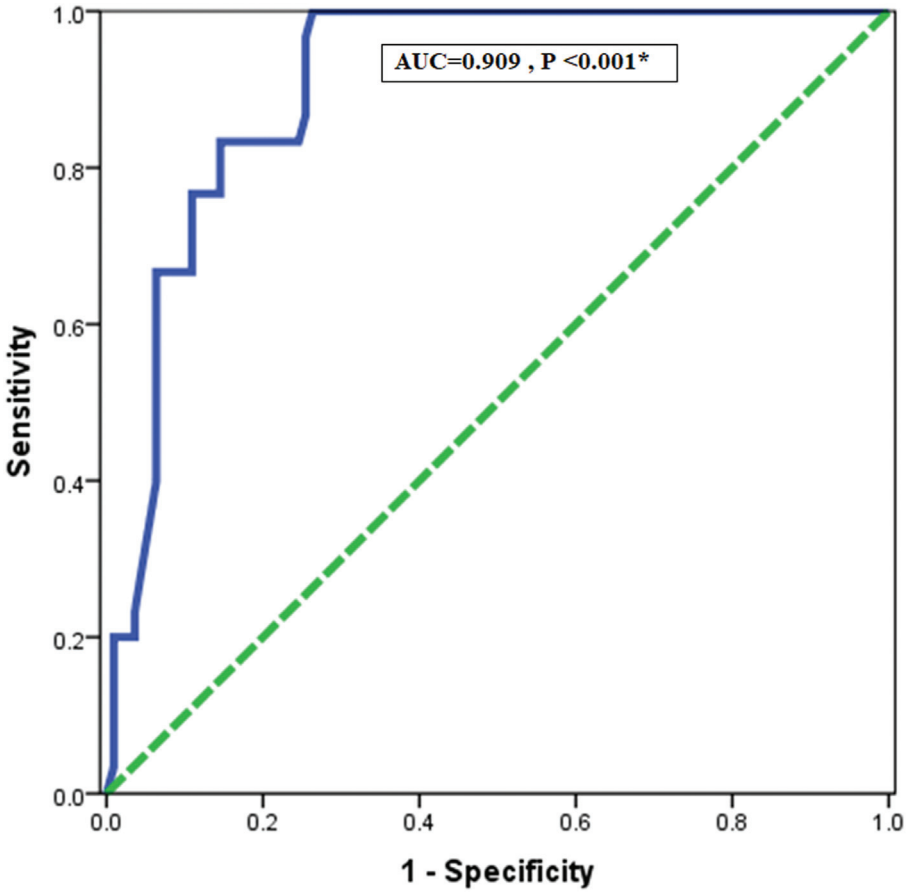
The results presented herein are innovative as this study performs a robust evaluation of serum RBP4

Table 4 Linear regression analyses in patients with ischemic stroke. to test the influence of the main independent variables against serum retinol-binding protein 4 (μg/ml) (dependent variable)

Model	Unstandardized coefficients	Standardized coefficients				95% CI	
	B	SE	Beta	t	*P*	Lower Bound	Upper Bound
Constant	2.759	0.205		13.477	<0.001*	2.352	3.166
Diastolic blood pressure	–0.00	0.003	–0.194	–2.270	<0.05*	–0.011	–0.001
Systolic blood pressure	–0.002	0.001	–0.186	–2.049	<0.05*	–0.004	0.000
Total cholesterol	–0.002	0.002	–0.013	–0.757	0.451	–0.006	0.003
Triglycerides	0.008	0.003	0.081	2.602	0.011	0.002	0.015
FT3 (pg/ml)	–1.297	0.440	–0.052	–2.950	0.004	–2.172	–0.422
TSH (μIU/ml)	0.978	0.034	0.894	28.554	0.000	0.910	1.046
Fasting plasma glucose	0.001	0.005	0.006	0.214	0.831	–0.009	0.001
Triglycerides	0.008	0.003	0.081	2.602	0.011	0.002	0.008
BMI	0.025	0.021	0.032	1.210	0.230	–0.016	0.025

FT3, free triidothyronine; FT4, free thyroxine; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein; RBP4, retinol-binding protein 4; TSH, thyroid-stimulating hormone. *$P<0.05$.

Figure 3



Accuracy of serum retinol-binding protein 4 for discriminating patients with hypothyroidism from control group by receiver operating characteristic curve analysis.

as a diagnostic biomarker of IS. Noteworthy, our results confirmed that serum RBP4 levels were significantly higher in IS compared with the non-IS group. Interestingly, they were positively correlated with other vascular and metabolic risks and TSH.

The results of Chaker *et al.* [28] found no association between subclinical hypothyroidism and overall risk of stroke events or fatal stroke. In stratified analyses, younger participants, particularly those under the age of 50 years, had increased stroke risk, although the number of events was small.

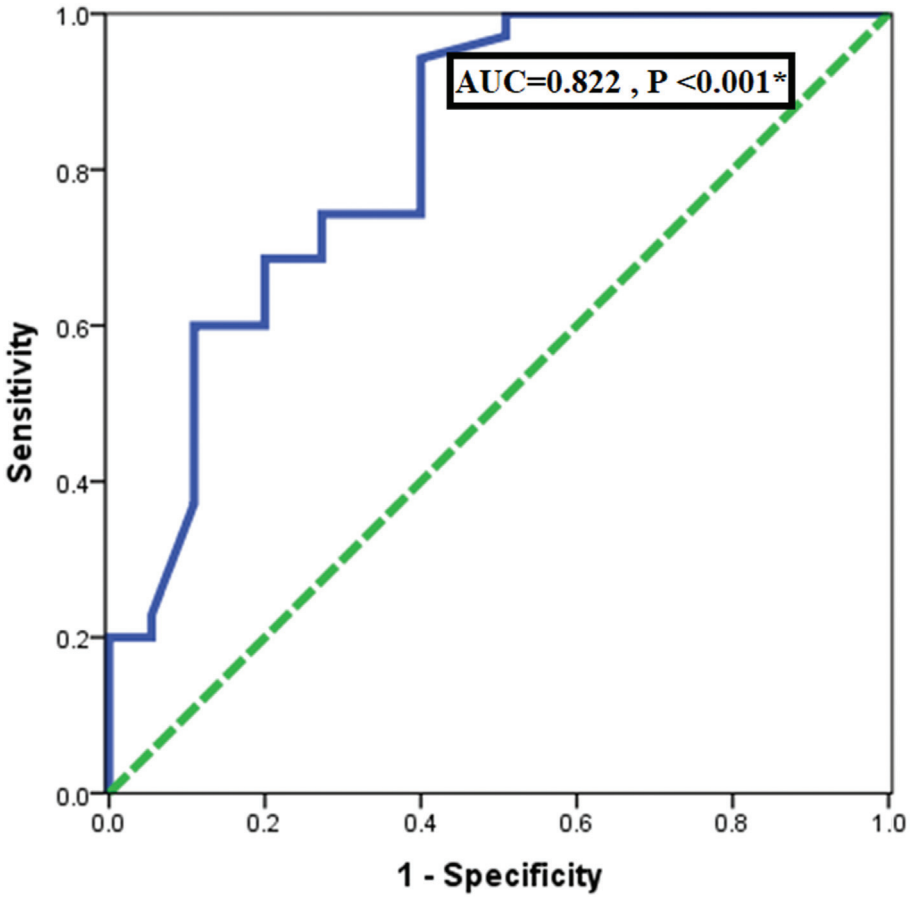
Interestingly, Chaker *et al.* [28] found that patients with a TSH level of 7.0–9.9 mIU/l also had an increased risk of fatal stroke compared with their euthyroid counterparts. We in this study attempted to pierce out the independent correlation with serum RBP4. Our results showed that there was a positive correlation between serum RBP4 and BMI, TC, TG, FPG, as well as TSH. However, there was a significantly negative correlation between serum RBP4 level with HDL and FT3 as well as FT4. Linear regression analysis test was done to assess the main independent parameters associated with serum RBP4. Our results showed that diastolic and systolic blood pressures, as well as postprandial glucose and HbA1c, were independently correlated with serum RBP4.

In Chaker *et al.* [23], RBP4 levels were positively correlated with TC, LDL-C, and BMI, which are all risk factors for CAD. Interestingly, other studies reported that increased circulating RBP4 is not only correlated with established cardiovascular risk factors such as obesity and dyslipidemia but also correlated with the prevalence of atherosclerotic diseases and CAD [13]. The exact mechanism by which increased circulating RBP4 promotes the development of CAD remains unclear. In endothelial cells, RBP4 could induce inflammation and mitochondrial dysfunction, both of which play key roles in atherogenesis [24].

Conclusion

The current study provides evidence of higher levels of serum RBP4 in hypothyroidism, especially in patients

Figure 4



Accuracy of serum retinol-binding protein 4 levels for discriminating ischemic stroke among patients with hypothyroidism by receiver operating characteristic curve analysis.

with IS, associated with metabolic and glucose abnormalities, and, thus, it could be a promising predictive biomarker of IS in hypothyroidism.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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