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Role of Zinc Alpha2-Glycoprotein and Retinol-Binding Protein-4 in Iraqi Women with Thyroid Dysfunctions

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Abstract

Thyroid dysfunction (TD) and diabetes mellitus (DM) are two of the most frequent chronic endocrine disorders. This study included 60 women with thyroid disorders: 30 with hypothyroidism and 30 with hyperthyroidism, aged between 20 and 40 years. This study used 30 healthy women aged 20-40 years as a control group. Anthropometric measurements of Body Mass Index (BMI), hematological analysis of glycated hemoglobin (HbA1c), biochemical parameters (Thyroid-stimulating hormone TSH, Free triiodothyronine FT3, Free thyroxine FT4, Fasting Blood Sugar FBS), and adipokine concentrations zinc-α2-glycoprotein (AZGP-1) and retinol-binding protein-4 (RBP-4) were performed for all participants. The results revealed that the serum levels of zinc-α2-glycoprotein and retinol-binding protein-4 are significantly higher in hyperthyroidism (P<0.0001) compared to both healthy and hypothyroid women. Besides that, zinc-α2-glycoprotein has a negative correlation with TSH in the hyperthyroidism group. In the hypothyroidism group, it correlates positively with retinol-binding protein-4. In addition to that, retinol-binding protein-4 is positively correlated with FT3 and FT4 and negatively correlated with BMI in the hyperthyroidism group. For this reason, we conclude that these adipokines can aid in the diagnosis and monitoring of thyroid disorders, thereby preventing the onset of disease complications associated with type 2 diabetes mellitus. It is important to highlight that women with hypothyroidism are more likely than those with hyperthyroidism to develop diabetes.

Keywords: Diabetes mellitus, Hyperthyroidism**,** Hypothyroidism, Retinol-binding protein-4, Zinc-α2-glycoprotein.

دور الخارصين الفا−2 كلايكوبروتين والريتينول المرتبط بالبروتين−4 في النساء العراقيات المصابات باختلال وظائف الدرقية

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الخلاصة

يعد ضعف الغدة الدرقية (TD) وداء السكري (DM) من أكثر اضطرابات الغدد الصماء المزمنة شيوعًا. تضمنت هذه الدراسة 60 امرأة تعاني من اضطرابات الغدة الدرقية: 30 مصابة بقصور الغدة الدرقية و 30 مصابة بفرط نشاط الغدة الدرقية ، تتراوح أعمارهن بين 20 و 40 عامًا. استخدمت هذه الدراسة 30 امرأة من الاصحاء تتراوح أعمارهن بين 20−40 سنة كمجموعة سيطرة . تم اجراء القياسات الأنثروبومترية لمؤشر كتلة

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الجسم (BMI) ، تحليل الدم للهيموجلوبين السكري (HbA1c) ، (المعلمات البيوكيميائية هرمون تحفيز الغدة الدرقية TSH ، ثلاثي أيودوثايرونين الحر FT3 ، هرمون الغدة الدرقية الحر FT4 ، سكر الدم الصائم FBS) ، وتركيزات الأديبوكين الخارصين الفا−2 كلايكوبروتين(AZGP−1) والبروتين المرتبط بالريتينول−4−RBP) (4لجميع المشاركين. أوضحت النتائج أن مستوى الخارصين ألفا−2 كلايكوبروتين و البروتين المرتبط بالريتينول− 4 أعلى بكثير في فرط نشاط الغدة الدرقية (P <0.0001) مقارنة بالنساء الأصحاء والنساء المصابات بقصور الغدة الدرقية. بالإضافة إلى ذلك ، فإن الخارصين ألفا−2 كلايكوبروتين له علاقة سلبية مع TSH في مجموعة فرط نشاط الغدة الدرقية. بينما في مجموعة قصور الغدة الدرقية ، فإنه يرتبط بشكل إيجابي ببروتين رابط الريتينول −4. بالإضافة إلى ذلك ، يرتبط البروتين المرتبط بالريتينول−4 ارتباطًا إيجابيًا بـ FT3 و FT4 ويرتبط سلبًا بمؤشر كتلة الجسم في مجموعة فرط نشاط الغدة الدرقية. لهذا السبب ، نستنتج أن هذه الأديبوكينات يمكن أن تساعد في تشخيص اضطرابات الغدة الدرقية ومراقبتها ، وبالتالي منع ظهور مضاعفات المرض المرتبطة بداء السكري من النوع 2. من المهم تسليط الضوء على أن النساء المصابات بقصور الغدة الدرقية أكثر عرضة للإصابة بمرض السكري من أولئك المصابات بفرط نشاط الغدة الدرقية.

الكلمات المفتاحية: داء السكري، فرط نشاط الغدة الدرقية، خمول الغدة الدرقية، البروتين المرتبط بالريتينول−4، الخارصين الفا−2 كلايكوبروتين.

1. Introduction

The thyroid gland is the largest endocrine organ in the human body [1]. It is responsible for the synthesis and release of thyroxine (T4) and triiodothyronine (T3) [2]. These hormones are essential to the development, growth, and function of an ever-expanding variety of tissues and cell types [3,4]. Hypothyroidism and hyperthyroidism are the two primary categories of thyroid disorders [5]. Hypothyroidism is a clinical syndrome caused by inadequate thyroid hormone secretion from the thyroid gland [6,7]. While hyperthyroidism is a clinical disease defined by increased thyroid hormone production and release by the thyroid gland [8,9]. In particular, hypothyroidism has several symptoms, including weight gain, higher blood cholesterol, impaired lipolysis, and gluconeogenesis, as well as lower resting energy expenditure. In contrast, hyperthyroidism is characterized by higher resting energy expenditure, weight loss, lower cholesterol levels, lipolysis, and higher glucose levels [10]**.** Adipose tissue (AT) is currently known as one of the most important endocrine organs in the human body, playing a crucial role in cellular responses and metabolic homeostasis instead of only serving as inactive tissue for energy storage [11]. Adipose tissue releases a diversity of bioactive compounds, including peptides/proteins, immune molecules, and inflammatory mediators, collectively known as adipokines [12]. First of all, retinol-binding protein-4 (RBP-4) is a new adipokine that is mostly made by fat cells and the liver, but also in smaller amounts by the lungs, kidneys, testes, retina, and brain [13]. RBP-4 has been identified as a factor involved in a range of human diseases, such as impaired vision and ocular diseases, disorders related to glucose and lipid homeostasis, and cardiovascular diseases [14]. Lack of thyroid hormones may raise one's risk for diabetes and insulin resistance. In hypothyroid people, RBP-4 is positively correlated with fasting blood sugar, insulin, and insulin resistance [15]. Consequently, RBP-4 is elevated in hypothyroidism patients and positively correlates with TSH levels [16]. Secondly, zinc-α-2-glycoprotein (AZGP-1) is a distinctive adipokine released by various organs, such as the lung, liver, breast, prostate, and, mainly, adipose tissue [17]. Being that AZGP-1 is found in various regions of the body, it is predicted that it has a wide range of functions in the body, including regulating the production of melanin, ribonuclease activity, cell adhesion, fertilization, and lipolysis [18]. Apart from its function in the mobilization of lipids, it also plays a significant role in the metabolism of glucose. AZGP-1 has been demonstrated to enhance glucose metabolism as well as modulate insulin sensitivity, thereby enhancing insulin sensitivity [19]. The expression of AZGP-1 in adipose tissue revealed variability in response to many factors. Several variables, including cancer cachexia, glucocorticoids, particularly 3-adrenergic

receptor agonists, growth hormone (GH), and thyroid hormones (THs), have been identified as contributors to the elevated expression. Conversely, chronic inflammation, elevated blood leptin levels, and a high level of body fat (obesity) may prevent the release of AZGP-1 in adipose tissue [20]. This study aims to investigate the role of zinc-α2-glycoprotein (AZGP-1) and retinol-binding protein-4 (RBP-4) in Iraqi women with hyperthyroidism and hypothyroidism. Additionally, this research aims to investigate the possible risk of diabetes mellitus development in these women.

2. Materials and method

2.1. Research participants

This research involved 60 women with thyroid disorders divided into two groups: 30 of whom had hypothyroidism and 30 had hyperthyroidism; both age ranges comprised women between the ages of 20 and 40 years. The control group for this research consisted of 30 healthy women ranging in age from 20 to 40 years. The participants were recruited from the National Diabetes Center/Al-Mustansiriya University, Baghdad City. This study was approved by the Department of Chemistry, College of Science, Al-Nahrain University, Baghdad.

2.2. Exclusion criteria

The present study excluded women with the following criteria due to potential effects on adipokine levels: pregnancy, smoking, neoplasms, hypertension, diabetes mellitus, heart failure, hepatic renal diseases, and women taking drugs other than levothyroxine or carbimazole.

2.3. Sample collection

The study included drawing 8 milliliters of venous blood from both patients with thyroid disorders and healthy women who had fasted overnight for 8 to 12 hours. Participants' blood samples were separated into two tubes: six milliliters in gel tubes left to coagulate for fifteen minutes and two milliliters for hematological analysis in ethylene diamine tetraacetic acid (EDTA) tubes. The blood samples were centrifuged at 3000 g for 15 minutes at room temperature to separate the serum. Aliquots of the extracted serum were frozen in a refrigerator at -60 ℃ until analysis.

2.4. Calculating Body Mass Index (BMI)

Each participant's BMI was calculated by dividing their weight (in kilograms, Kg) by their height (in meters squared, m^2) [21].

BMI = Weight (Kg)/ height (m^2)

2.5. Hematological analysis

Glycated hemoglobin (HbA1c) was estimated using the semi-automated NycoCard Reader II (Abbott, USA).

2.6. Biochemical analysis

On a hormonal analyzer (autoanalyzer Cobas E411, Roche, Germany), the ElectroChemiLuminescence method was used to test serum total thyroid function. In addition, serum fasting blood glucose concentrations were measured with the semi-auto chemistry analyzer (Humalyzer primus, Human, Germany). While serum adipokine levels were evaluated using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions utilizing one kit, the human RBP-4 (Catalog No: E-EL-H1581) and AZGP-1 (Catalog No: E-EL-H1541) ELISA kits were supplied by Elabscience (United States) [22].

2.7. Statistical analysis

GraphPad Prism software version 7.04 (San Diego, California, USA) was used to analyze the demographic and biochemical data in the current study. The mean, standard deviation (SD), and statistically significant differences (*P*-value) between the means of the three study groups were analyzed using one-way ANOVA. The present study utilized Pearson's correlation coefficient (r) to estimate the associations between variables. A *P*-value less than 0.05 was regarded as statistically significant, and a *P*-value less than 0.0001 was highly significant. The presence of three letters (a, b, and c) in the same row in the tables indicates significant differences in a parameter among groups.

3. Results

Table 1 summarizes the findings of our research on demographic data and biochemical parameters in healthy women (Group 1), hypothyroid women (Group 2), and hyperthyroid women (Group 3). The preliminary analysis findings of the demographic data reported in Table 1 showed that there were highly significant differences in BMI between the hypothyroidism group and the control or hyperthyroidism group ($p < 0.0001$). According to our results from the laboratory data for the three groups, there were significant differences ($p < 0.0001$) in the TSH, FT3, and FT4 between all three groups. Furthermore, there were significant differences ($p < 0.01$) in FBS and HbA1c levels between the hypothyroidism group and the hyperthyroidism or control groups. Table 2 shows the levels of adipokines in each of the three groups. Statistical analysis indicated that serum AZGP-1 levels in hyperthyroidism were shown to be significantly higher ($p < 0.0001$) than in hypothyroidism or control groups. In contrast, it had fewer significant differences ($p < 0.0001$) in hypothyroidism compared to the control group. Furthermore, serum RBP-4 levels in hyperthyroidism were significantly higher ($p < 0.0001$) than in hypothyroidism or control groups. Contrariwise, it had fewer significant differences ($p = 0.0017$) in hypothyroidism compared to the control group. The present study identified Pearson's correlation coefficient between the different research variables. In female hyperthyroid patients, the investigation's findings showed AZGP-1 had a negative correlation with TSH (r = -0.385, $P = 0.036$). RBP-4 demonstrated a positive correlation with FT3 (r = 0.508, $P = 0.004$) and FT4 (r = 0.411, $P = 0.024$). despite having a negative correlation with BMI (r = -0.423, $P = 0.020$). Also, it was found that in female hypothyroid patients, AZGP-1 correlated positively with RBP-4 (r = 0.493, $P = 0.006$). Additionally, a significant positive correlation was found between FBS and HbA1c in both hypo and hyperthyroidism groups (r = 0.825, $p < 0.0001$), (r = 0.716, $p < 0.0001$) respectively.

Table 1: The demographic data and biochemical values for the groups with hypothyroidism, hyperthyroidism, and controls

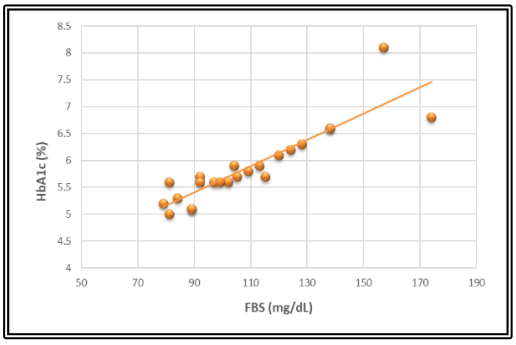
Parameter	Control	Hypothyroidism	Hyperthyroidism	p-value	Sign
BMI (kg/m^2)	24.39±3.40 [a]	28.23±4.63 [b]	21.38±2.26 [c]	<0.0001	HS
TSH (uIU/mL)	2.19±0.52 [a]	9.15±2.37 [b]	0.12±0.02 [c]	<0.0001	HS
FT3 (pg/mL)	2.46±0.30 [a]	1.26±0.17 [b]	4.44±0.61 [c]	<0.0001	HS
FT4 (ng/dL)	1.15±0.17 [a]	0.41±0.13 [b]	2.08±0.64 [c]	<0.0001	HS
FBS (mg/dL)	86.95±11.18 [a]	105.30±23.40 [b]	99.77±14.09 [b]	0.0023	S
HbA1c (%)	5.23±0.34 [a]	5.80±0.70 [b]	5.63±0.42 [b]	0.0044	S

The mean and standard deviation (SD) for each value are shown; *p*-values are denoted as **S**: *p*-value < 0.05 (significant), **NS**: *P*-value > 0.05 (non-significant), **HS**: *p*-value < 0.0001 (highly significant). The three letters (a, b, and c) in the same row of the tables indicate statistically significant differences in a parameter among groups. As for the groups that contain the same letter, there are no significant differences between them.

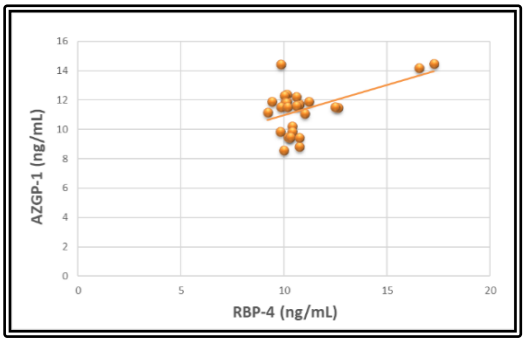
Table 2: Comparison of the adipokine concentrations for the control, hypothyroidism, and hyperthyroidism groups

Parameter	Control	Hypothyroidism	Hyperthyroidism	*P*-value	Sign
AZGP-1 (ng/mL)	15.13±1.32 [a]	10.86±1.79 [b]	28.60±4.49 [c]	<0.0001	HS
RBP-4 (ng/mL)	13.94±0.76 [a]	11.32±1.50 [b]	27.51±3.80 [c]	<0.0001	HS

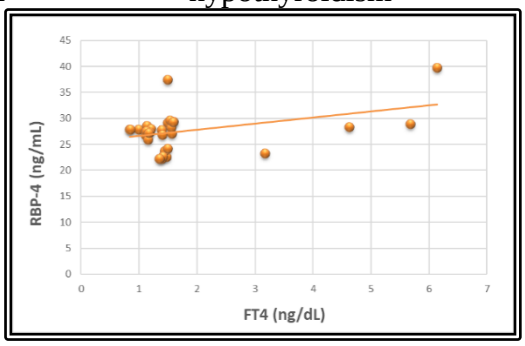
The mean and standard deviation (SD) for each value are shown; *p*-values are denoted as **S**: *p*-value < 0.05 (significant), **NS**: *P*-value > 0.05 (non-significant), **HS**: *p*-value < 0.0001 (highly significant). The three letters (a, b, and c) in the same row of the tables indicate statistically significant differences in a parameter among groups. As for the groups that contain the same letter, there are no significant differences between them.



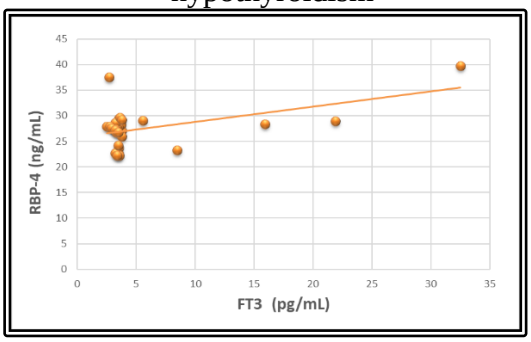
Correlation between FBS and HbA1c in in hypothyroidism



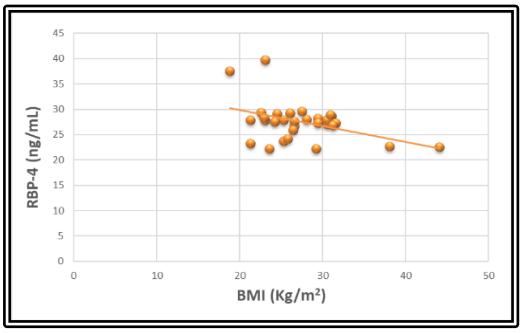
Correlation between AZGP-1 and RBP-4 hypothyroidism



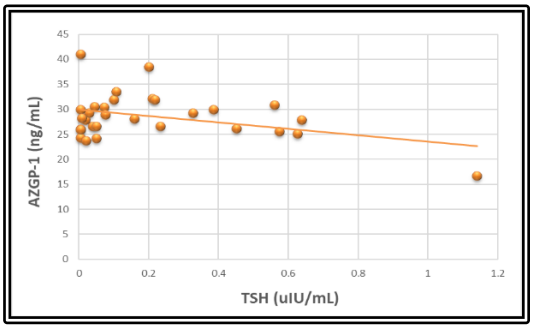
Correlation between FT4 and RBP-4 in Hyperthyroidism



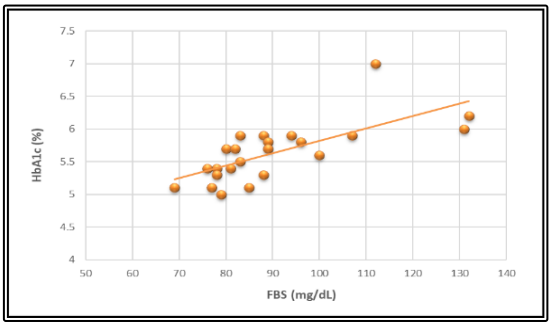
Correlation between FT3 and RBP-4 in Hyperthyroidism



Correlation between BMI and RBP-4 Hyperthyroidism



Correlation between AZGP-1 and TSH in Hyperthyroidism



Correlation between FBS and HbA1c in hyperthyroidism

4. Discussion

The purpose of this study was to evaluate the serum AZGP-1 and RBP-4 levels in women with hypothyroidism and hyperthyroidism in order to ascertain the role that adipokines play in this type of disease. We observed that hyperthyroid women had significantly lower TSH levels than the control group, as well as significantly higher FT3 and FT4 levels. Compared to the control group, women with hypothyroidism had significantly lower levels of FT3 and FT4 and significantly higher levels of TSH. The findings of a previous study [23] are consistent with our findings. This may be because the thyroid hormones thyroxine (T4) and *tri*-iodothyronine (T3) interact in a complicated way with the pituitary hormone thyrotropin (TSH). Thyroid stimulating hormone and thyroid hormones have a negative feedback system. As a result, TSH levels prove to be the most sensitive indicator of thyroid function [24]. The results of our investigation indicate that the mean value of AZGP-1 is significantly greater in the hyperthyroidism group compared to the control and hypothyroidism groups, suggesting that hyperthyroidism affects serum AZGP-1 concentration and that this adipokine is associated with thyroid hormones. These findings further support the ideas of previous research [25]. One reason could be that thyroid hormones (THs) may control what adipocytes do because adipocytes have a lot of thyroid hormones (THs) and TSH receptors, which work like thyroid receptors [26]. Thereby, excess thyroid hormones may influence the secretion of adipokines. According to this research, AZGP-1 may decrease obesity, increase insulin sensitivity, and reduce the risk of developing diabetes through its ability to inhibit the accumulation of lipids in skeletal muscle [27]. Regrettably, this is the first study that has examined the relationship between thyroid hormone and zinc-α2-glycoprotein in hypothyroidism. Our study found that female patients with hypothyroidism had significantly lower blood levels of zinc-α2-glycoprotein (AZGP-1) than the control and hyperthyroidism groups. The link between hypothyroidism and hypometabolism might be responsible for this result. According to studies, individuals with excessive body fat have lower blood levels of zinc and AZGP-1. and their deficiency contributes to the development of obesity and type 2 diabetes [20]. RBP-4 has been identified as a major adipokine that is involved in the development of insulin resistance and can link adipose tissue dysfunction to T2D [28]. Thyroid hormones inhibit RBP-4 and reduce insulin resistance by decreasing inflammation in adipose tissue. A lack of thyroid hormones raises the risk of insulin resistance and diabetes type 2 [29]. The present investigation found that RBP-4 levels were significantly lower in hypothyroid female patients than in healthy controls. The findings of the current investigation were consistent with those of a previous study [30]. The fact that transthyretin (TTR), a protein transporter, transports both RBP-4 and the thyroid hormone thyroxine (T4) through the circulatory system may be used to explain the reported findings. RBP-4 renal clearance is lowered and glomerular filtration is inhibited when RBP-4 binds to TTR, forming a protein complex. Lowering TTR might thus decrease circulating RBP-4 levels by enhancing its renal clearance [31]. Additionally, medical treatments that enhance thyroid function may reduce RBP-4 levels in the blood. The concentration of RBP-4 in the present study's hyperthyroid female patients was significantly greater than in the control

group. This outcome corresponds with an earlier study [32]. Indicating that serum RBP-4 levels are raised in people with Graves disease and rise as the condition worsens. Additionally, inflammatory markers like CXCL-13 and interleukin-6 (IL-6) were found to have a positive correlation with RBP-4 levels. The subsequent investigation revealed the cause of the elevated RBP-4 levels in hyperthyroidism: RBP-4 has been linked to the control of inflammation. As a result, cytokines and other inflammatory mediators may increase the levels of RBP-4. This increase may be the result of inflammation brought on by hyperthyroidism [33]. Thyroid hormones are important metabolic regulators. Through their interactions with several organs, they have a direct effect on insulin sensitivity, insulin secretion, blood glucose, and carbohydrate metabolism [34]. Both hypothyroidism and hyperthyroidism might affect insulin resistance and glucose metabolism, thereby increasing the incidence of type 2 diabetes [35]. In the case of hypothyroidism, altered lipid metabolism in adipose tissue, which is seen in obesity, reduces insulin's ability to bind to insulin receptors. Moreover, there is decreased translocation of GLUT-4 glucose transporters on the plasma membrane, which causes a reduction in glucose absorption in muscles and adipose tissue. The combination of impaired insulin binding and reduced glucose absorption leads to a rise in blood glucose levels. Consequently, diabetes risk is increasing [36]. Concerning hyperthyroidism, excessive thyroid hormones may increase glucose absorption in the gastrointestinal tract, thereby promoting endogenous glucose synthesis and exacerbating hyperglycemia. Moreover, hyperthyroidism can increase the liver's synthesis of glucose and decrease the ability of peripheral tissues to utilize it, resulting in higher blood glucose levels [37]. Insulin secretion is influenced directly by each of the aforementioned thyroid hormones. Hypothyroidism lowered beta-cell insulin production, but hyperthyroidism enhanced beta-cell responsiveness to glucose owing to increased beta-cell mass. This may lead to insulin resistance and an increased risk of developing type 2 diabetes [38].

5. Conclusion

According to the present study's findings, we conclude that these adipokines can aid in the diagnosis and monitoring of thyroid disorders, thereby preventing the onset of disease complications associated with type 2 diabetes mellitus. It is important to highlight that women with hypothyroidism are more likely to develop diabetes than those with hyperthyroidism.

Ethics clearance

The research ethical committee at scientific research has the ethical approval of environmental, health, higher education, and scientific research ministries in Iraq.

References

[1] P. Poudel, A. Illanes, E. J. G. Ataide, N. Esmaeili, S. Balakrishnan, and M. Friebe, “Thyroid ultrasound texture classification using autoregressive features in conjunction with machine learning approaches”, *IEEE Access*, vol. 7, no. Ml, pp. 79354-79365, 2019.

[2] C. Bereketoglu and A. Pradhan, “Plasticizers: negative impacts on the thyroid hormone system”, *Environmental Science and Pollution Research*, vol. 29, no. 26, pp. 38912-38927, 2022.

[3] A. H. Van Der Spek, E. Fliers, and A. Boelen, “Thyroid hormone and deiodination in innate immune cells”, *Endocrinology*, vol. 162, no. 1, pp. 1-15, 2021.

[4] Z. K. Hussain, “Study of possible changes in lipid profiles between premenopausal and postmenopausal women with hyperthyroidism and others with hypothyroidism”, *Iraqi Journal of Science*, vol. 63, no. 12, pp. 5139-5146, 2022.

[5] H. A. Ur Rehman, C. Y. Lin, Z. Mushtaq, and S. F. Su, “Performance analysis of machine learning algorithms for thyroid disease”, *Arabian Journal for Science and Engineering*, vol. 46, pp. 9437-9449, 2021.

[6] A. C. Y. Al-Fatlawi, “An evaluation of blood glucose and lipid profile in female hypothyroidism patients in Kerbala province, Iraq”, *Biomedicine*, vol. 42, no. 3, pp. 556–560, 2022.

[7] A. H. Yassin, A. K. A. Al-Kazaz, A. M. Rahmah, and T. Y. Ibrahim, “Association of CTLA-4

single nucleotide polymorphisms with autoimmune hypothyroidism in Iraqi patients", *Iraqi Journal of Science*, vol. 63, no. 7, pp. 2891-2899, 2022.

[8] Z. A. Maaroof, S. R. Ibraheem, and A. H. Ibrahim, "A correlation study between hyperthyroidism and some apoptosis markers among Iraqi patients", *Iraqi Journal of Science*, vol. 62, no. 5, pp. 1484-1493, 2021.

[9] D. K. Hussein, S. A. K. Al-Jowari, and A. M. Rahmah, "Determination of the level of IL-6 and vaspin in hyperthyroid patients treated with carbimazole", *Iraqi Journal of Science*, vol. 63, no. 5, pp. 1909-1917, 2022.

[10] S. Kalra, S. Aggarwal, and D. Khandelwal, "Thyroid dysfunction and dysmetabolic syndrome: The need for enhanced thyrovigilance strategies", *International Journal of Endocrinology*, vol. 2021, Article no. 9641846, 2021.

[11] I. Huhtaniemi and L. Martini, "Encyclopedia of endocrine diseases", Academic Press, vol. 1, p. 370, 2018.

[12] D. Azamar-Llamas, G. Hernandez-Molina, B. Ramos-Avalos, and J. Furuzawa-Carballeda, "Adipokine contribution to the pathogenesis of osteoarthritis", *Mediators of Inflammation*, vol. 2017, Article no. 5468023, 2017.

[13] P. A. N. Nankam and M. Blüher, "Retinol-binding protein 4 in obesity and metabolic dysfunctions", *Molecular and Cellular Endocrinology*, vol. 531, p. 111312, 2021.

[14] J. S. Steinhoff, A. Lass, and M. Schupp, "Biological functions of RBP4 and its relevance for human diseases", *Frontiers in Physiology*, vol. 12, Article no. 659977, 2021.

[15] D. Dadej, E. Szczepanek-Parulska, and M. Ruchała, "Interplay between fatty acid binding protein 4, fetuin-a, retinol binding protein 4 and thyroid function in metabolic dysregulation", *Metabolites*, vol. 12, no. 4, p. 300, 2022.

[16] X. Chang, H. Yan, H. Bian, M. Xia, L. Zhang, J. Gao, and X. Gao, "Serum retinol binding protein 4 is associated with visceral fat in human with nonalcoholic fatty liver disease without known diabetes: a cross-sectional study", *Lipids in Health and Disease*, vol. 14, no. 28, pp. 1-8, 2015.

[17] J. S. Severo, J. B. S. Morais, J. B. Beserra, L. R. dos Santos, S. R. de Sousa Melo, G. S. de Sousa, E. M. de Matos Neto, G. S. Henriques, and D. do Nascimento Marreiro, "Role of zinc in zinc-α2-glycoprotein metabolism in obesity: a review of literature", *Biological Trace Element Research*, vol. 193, no. 1, pp. 81-88, 2020.

[18] H. M. Pearsey, J. Henson, J. A. Sargeant, M. J. Davies, K. Khunti, T. Suzuki, K. A. Bowden-Davies, D. J. Cuthbertson, and T. E. Yates, "Zinc-alpha2-glycoprotein, dysglycaemia and insulin resistance: a systematic review and meta-analysis", *Reviews in Endocrine and Metabolic Disorders*, vol. 21, no. 4, pp. 569-575, 2020.

[19] X. Wei, X. Liu, C. Tan, L. Mo, H. Wang, X. Peng, F. Deng, and L. Chen, "Expression and function of zinc-α2-glycoprotein", *Neuroscience Bulletin*, vol. 35, no. 3, pp. 540-550, 2019.

[20] M. Banaszak, I. Górna, and J. Przysławski, "Zinc and the innovative zinc-α2-glycoprotein adipokine play an important role in lipid metabolism: A critical review", *Nutrients*, vol. 13, no. 6, Article no. 2023, 2021.

[21] A. R. Rahbar , M. Kalantarhormozi, F. Izadi, E. Arkia, M. Rashidi, F. Pourbehi, F. Daneshifard, and A. Rahbar, "Relationship between body mass index, waist-to-hip ratio, and serum lipid concentrations and thyroid-stimulating hormone in the euthyroid adult population", *Iranian Journal of Medical Sciences*, vol. 42, no. 3, pp. 301-305, 2017.

[22] J. R. Crowther, "Basic principles of ELISA", *Elisa: Theory and Practice*, vol. 42, pp. 35-61, 1995.

[23] P. Feng, D. Wei, Y. Zhang, Y. Zhang, H. Zheng, G. Suo, and X. Li, "Comparison on the consistency of mindray and siemens chemiluminescence analyzers for detecting FT3, FT4 and TSH in patients with hyper- and hypothyroidism", *Annals of Translational Medicine*, vol. 10, no. 20, Article no. 1133, 2022.

[24] A. H. Jawad, R. Alsayed, A. E. Ibrahim, Z. Hallab, Z. Al-Qaisi, and E. Yousif, "Thyroid gland and its rule in human body", *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 7, no. 6, pp. 1336-1343, 2016.

[25] A. N. Ali, I. M. Salem, A. G. Hussein, and M. G. Hamed, "Association between serum zinc-alpha-2- glycoprotein with thyroid hormone in newly diagnosed hyperthyroidism", *European Journal of Molecular and Clinical Medicine*, vol. 8, no. 3, pp. 4669-4678, 2021.

[26] M. Nannipieri, F. Cecchetti, M. Anselmino, S. Camastra, P. Niccolini, M. Lamacchia,M. Rossi, G.

Iervasi, and E. Ferrannini, "Expression of thyrotropin and thyroid hormone receptors in adipose tissue of patients with morbid obesity and/or type 2 diabetes: effects of weight loss", *International Journal of Obesity*, vol. 33, no. 9, pp. 1001-1006, 2009.

[27] S. X. Gao, J. Guo, G. Q. Fan, Y. Qiao, R. Q. Zhao, and X. J. Yang, "ZAG alleviates HFD-induced insulin resistance accompanied with decreased lipid depot in skeletal muscle in mice", *Journal of Lipid Research*, vol. 59, pp. 2277-2286, 2018.

[28] H. A. Najeeb, D. J. Al-Timimi, B. A. Qasim, and A. A. Mohammed, "Parental history of coronary artery disease among adults with hypothyroidism: Case controlled study", *Annals of Medicine and Surgery*, vol. 60, pp. 92-101, 2020.

[29] D. Dadej, E. Szczepanek-Parulska, and M. Ruchała, "Interplay between fatty acid binding protein 4, Fetuin-A, retinol binding protein 4 and thyroid function in metabolic dysregulation", *Metabolites*, vol. 12, Article no. 300, 2022.

[30] E. A. A. Abass, W. T. Al-Sa'adsi, and M. N. Moslem, "A comparative study of retinol-binding protein-4 and progranulin in iraqi women with thyroid disorder", *International Journal Drug Delivery Technology*, vol. 11, no. 1, pp. 36-41, 2021.

[31] H. X. Sun, H. H. Ji, X. L. Chen, L. Wang, Y. Wang, X. Y. Shen, X. Lu, W. Gao, and L. S. Wang, "Serum retinol-binding protein 4 is associated with the presence and severity of coronary artery disease in patients with subclinical hypothyroidism", *Aging (Albany NY)*, vol. 11, no. 13, pp. 4510-4520, 2019.

[32] Y. Hu, Y. Sun,Y. Huang, Q. Liu, and F. Ren, "Serum levels of CXCL-13, RBP-4, and IL-6, and correlation analysis of patients with graves' disease", *Emergency Medicine International*, vol. 2022, Article no. 5131846, 2022.

[33] Y. A. Flores-Cortez, M. I. Barragán-Bonilla, J. M. Mendoza-Bello, C. González-Calixto, E. Flores-Alfaro, and M. Espinoza-Rojo, "Interplay of retinol binding protein 4 with obesity and associated chronic alterations (review)", *Molecular Medicine Reports*, vol. 26, no. 244, pp. 1-12, 2022.

[34] E. Dayakar, C. S. Sree, and E. Sanjay, "Study on the prevalence of dyslipidemia in type 2 diabetes mellitus", *International Journal of Advances in Medicine*, vol. 6, no. 3, pp. 786-789, 2019.

[35] F. Rong, H. Dai, Y. Wu, J. Li, G. Liu, H. Chen, and X. Zhang, "Association between thyroid dysfunction and type 2 diabetes: a meta-analysis of prospective observational studies", *BMC Medicine*, vol. 19, no. 257, pp. 1-13, 2021.

[36] O. H. Roa Dueñas, A. C. Van der Burgh, T. Ittermann, S. Ligthart, M. A. Ikram, R. Peeters, and L. Chaker, "Function and the risk of prediabetes and type 2 diabete thyroids", *Journal of Clinical Endocrinology Metabolism*, vol. 107, no. 6, pp. 1789-1798, 2022.

[37] Y. S. Eom, J. R. Wilson, and V. J. Bernet, "Links between thyroid disorders and glucose homeostasis", *Diabetes and Metabolism Journal*, vol. 46, no. 2, pp. 239-256, 2022.

[38] S. M. M. Hussein and R. M. AbdElmageed, "The relationship between type 2 diabetes mellitus and related thyroid diseases", *Cureus*, vol. 13, no. 12, pp. 1-5, 2021.