

FETUIN-A LEVEL IN PATIENTS WITH UNTREATED THYROID DYSFUNCTION

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Fetuin-A, a plasma glycoprotein, has been demonstrated to play an essential role in the pathogenesis of several metabolic disorders. This study aimed to estimate fetuin-A serum level in patients with newly diagnosed primary hyperthyroidism (PHT) and subclinical hypothyroidism (SCH) and to examine its correlation with thyroid hormones level, age and sex of patients. The study involved 90 patients with untreated thyroid dysfunction verified with thyroid function test (45 with PHT and 45 with SCH) and 90 control subjects. Triiodothyronin (T3), tetraiodothyronin (T4), and thyroid stimulating hormone (TSH) serum concentrations were measured with enzyme-linked fluorescent assay (ELFA), fetuin-A concentration was measured with enzyme-linked immunosorbent assay (ELISA). It was demonstrated that the level of fetuin-A was significantly higher in the PHT group as compared with the control group and showed a significant positive correlation with the T3 level. In the SCH group, the level of fetuin-A was significantly lower and showed a negative correlation with TSH level. Fetuin-A level rose with age in the PHT group and was unaffected by sex in all studied groups. The perfect AUC value obtained for fetuin-A in the comparison between PHT and SCH groups suggests its potential use as a reliable diagnostic marker to differentiate between these two thyroid conditions.

Key words: *fetuin-A, thyroid hormones, primary hyperthyroidism, subclinical hypothyroidism, correlation analysis, AUC value.*

Thyroid dysfunction (hyperthyroidism and hypothyroidism) is a common endocrine pathology in iodine-sufficient regions [1]. When there is an abnormality in the thyroid gland, it will not function properly and thus may affect the whole body. Therefore, if the hormone is overproduced, an imbalance called hyperthyroidism occurs, and when the hormone secretion decreases, a defect called hypothyroidism will result. Both conditions are dangerous for humans, and they must be treated, and a balanced hormonal secretion must be maintained [2]. Hyperthyroidism is a condition that is caused by a complex interaction between inherited predisposition and environmental factors, including the degree of iodine consumption and the extent of smoking. Unrelated to its aetiology, whether Graves' disease or nodular toxic goitre, hyperthyroidism is correlated with various diseases, including atrial fibrillation, heart failure [3], hypercoagulopathy [4] and pulmonary embolism which theoretically can lead to increased mortality if it is not investigated and managed promptly [5].

The American Thyroid Association reported that 20 million Americans have some form of thyroid disease and that more than 12% of the US population will develop a thyroid condition during their lifetime [6]. Most cases of hyperthyroidism occur in women with a ratio of women and men is 1:5 [7]. In the US, the total prevalence of hyperthyroidism is 1.2% which divided into 0.5% and 0.7% for both overt hyperthyroidism and subclinical hyperthyroidism respectively [8]. The prevalence data in elderly persons show a wide range between 0.4-2% and a higher prevalence is seen in iodine – deficient areas [9]. According to subclinical hyperthyroidism, it was shown that individuals of black ethnicity had a notably lower average blood thyroid stimulating hormone (TSH) concentration, resulting in a higher occurrence of subclinical hyperthyroidism (0.4%) compared to individuals of white ethnicity (0.1%) or Mexican American ethnicity (0.3%). Additionally, the occurrence of this condition is more common in populations with iodine deficiency, as it is associated with functional autonomy resulting from nodular

goiters, with a prevalence ranging from 6% to 10% [10].

The occurrence of subclinical hyperthyroidism is commonly observed in the general populace; nevertheless its incidence varies among different regions. The observed variations can be attributed to factors such as age, gender, race, genetic susceptibility, iodine levels, and the varying definitions of subclinical hyperthyroidism employed across different research. In the context of Spain, the general prevalence of thyroid dysfunction is reported to be 8.9%, with a higher representation of females (71.2%). Additionally, the occurrence of subclinical hyperthyroidism is estimated to be 1.3%. In the Jutland region, there was an observed incidence of low TSH levels, amounting to 10%. This prevalence was notably higher among females. The frequency of undetected subclinical hyperthyroidism in Italy was found to be 2.4%. The prevalence of subclinical hyperthyroidism tends to be more pronounced among those in advanced age, with rates reaching up to 15.4% in patients aged 75 years or older. The incidence of subclinical hyperthyroidism in the United States is estimated to range from 2 to 5% among the general population [11].

Graves' disease, toxic nodular goiter, toxic adenoma, and painless thyroiditis are frequent endogenous causes of primary hyperthyroidism. Graves' disease is the most common cause of hyperthyroidism [12, 13]. Graves' disease is associated with certain risk factors, namely being female and having a personal or family history of autoimmune diseases [14]. Toxic multi-nodular goiter is the second most common cause of hyperthyroidism and the prevailing aetiology among elderly individuals residing in regions with inadequate iodine levels [14]. Over a period of time, the formation of nodules occurs as a result of the repeated replication of clonogenic cells, which subsequently leads to a somatic triggering mutation of TSH receptors [15]. A toxic nodular goiter is also another cause of primary hyperthyroidism that is called a toxic adenoma or Plummer disease [16].

Subclinical hypothyroidism is a predominantly asymptomatic medical disease that is identified through laboratory testing, with thyrotropin serving as the primary diagnostic marker. The rising prevalence of subclinical hypothyroidism is a cause for concern, as it has become a significant public health issue. This is due to the potential adverse metabolic consequences, particularly in relation to cardiovascular health, as well as the risk of

progression to primary hypothyroidism. Currently, prominent American and European thyroid guidelines have undertaken numerous experiments that have yielded valuable suggestions for identifying patients who would derive advantages from pharmaceutical therapy [17]. Subclinical hypothyroidism and overt hypothyroidism have almost the same causes, as iodine insufficiency is the central reason of thyroid disease in all parts of the world, and there is another cause represented by immune and autoimmune factors known as (Hashimoto), and there are other side causes represented by the surgical removal of the thyroid gland, tumors and the effect of some types of drugs [18]. Fetuin-A is a multifunctional protein mainly produced by the liver [19]. Animal and human data also suggest that adipocytes produce and secrete fetuin-A [20, 21]. In healthy conditions, fetuin-A is involved in bone metabolism (mineralization, calcification inhibition), cardiovascular, and central nervous systems [22]. Fetuin-A is a plasma-binding protein that facilitates the transportation and systemic availability of many chemicals inside the circulatory system. The negatively charged glycoprotein circulates through blood and extracellular fluid. It exhibits a half-life spanning many days [23]. The 3q27 human chromosome locus 2-Heremans-Schmid glycoprotein (AHSG) gene encodes fetuin-A with seven exons and six introns that are roughly 8.2 kb long [24]. This gene's transcription produces one mRNA copy of a 367-amino-acid human fetuin-A preprotein chain [25]. Several CCAAT enhancer-binding protein (C/EBP) and nuclear factor (NF)-1 binding sites regulate promoter transcription [26]. Pre-fetuin-A has two polypeptide chains: heavy A-chain and light B-chain [27]. The B-chain has 27 amino acid residues, while the A-chain has 282 amino acid residues. A signal sequence (SS) of 18 amino acids at the N-terminus and a connecting peptide (CP) of 40 amino acids between the two chains make up this precursor protein [27]. The fetuin-A posttranslational modification (PTM) is poorly understood and variable. Numerous studies show that glycosylation, proteolytic cleavage, folding, and phosphorylation occur in both the A and B polypeptide chains of human fetuin-A, forming active fetuin-A [28]. Fetuin-A is ultimately liberated from the liver and enters the bloodstream, despite being subject to many positive and negative influences. High blood glucose and free fatty acids promote fetuin-A release. Elevated blood glucose levels promote fetuin-A secretion by triggering extracellular signal-regulated kinase 1/2, elevated blood-free fatty

acid levels promote fetuin-A secretion by enhancing NF- κ B activity [29]. Numerous studies have demonstrated that the consumption of exogenous substances, including curcumin, resveratrol, dairy products, niacin, alcohol, and caffeine, can effectively impede the production of fetuin-A and their subsequent accumulation in the bloodstream [30, 31]. On the contrary, It has been shown that omega-3 fatty acid consumption increases hepatic fetuin-A secretion [32]. The mechanism by which a number of these factors affect fetuin-A release is not well understood, but hypothesised mechanisms exist for some of them.

Thyroid hormones collaborate with fetuin-A to prevent calcification of soft tissue. Triiodothyronine (T3), at physiological concentrations, has been shown to rise the expression of Matrix Gla Protein (MGP), a potent inhibitor of calcification in VSMC, which prevents vascular calcification, via thyroid hormone nuclear receptors [33]. In euthyroid subjects, low thyroxine (T4) was associated with a high progression risk for coronary artery calcification [34]. Several studies found an association between reduced TSH and elevated CAC in euthyroidism and subclinical hyperthyroidism [35, 36]. Even while cellular and cytokine processes are poorly understood, it is well-established that thyroid dysfunction increases cardiovascular risk, including the risk of coronary artery disease [37]. An *in vitro* investigation demonstrated the enhancing impact of T3 on the expression of liver cell line fetuin-A. The molecular mechanism involves T3 binding to thyroid receptor 1, which then affects gene promoter regions to modulate the transcription of numerous proteins, including fetuin-A [38]. In accordance with this hypothesis, administration of T3 to hypophysectomized rodents increased serum fetuin-A levels [39]. In a human investigation, it was shown that the administration of recombinant human thyroid-stimulating hormone (rhTSH) to patients as a means of screening for thyroid cancer recurrence did not result in any significant alteration in serum fetuin-A levels [40]. The justification for this research lies in the understanding that thyroid disorders can have a profound impact on overall body metabolism and organ homeostasis, and therefore assessing fetuin-A could provide valuable insights into the disease process and its potential complications.

Materials and Methods

This study was performed in the laboratories of the Biochemistry Department, College of Medicine/ Al-Nahrain University. The collection of samples

was done during the period from the 1st of March 2023 until the 30th of July 2023. Ninety (90) patients participated in the present study 45 of them with primary hyperthyroidism (PHT) aged 42.71 ± 12.29 years (this group comprised males 48.9% and females 51.1%) and the other 45 patients with subclinical hypothyroidism (SCH) aged 41.49 ± 13.44 years (this group comprised males 46.7% and females 53.3%). All of these individuals were initially diagnosed by an endocrinologist at Al-Imamain Al-Kadhimin Medical City in Baghdad, and their diagnoses were later confirmed by thyroid function tests (TFT). The study also included 90 apparently healthy people as a control group, aged 40.06 ± 11.72 years (this group comprised males 51.1% and females 48.9%). Each member of the control group underwent a complete medical history and physical examination, including age, gender, residence, smoking, past medical history, family history, and medications.

Including and excluding criteria. Adult (>18 years) Patients with a new diagnosis (without treatment) of primary hyperthyroidism, defined as an increase in T3 and T4 concentrations with a decrease in TSH concentration, and patients with a new diagnosis (without treatment) of subclinical hypothyroidism, defined as normal T3 and T4 concentrations with an increased TSH concentration, will be included in this study. Any subject with cardiovascular disease, hypertension, renal failure, liver disease, pregnancy, malignant tumors, diabetes mellitus and obesity will be excluded from this study.

Evaluation of serum levels of T3, T4, TSH, and fetuin-A. The concentrations of T3, T4, TSH, and fetuin-A were measured using the sera obtained from the blood of all study subjects. Biomérieux SA (REF 30 403/T3, REF 30 404/T4, and REF 400/TSH) provided enzyme-linked fluorescent assay (ELFA)-based human T3, T4, and TSH reagents. The human fetuin-A assay was conducted using a Sandwich-ELISA method kit from Elabscience® (catalogue number E-EL-H0386).

Results

As indicated in the previous section, there were non-significant variances in age between the control group, PHT group, and SCH group ($P > 0.05$). These findings suggest that there was no significant difference in age observed among the groups, indicating a well-matched distribution of participants in terms of age.

In the present study, nearly equal numbers of males and females are chosen to control the effect of age and sex on the levels of parameters. Fig. 1 presents the distribution of participants according to sex in each group, including the control group, PHT group, and SCH group. Overall, the chi-squared test revealed no statistically significant difference in sex distribution among the three groups ($\chi^2 = 0.244$, $df = 2$, $P = 0.884$). These findings suggest that the sex distribution was comparable across the control group, PHT group, and SCH group, indicating a balanced representation of both females and males in the study population.

Table 1 illustrates the distribution of participants according to the severity of thyroid dysfunction within the SCH group. The severity was categorized as “less than 10” or “greater than or equal to 10”, indicating the level of deviation from the normal range. Among the 45 participants in the SCH group, 33 individuals (73.3%) had a severity level of less than 10, while 12 individuals (26.7%) had a severity level of greater than or equal to 10. This distribution indicates that the majority of participants with subclinical hypothyroidism (73.3%) had a milder form of the condition, characterized by a smaller deviation from the normal range. Conversely, a smaller proportion of participants (26.7%) had a more severe form of subclinical hypothyroidism, with a greater deviation from the normal range. These findings highlight the variability in the severity and type of thyroid dysfunction within the subclinical hypothyroidism group, suggesting that the condition can manifest in different degrees of impairment.

Also the Table 1 displays the distribution of participants within the PHT group based on the type of thyroid dysfunction, specifically Graves’ disease and toxic multinodular goiter (TMNG). Among

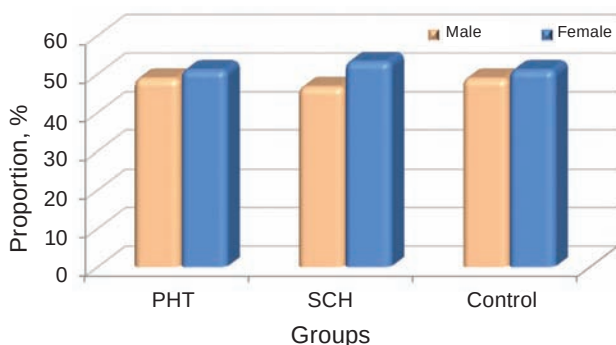


Fig. 1. Proportional distribution of sex in the studied group

the 45 participants in the PHT group, 32 individuals (71.1%) were diagnosed with Graves’ disease, while 13 individuals (28.9%) had toxic multinodular goiter (TMNG). These results indicate that Graves’ disease is the predominant type of thyroid dysfunction within the primary hyperthyroidism group, accounting for the majority of cases. These findings highlight the prevalence of Graves’ disease as the primary cause of hyperthyroidism in the studied population, while toxic multinodular goiter represents a smaller proportion of cases.

Table 2 presents the serum concentrations of thyroid hormones, including T3, T4, TSH, and fetuin-A in the PHT group, subclinical hypothyroidism group and control group. The mean values and standard deviations are provided, and statistical analysis was conducted to compare the results among the groups.

In the control group, the mean T3, T4, and TSH concentrations were (1.78 ± 0.33 nmol/ml, 90.50 ± 8.24 nmol/ml, and 1.93 ± 0.69 μ IU/ml respectively). Comparatively, the PHT group had a significantly higher mean T3 and T4 concentrations (4.42 ± 1.39 and 194.58 ± 38.89 nmol/ml respectively, $P < 0.001$), while TSH had a significantly lower mean concentration 0.05 ± 0.04 μ IU/ml ($P < 0.001$). The SCH group had a significantly lower mean T3 and T4 concentrations (1.36 ± 0.27 and 87.87 ± 13.00 nmol/ml, respectively, $P < 0.001$) and significantly higher mean TSH concentration 8.54 ± 1.87 μ IU/ml ($P < 0.001$) compared to control group. These findings indicate that primary hyperthyroidism is associated with alterations in serum thyroid hormones, and TSH levels compared to the

Table 1. The distribution of participants according to the severity and type of thyroid dysfunction within the SCH and PHT groups, respectively

Groups	Severity		
	< 10	≥ 10	
SCH	33 (73.3%)	12 (26.7%)	45 (100%)
Type			
	GD	TMNG	
PHT	32 (71.1%)	13 (28.9%)	45 (100%)

SCH – subclinical hypothyroidism, PHT – primary hyperthyroidism; GD – Graves’ disease, TMNG – toxic multinodular goiter

Table 2. Serum concentrations of parameters among the studied groups

Parameter	Groups	N	Median	mean $\pm$ SD	SEM
T3	Control	90	1.87	1.78 ± 0.33	0.03
	PHT	45	4.10	4.42 ± 1.39	0.21
	SCH	45	1.43	1.36 ± 0.27	0.04
T4	Control	90	90.00	90.50 ± 8.24	0.87
	PHT	45	199.00	194.58 ± 38.89	5.80
	SCH	45	90.00	87.87 ± 13.00	1.94
TSH	Control	90	1.84	1.93 ± 0.69	0.07
	PHT	45	0.05	0.05 ± 0.04	0.01
	SCH	45	8.36	8.54 ± 1.87	0.28
Fetuin-A	Control	90	215.50	236.62 ± 51.82	5.46
	PHT	45	401.00	401.84 ± 48.58	7.24
	SCH	45	222.00	228.09 ± 32.75	4.88

SCH – subclinical hypothyroidism, PHT – primary hyperthyroidism, SEM –standard error of the mean, $P < 0.001$

control group. The statistical analysis demonstrates important differences in serum concentrations of T3, T4, and TSH among the control group and PHT group, and subclinical hypothyroidism group indicating the impact of thyroid dysfunction on the levels of these hormones.

In the control group, the mean serum concentration of fetuin-A was 236.62 ± 51.82 ng/ml. comparatively; the PHT group exhibited a significantly higher mean fetuin-A concentration of 401.84 ± 48.58 ng/ml ($P < 0.001$). Conversely, the SCH group had a significantly lower mean fetuin-A concentration of 228.09 ± 32.75 mg/l ($P < 0.001$) compared to the control group. These results indicate that both primary hyperthyroidism and subclinical hypothyroidism are associated with alterations in serum fetuin-A levels compared to the control group (Fig. 2).

Table 3 presents the Pearson correlation coefficients and significance levels for various parameters within the PHT group. Fetuin-A showed a significant positive correlation with T3 levels ($r = 0.49$, $P < 0.001$). Thyroid hormones T3 and T4 demonstrated significant positive correlations with each other ($r = 0.617$, $P < 0.001$). TSH exhibited significant negative correlation with T3 and T4 ($P < 0.001$). Additionally, there was a weak negative correlation with fetuin-A ($P > 0.05$).

Table 4 shows the correlation within the SCH group. Fetuin-A showed a negative correlation with TSH levels and positive correlation with other thy-

roid hormones, it was not statistically significant ($P > 0.05$). Thyroid hormones T3 and T4 demonstrated a significant positive correlation with each other ($r = 0.822$, $P < 0.001$). T3 also showed a significant negative correlation with TSH ($r = -0.783$, $P < 0.001$). T4 exhibited a strong negative correlation with TSH ($r = -0.732$, $P < 0.001$). TSH shows significant negative correlation with T3 and T4 ($P < 0.001$).

Table 5 presents the correlation within the control group; fetuin-A showed no significant correlations with any of the parameters. Thyroid hormones T3, T4 and TSH did not exhibit significant correlation with fetuin-A within the control group.

Table 6 presents the Pearson correlation coefficients and significance levels for various parameters within the PHT, SCH and control groups. Within the PHT group, fetuin-A showed a significant positive correlation with age ($P < 0.001$). Within the SCH group and control group, there were no important correlations between age and parameters.

As it is shown in Table 7, the mean difference of Fetuin-A between males and females is statistically insignificant in all groups.

Overall, these correlation results shed light on the relationships between various parameters within the PHT, SCH, and control groups, implying potential associations and interactions.

As shown in Table 8 and Fig. 3, 4 and 5, receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic accuracy of the variables for distinguishing between different

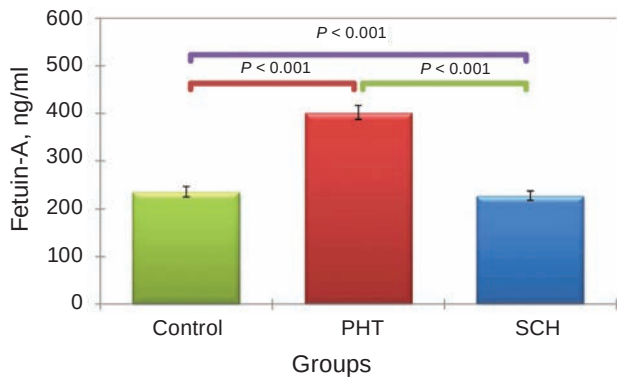


Fig. 2. Comparison of fetuin-A levels among the studied groups

Table 3. Pearson correlation coefficients and significance levels for various parameters within the primary hyperthyroidism group

		FA	T3	T4	TSH
FA	<i>r</i>		0.49	0.25	-0.28
	<i>P</i>		<0.001	0.09	0.06
T3	<i>r</i>	0.49		0.62	-0.83
	<i>P</i>	0.00		<0.001	<0.001
T4	<i>r</i>	0.25	0.62		-0.69
	<i>P</i>	0.09	<0.001		<0.001
TSH	<i>r</i>	-0.28	-0.83	-0.69	
	<i>P</i>	0.06	<0.001	<0.001	

FA – fetuin-A

groups. For the comparison between patients with PHT and the control group, fetuin-A exhibited a high area under the curve (AUC) of 0.988. The optimal cutoff value was greater than 322, and it showed a sensitivity of 100% and a specificity of 95.56%. The positive likelihood ratio (+LR) was 22.5, indicating a strong positive association, while the negative likelihood ratio (-LR) was 0.0, suggesting a lack of association. For the comparison between patients with SCH and the control group, fetuin-A demonstrated a low AUC of 0.512. The optimal cutoff value was greater than 292, and it showed a sensitivity of 44.4% and a specificity of 75.56%. The +LR was 0.18, indicating a weak positive association, while the -LR was 1.26, suggesting a weak negative as-

Table 4. Pearson correlation coefficients and significance levels for various parameters within the subclinical hypothyroidism group

		FA	T3	T4	TSH
FA	<i>r</i>		0.271	0.27	-0.246
	<i>P</i>		0.0722	0.0731	0.1034
T3	<i>r</i>	0.271		0.822	-0.783
	<i>P</i>	0.0722		<0.001	<0.001
T4	<i>r</i>	0.27	0.822		-0.732
	<i>P</i>	0.0731	<0.001		<0.001
TSH	<i>r</i>	-0.246	-0.783	-0.732	
	<i>P</i>	0.1034	<0.001	<0.001	

FA – fetuin-A

Table 5. Pearson correlation coefficients and significance levels for various parameters within the control group

		FA	T3	T4	TSH
FA	<i>r</i>		0.25	-0.14	0.28
	<i>P</i>		0.07	0.18	0.06
T3	<i>r</i>	0.25		-0.11	-0.07
	<i>P</i>	0.09		0.33	0.54
T4	<i>r</i>	-0.14	-0.11		0.15
	<i>P</i>	0.18	0.33		0.16
TSH	<i>r</i>	0.28	-0.07	0.15	
	<i>P</i>	0.06	0.54	0.16	

FA – fetuin-A

Table 6. Pearson correlation coefficients and significance levels for various parameters within the study groups

Age	Groups		FA	T3	T4	TSH
	PHT	<i>r</i>	0.77	0.31	0.09	0.02
		<i>P</i>	<0.001	0.04	0.57	0.88
	SCH	<i>r</i>	0.098	-0.084	-0.082	0.51
		<i>P</i>	0.521	0.584	0.593	0.301
	Control	<i>r</i>	0.00	0.14	0.15	0.20
		<i>P</i>	0.97	0.19	0.17	0.06

FA – fetuin-A

Table 7. Association of fetuin-A levels with sex

	Groups		
	PHT	SCH	Control
Female	405.20±50.25	230.0 ± 31.41	237.92± 52.45
Male	398.48±46.91	226.17±34.20	235.32 ± 51.20
P value male vs. female	0.86	0.71	0.38

P value < 0.05 significant. P value > 0.05 no significant

Table 8. Receiver operating characteristic analysis criteria evaluating the diagnostic accuracy of the study parameters for distinguishing between different groups

		AUC	SE	95% CI	Cutoff	Sens.	Spec.	+LR	-LR
PHT vs Control	FA	0.988	0.006	0.95 to 0.99	>322	100.0	95.56	22.5	0.0
SCH vs Control	FA	0.512	0.049	0.42 to 0.59	>292	44.4	75.56	0.18	1.26
PHT vs SCH	FA	1.000	0.000	0.96 to 1.00	>304	100.0	100.0		0.00

AUC – Area under the curve; SE – standard error; 95% CI – 95% confidence interval; Sens. – sensitivity; Spec. – specificity; +LR – positive likelihood ratio; -LR – negative likelihood ratio; FA – fetuin-A

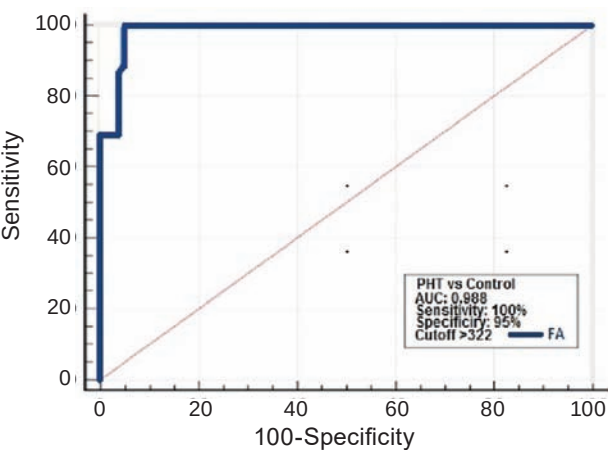


Fig. 3. Receiver operating characteristic analysis showing diagnostic accuracy of fetuin-A for distinguishing between PHT and control groups

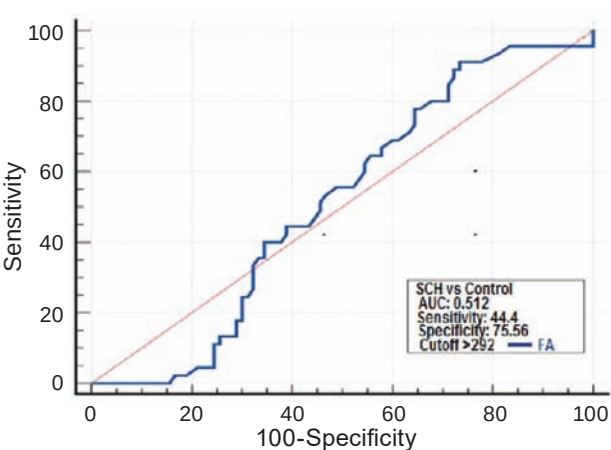


Fig. 4. Receiver operating characteristic analysis showing diagnostic accuracy of fetuin-A for distinguishing between SCH and control groups

sociation. For the comparison between patients with PHT and SCH, fetuin-A showed perfect AUC of 1.000. The optimal cutoff value was greater than 304, sensitivity and specificity were 100.00%. The +LR and -LR values were not reported.

Discussion

Thyroid disorders have a significant influence on whole-body metabolism and organ homeostasis. This study aimed to assess the serum concentrations of fetuin-A in patients with subclinical hypothyroi-

dism and primary hyperthyroidism. Moreover, the study aimed to examine the correlation of fetuin-A with external factors such as age, sex, and biochemical variables, as well as to define cutoff values for fetuin-A in patients with subclinical thyroid disease and primary hyperthyroidism.

The results of this study revealed significant differences in the serum concentrations of fetuin-A among the control group, PHT group, and SCH group. These findings suggest that fetuin-A may be altered in the presence of thyroid dysfunction

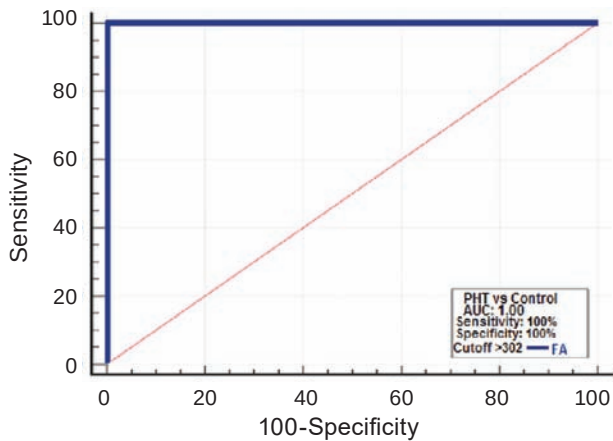


Fig. 5. Receiver operating characteristic analysis showing diagnostic accuracy of fetuin-A for distinguishing between PHT and SCH groups

and could potentially serve as indicators of thyroid-related complications. Fetuin-A levels were considerably higher in the PHT group and lower in the SCH group, compared to the control group. This indicates that fetuin-A concentrations are impacted by thyroid dysfunction and could potentially signal the presence and seriousness of the disease. Significantly higher levels of serum fetuin-A were noted in newly diagnosed patients with hyperthyroidism before the initiation of any treatment compared with the control group ($P < 0.001$) [41]. Certain studies determined that pre-treatment hyperthyroidism had an impact on fetuin-A levels. Additionally, there was a substantial positive link between fetuin-A levels and thyroid hormones ($P < 0.05$), as well as a significant negative correlation between fetuin-A levels and TSH ($r = -0.553$, $P < 0.001$) [42]. Thyroid hormones have been seen to elevate bone turnover indicators, and it has been noted that Hyperthyroidism is linked to accelerated bone turnover. The levels of fetuin-A have been observed to be elevated in individuals with hyperthyroidism, and this occurrence is likely associated with bone metabolism. Moreover, hypercalcemia is a widely recognized problem associated with hyperthyroidism. Consequently, the stimulation of fetuin-A synthesis could serve as an adaptive mechanism to mitigate the risk of ectopic tissue calcification in individuals with hyperthyroidism [43]. The study revealed a notable and positive relationship between the levels of fT3 and fetuin-A. This correlation suggests that fT3 may have a regulatory impact on metabolism and hepatokine levels. In the middle-aged and old population of China, the

concentration of fetuin in the serum is seen. A favorable correlation was observed between fetuin-A levels and fT3 levels [44]. A recent study revealed an adverse correlation between the fetuin-A level and TSH [45]. Significantly higher levels of serum fetuin-A were noted in the patient with hyperthyroidism group before treatment compared with the control group. Fetuin-A was linked positively with thyroid hormones, whereas TSH and fetuin-A had a negative connection [46]. Previous research stated that fetuin-A was significantly lower in hypothyroidism compared to control groups ($P = 0.00$), T4 and FT4 were considerably low in hypothyroid compared to control ($P = 0.00$). TSH was high in patients compared to control ($P = 0.00$) [47]. Another study reported no significant correlations between fetuin-A and T4, FT4, and TSH ($P > 0.05$). However, no significant correlation was observed between plasma fetuin-A and thyroid hormone or TSH in hypothyroidism and control groups as that observed by a previous study [48] stated that there was a negative connection was observed between plasma TSH and fetuin-A levels in patients diagnosed with hypothyroidism. The study observed that individuals diagnosed with Hashimoto thyroiditis and subclinical hypothyroidism exhibited decreased levels of fetuin-A compared to the control group [49].

In contrast, an earlier study [50] revealed that the levels of fetuin-A were elevated in patients diagnosed with subclinical hypothyroidism compared to the control group. Our findings disagreed with the authors' results [51], who reported that the concentration of fetuin-A was significantly greater in both hyper and hypothyroidism compared to the control group ($P < 0.001$). The study included 45 thyroid patients at AL-Najaf's endocrine and diabetes center and 45 healthy people.

An *in vitro* experiment was conducted to investigate the impact of direct interaction between the promoter region of important liver-synthesized proteins, including fetuin-A, and the α -1 thyroid receptors with the effects of T3. The study successfully demonstrated an increase in protein synthesis due to this connection. The findings of these investigations offer potential insights into the underlying reasons for the reduced levels of fetuin-A observed in individuals with hypothyroidism, as well as the potential for restoring normal levels through maintaining euthyroidism [38]. A recent study examining plasma Fetuin-A levels in patients who had undergone thyroidectomy, reported negligible fetuin-A

modification using recombinant TSH. This outcome validates fetuin creation had a greater impact on thyroid hormone status than on serum TSH levels [40]. An additional recent clinical study [42] found that patients with hyperthyroidism had elevated fetuin-A levels, which returned to normal upon achieving euthyroidism. Due to the lack of a clinical investigation examining fetuin-A levels in hypothyroidism patients, our study is unique in the medical literature and comparisons with other human studies.

The results of the present investigation indicate a notable positive association between fetuin-A levels and age among individuals in the PHT group. In contrast SCH, and the control group exhibited no significant correlations between age and fetuin-A. The current results, in accordance with the previous study, found that age would be positively associated with elevated fetuin-A levels [52]. In contrast to our findings, age is inversely associated with Fetuin-A levels [53]. During aging, our body produces and secretes less fetuin-A, and there was an adverse link between age and fetuin-A levels [54]. As reported before, age is associated with the strongest negative correlation with fetuin-A [55]. In comparison with the earlier study reported that fetuin-A increased with age in patients and control significantly [56], our study agreed to an increase in fetuin-A with increasing age in patients with PHT but disagreed to an increase in fetuin-A with age in healthy subjects.

The mean difference of fetuin-A between males and females is statistically insignificant in all groups. Our findings in accordance with the authors findings, who reported that there was no important variance in fetuin-A between males and females ($P > 0.05$) in patient and control groups [56].

Another important aspect of this study was to define cutoff values for fetuin-A in patients with subclinical thyroid disease and primary hyperthyroidism. The obtained results provide valuable information for clinicians to establish reference ranges and aid in identifying and managing thyroid dysfunction. These cutoff values can contribute to the identification of individuals with abnormal biomarker levels who may require further evaluation and treatment. The rationale behind this study was rooted in the understanding that thyroid disorders can significantly impact overall body metabolism and organ homeostasis. Therefore, measuring specific biomarkers such as fetuin-A may provide valuable insights into the pathophysiology and complications related to thyroid dysfunction.

The findings of this study revealed interesting results regarding the serum concentrations of fetuin-A in patients with thyroid dysfunction compared to the healthy control group. In comparing PHT and the control group, fetuin-A exhibited a high AUC of 0.988, suggesting its potential as a reliable biomarker for distinguishing between these two groups. In comparing SCH and the control group, fetuin-A showed a low AUC of 0.512, suggesting its limited usefulness in distinguishing between these two groups. Regarding the comparison between PHT and SCH, fetuin-A displayed a perfect AUC value of 1.000, implying its potential as a highly accurate diagnostic marker for differentiating between these two groups. These findings suggest that fetuin-A could serve as a valuable biomarker for evaluating thyroid dysfunction. The high AUC value observed for fetuin-A in differentiating PHT from the control group indicates its strong discriminatory power. Additionally, the perfect AUC value obtained for fetuin-A in the comparison between PHT and SCH suggests its potential use as a reliable diagnostic marker to differentiate between these two thyroid conditions. Defining cutoff values for fetuin-A in patients with subclinical thyroid disease and primary hyperthyroidism is crucial for their clinical utility. These cutoff values can help clinicians make accurate diagnoses and determine appropriate treatment strategies for patients with thyroid dysfunction. T3 and T4 did not exhibit significant correlations with any of the evaluated parameters within the control group, but they exhibited significant correlations with fetuin-A in the thyroid dysfunction groups. These results suggest that the levels of T3 and T4 may directly influence fetuin-A in an abnormal thyroid condition. Further investigations are necessary to elucidate the complex interactions between thyroid hormones and fetuin-A under examination.

In conclusion, this study provides valuable insights into evaluating fetuin-A as a serum biomarker in patients with thyroid dysfunction. The results highlight the potential diagnostic accuracy of fetuin-A in distinguishing between different groups and its possible use in clinical practice. Nevertheless, additional research is required to substantiate these findings and investigate the underlying processes through which fetuin-A may contribute to thyroid dysfunction.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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РІВЕНЬ ФЕТУЇНУ-А У ПАЦІЄНТІВ ІЗ НЕЛІКОВАНОЮ ДИСФУНКЦІЄЮ ЩИТОПОДІБНОЇ ЗАЛОЗИ

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Відомо, що глікопротеїн плазми крові фетуїн-А, відіграє важливу роль у патогенезі багатьох метаболічних порушень. Метою цього дослідження було визначити рівень фетуїну-А в сироватці крові пацієнтів із вперше діагностованим первинним гіпертиреозом (ПГТ) та субклінічним гіпотиреозом (СГТ), а також дослідити кореляцію цього показника з рівнем тиреоїдних гормонів, віком та статтю пацієнтів. У дослідженні взяли участь 90 пацієнтів із нелікованою дисфункцією щитоподібної залози, за даними тиреоїдного тесту 45 з ПГТ і 45 з СГТ, та 90 осіб контрольної групи. Концентрації трийодтироніну (Т3), тетрайодтироніну (Т4) та тиреотропного гормону (ТТГ) у сироватці крові визначали за допомогою імунофлуоресцентного аналізу (ELFA), концентрацію фетуїну-А – за допомогою імуноензимного аналізу (ELISA). Встановлено, що рівень фетуїну-А був значно вищим у групі ПГТ порівняно з контрольною групою і мав достовірну позитивну кореляцію з рівнем Т3. У групі СГТ рівень фетуїну-А був достовірно нижчим і мав негативну кореляцію з рівнем ТТГ. Рівень фетуїну-А підвищувався з віком у групі ПГТ і не залежав від статі в усіх досліджуваних групах. Ідеальне значення AUC, отримане для фетуїну-А у порівнянні між групами ПГТ і СГТ, свідчить про можливість його використання як діагностичного маркера для диференціації між цими двома захворюваннями щитоподібної залози.

Ключові слова: фетуїн-А, тиреоїдні гормони, первинний гіпертиреоз, субклінічний гіпотиреоз, кореляційний аналіз, значення AUC.

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