Study of Fetuin-A Protein as A Predictive Marker for Thyroid Patients in Al-Najaf City

Murtadha M. Jawad[1], Asmaa H. Hussein[2]
[1,2]College of Health and Medical Techniques, Kufa, Al-Furat Al-Awsat University/Iraq
Email1: kuh.dr.mur@atu.edu.iq

Abstract
Objectives: The aim of this study is to detect thyroid diseases through the examination of novel biomarkers parameter in blood serum, including (Fetuin-A) by using ELISA method with hormonal tests (T3,T4 and TSH) by using Vidas methodMethadology: The current study was conducted on 45 patients with thyroid disease was attending in endocrine and diabetic center in the Al-Sadder Teaching Hospital and Al-Hakim General Hospital in AL-Najaf Province, and 45 healthy person, the samples collected during the period from September 2021 to 2`January.Results: The result of hormonal markers shows high significant with p-value = <0.001 in the level of T3 was higher in Hypothyroidism compared to Hyperthyroidism and the control group . On the other hand, this result was high significant with p-value = <0.001 in the level of T4 was higher in Hypothyroidism compared to Hyperthyroidism and the control group. In addition, This result was high significant with p-value = <0.001 in the level of TSH was higher in Hyperthyroidism compared to Hypothyroidism and the control group.The results of biochemical marker of Fetuin-A was higher in Hyperthyroidism and Hypothyroidism (0.68 ± 0.18 and 0.74 ± 0.08 respectively) compared to and the control group (0.49 ± 0.21). This result was statistically high significant with p-value ≤ 0. 001.Conclusion: Fetuin-A serum concentration level was higher in thyroid disorders patients rather than control group and this mean that Fetuin-A have effect in severity of thyroid disease.
Key words: Hyperthyroidism, Hypothyroidism, Fetuin-A.

1. Introduction

The thyroid gland is one of the largest and most important endocrine glands in humans and vertebrates (Kloas and Kirschbaum, 2019). The thyroid is essential for the functions of nearly all body organs, such as the kidneys, lungs, liver, heart, and reproductive organs. The thyroid gland produces hormones that regulate numerous physiological systems and improve mood. The thyroid gland secretes three hormones into the bloodstream: Thyrocalcitonin (calcitonin), triiodothyronine (T3), and tetraiodothyronine or thyroxine (T4) (Moini et al., 2020).

T4 regulates body temperature, metabolism, and mood, among other functions. The thyroid gland synthesis of T3 is made by removing iodine from T4 in other parts of the body. This hormone is responsible for metabolic and digestive function, as well as bone health. While calcitonin aids in the regulation of phosphate and calcium levels in the blood, it also decreases blood calcium levels (Solórzano et al., 2021).

Thyroid hormone (TSH) is secreted by the pituitary gland and is primarily responsible for thyroid gland production and iodine acquisition (Nostrand, 2016).

TH synthesis requires iodine, copper, zinc, magnesium, B2, B3, and B6 vitamins. When the thyroid gland's production is disrupted, it can cause various disorders. Hyperthyroidism arises when secretion is high, and hypothyroidism develops when insufficient secretion (Ihsan et al., 2021).

Fetuin-A is a 46 kDa hepatocyte-derived protein (hepatokine). The liver produces fetuin-A, which is released into the bloodstream. Fetuin-A is one of the main transporters of free fatty acids in the circulatory system (Pal et al., 2012).

Fetuin-A plays an important and essential role in insulin resistance caused by free fatty acids in the liver; increased fetuin-A levels in pre-diabetic individuals are linked to a higher risk of developing diabetes and a worse chance of reversing normoglycemia (Frayn, 2019).

As a result, circulating Fetuin-A is an indicator of glycemic outcomes in pre-diabetes; obese persons have higher levels of Fetuin-A in their blood, which can be lowered with exercise, weight reduction, and metformin. Increased fetuin-A levels have also been related to a higher risk of cardiovascular events and hepatic steatosis. The inflammatory effects of Fetuin-A are believed to be the elevated risk levels for vascular and liver disease (Samadi et al., 2020).

In contrast, Fetuin-A has been shown to have anti-inflammatory properties. Fetuin-A is an acute-phase passive reactant for endotoxemia and sepsis, promotes wound healing as well as a neuroprotective in Alzheimer's patients. Low fetuin-A levels correlate with high disease activity in Crohn's disease, microscopic colitis, and obstructive lung disorders (Aguer et al., 2020).

The aim of this study is to detect thyroid diseases through the examination of novel biomarkers parameter in blood serum, including (Fetuin-A) and to study the Relationship between Fetuin-A and the severity of thyroid disease by using ELISA method with hormonal tests (T3, T4 and TSH) by using Vidas method.

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2. Methadology

Patients and Control Group

This prospective study was conducted on patients attending to endocrine and diabetic center, Alhakim General Hospital and many private sector laboratories in AL-Najaf province during September 2021 to 2`January 2022. The blood specimens were collected from (45) patients with thyroid disease, In addition 45 apparently healthy subjects as control group.

Samples Collection

Five ml of venous blood were withdrawn from each subjects by vein puncture using sterile syringe with needle gauge 23, than the blood sample was transfer in to coagulate gel tubes, then centrifuged for 5 minutes at 4000 (rpm) to separate serum were transferred to another sterile Eppendorf tubes, labeled with Serial Number together with the patient name, and frozen at (-20°C) until used (Lesser *et al.*, 2020).

Hormone Analysis

Thyroxin (T4), Triiodothyronine (T3) and Thyroid stimulating hormone (TSH) are an automated quantitative test for use on the VIDAS instruments, for the enzyme immunoassay determination of human T4, T3 and TSH in human serum \ using minivads technique.

Determination level of (T3), (T4) and (TSH) was measured according to the standards required by the manufacturer company Bio merieux/ France.

Biomarker Tests

The procedure of determination of Human Fetuin-A was measured by ELISA Technique according to BT LAB / China protocol.

Statistical Methods

Data were analyzed using SPSS program version 16 and Microsoft Office Excel 2007. Numeric variables were expressed as mean +SD while nominal variables were expressed as number and percentage. Independent sample t-test was used to study difference in mean between any two groups while chi-square was used to study association between any two variables. P-value was considered significant when it was less than or equal to 0.05.

3. Results and Discussion

1. Hormonal markers in Hyperthyroidism, Hypothyroidism in comparison with Control.

The results in Table (1) shows levels of hormones among the study groups, where the level of T3 was higher in Hypothyroidism (2.29 ± 0.74) compared to Hyperthyroidism and the control group (1.21 ± 0.68 and 1.47 ± 0.33) respectively. This result was statistically high significant with p-value ≤ 0.001.

On the other hand, the level of T4 was higher in Hypothyroidism (142.2 ± 41.2) compared to Hyperthyroidism and the control group (71.49 ± 28.1 and 91.5 ± 12.1) respectively. This result was statistically high significant with p-value ≤ 0.001.

In addition, the level of TSH was higher in Hyperthyroidism (13.8 ± 9.84) compared to Hypothyroidism and the control group (0.07 ± 0.04 and 1.65 ± 0.93) respectively. This result was statistically high significant with p-value ≤ 0.001.

Thyroid dysfunction is a common endocrine disorder affecting around 300 million people worldwide and it is presumed that more than half are unaware of their condition. The major thyroid disorders are hyperthyroidism and hypothyroidism, with 1.6 billion people at risk in more than 110 countries around the world (Yadav et al., 2013).

Higher-than-normal T3 levels typically indicate hyperthyroidism (overactive thyroid) and this has several causes, including Graves' disease (an autoimmune condition), thyroid nodules and thyroiditis (inflammation of your thyroid gland) (Mohammadi et al., 2021). Hyperthyroidism speeds up metabolism, which can be dangerous also, cause unexplained weight loss, Feeling shaky and/or nervous, ncreased bowel movements, rapid or irregular heartbeat (arrhythmia) (Devereaux and Tewelde, 2014).

Thyrotropin (TSH), traditionally seen as a pituitary hormone that regulates thyroid glands and produced in the brain and travels to the thyroid gland to stimulate the thyroid to produce and release more thyroid hormone. A high TSH level indicates that the body does not have enough thyroid hormone (Livingston, 2019). Autoimmune thyroiditis is the most common cause of hypothyroidism via gradually destroys the thyroid tissue and leads to a decrease in circulating thyroid hormone levels, pituitary secretion of TSH increases (Asvold et al., 2012).

Kravets, 2016 mentioned the benign pituitary gland tumor may overproduce thyroid stimulating hormone (TSH), which causes hyperthyroidism.

Asmelash et al., 2019 reported that the prevalence of hyperthyroidism and hypothyroidism were 14.6% and 1.6% respectively. While H Mosli and M Attar, 2014 revealed on the prevalence of hyperthyroidism and hypothyroidism in Saudi Arabia were (2.6%) and (19%) respectively. The results of our study revealed on high level of T3 in hypothyroidism patients and this results in agreement with result of (Hennessey, 2015) where repoeted the total of hypothyroidism patients had higher T3.

Table (1): Hormonal markers in Hyperthyroidism (O), Hypothyroidism (R) in comparison with Control (N).

Hormonal Level	Hyperthyroidism n=23	Hypothyr-Oidism n=22	Control n=45	Statistics	*p* value	*Post-hoc* analysis
	Mean ± SD	Mean ± SD	Mean ± SD			
T3	1.21 ± 0.68	2.29 ± 0.74	1.47 ± 0.33	X^2 = 25.63, df = 2	<0.001	R > O , N [ab]
T4	71.49 ± 28.1	142.2 ± 41.2	91.5 ± 12.1	X^2 = 39.93, df = 2	<0.001	O < R > N [ab]
TSH	13.8 ± 9.84	0.07 ± 0.04	1.65 ± 0.93	X^2 = 75.38, df = 2	<0.001	R < O > N [ab]

Abbreviations: a=Those data were analyzed by using the Kruskal–Wallis test; b=The Dunnett C method was used for post hoc comparisons; and SD=standard deviation.

2. Biochemical markers in Hyperthyroidism, Hypothyroidism in comparison with Control

The results in Table (2) shows levels of Fetuine among the study groups, where the level of Fetuine was higher in Hyperthyroidism and Hypothyroidism (0.68 ± 0.18 and 0.74 ± 0.08 respectively) compared to and the control group (0.49 ± 0.21). This result was statistically high significant with p-value ≤ 0.001.

Fetuin-A is a liver-derived blood protein that acts as a potent inhibitor of ectopic mineralization. Monomeric fetuin A protein binds to small clusters of calcium and phosphate (Ricken et al., 2022).

In vitro, fetuin-A can inhibit or stimulate osteogenesis, depending on its concentrations. Thyroid hormones increase bone turnover markers, and hyperthyroidism increases bone turnover. Fetuin-A may be increased in hyperthyroidism through a mechanism related to bone metabolism (Pamuk et al., 2013).

Hypercalcemia is a well-known complication of hyperthyroidism and stimulating fetuin-A synthesis may be an adaptation to prevent ectopic tissue calcification in hyperthyroidism. In addition to its functions as an inhibitor of tissue calcification, fetuin-A is an endogenous inhibitor of the insulin receptor and fetuin-A plays a protective role in systemic inflammation. Hyperthyroidism may also initiate an inflammatory cascade and eventually stimulate fetuin-A synthesis (Ammar et al., 2017).

Insulin resistance in hyperthyroidism may also be related to the increased levels of fetuin-A (Pamuk et al.,2013).

Bakiner et al., 2014 reported that who documented that elevated Fetuin-A levels were associated with decreased TSH levels (P=0.001).

Pamuk et al.,2013 who reported that elevated Fetuin-A levels were associated with increased T3 (P=0.001), T4 (P=0.002).

Tseng et al., 2018 and Steinhoff et al., 2021 revealed that the level of Fetuin-A was higher in Hyperthyroidism than in hypothyroidism, thus these results uncompatible with our results

Also, our results disagreement with one studies that conducted recently in Iraq, where mentioned fetuin-A levels shown to be significantly lower in hypothyroid Iraqi women patients (Albayati and Hussein, 2020).

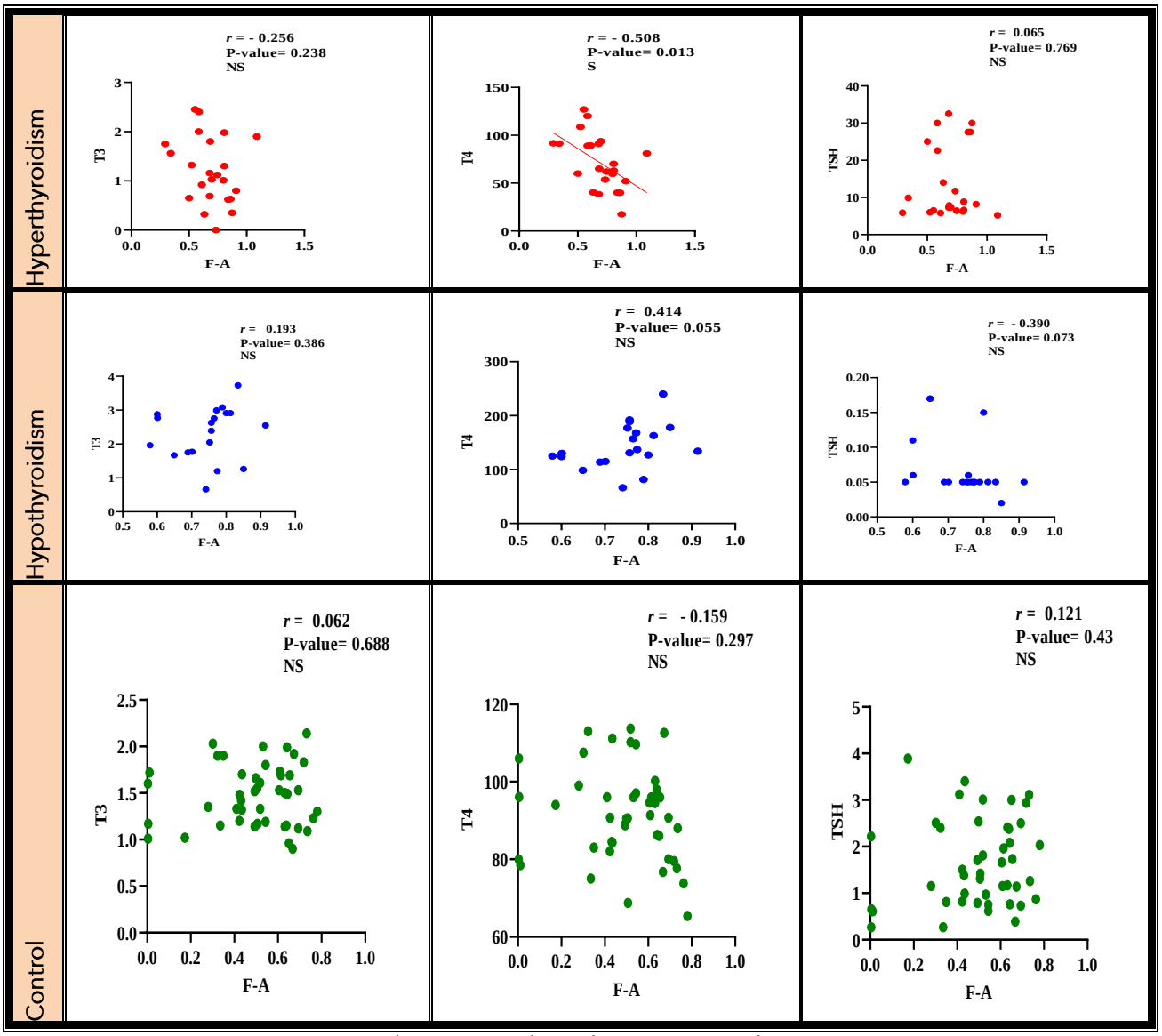

Figure (1): showing Correlation between F-A and T3, T4, TSH.

Table (2): Biochemical markers in Hyperthyroidism (O), Hypothyroidism (R) in comparison with Control (N).						
Biochemical Markers	Hyperthyr-oidism n=23	Hypothyr-oidism n=22	Control n=45	Statistics	p value	Post-hoc analysis
	Mean ± SD	Mean ± SD	Mean ± SD			
Fetuine (FA)	0.68 ± 0.18	0.74 ± 0.08	0.49 ± 0.21	X2 = 30.587, df = 2	<0.001	O, R > N [ab]
Abbreviations: [a]=Those data were analyzed by using the Kruskal–Wallis test; [b]=The Dunnett C method was used for *post hoc* comparisons; [c]=The Bonferroni method was used for *post hoc* comparisons; and SD=standard deviation.						

3. Correlation between Fetuine and thyroid hormones in hyperthyroidism, hypothyroidism, and control groups

Figure (1) shows that there are no correlations among Fetuine and thyroid hormones in each of the studied groups; (hyper, hypo, and control groups), except there is a statistically significant negative correlation between level of Fetuine and T4 in hyper group; (r=0.508 and p-value was 0.013).

4. Conclusions

- TSH was higher in Hyperthyroidism compared to Hypothyroidism and the control group.
- T3 and T4 were higher in Hypothyroidism compared to Hyperthyroidism and the control group.
- 3- Fetuin-A serum concentration level was higher in thyroid disorders patients rather than control group and this mean that Fetuin-A have effect in severity of thyroid disease.

Recommendations

It was recommended to study the immunohistological relationship between Fetuin-A and thyroid disease patients.

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3 Ethical committee

This study was approved by the Ethics Committee of the Faculty of health and medical technologies /Kufa and Medical Ethics Committee of the Ministry of Health in Iraq. In addition the Ethical approval of all patients included in the research study was taken.

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