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Study of midkine protein as prognostic marker for thyroid disease in Al-Najaf City

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Abstract--Objectives: The aim of this study is to detection of various thyroid diseases in the early stages and their impact on the metabolic state of the body through the estimation of the Midkine by using ELISA method. Methodology: 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 Midkine was higher in Hyperthyroidism and Hypothyroidism (499.6 ± 120.2 and 489.1 ± 108.1 respectively) compared to and the control group (372.9 ± 92.5). This result was statistically high significant with p-value ≤ 0.001 . The level of Midkine was higher in both the hyperthyroidism and control group in males compared to females, while in the hypothyroidism group was lower in males compared to females. However, these results were not statistically significant (p-value was > 0.05). Also the level of Midkine was higher in the hyperthyroidism group with no fertility with abortion category compared to others, this result was not significant (p-value was > 0.05 . Conclusion: The level of Midkine was higher in Hyperthyroidism and Hypothyroidism in comparision with control group. Thus it can be used as potential marker of thyroid disease.

Keywords--Hyperthyroidism, Hypothyroidism, MidKine.

Introduction

Hyperthyroidism and hypothyroidism are prevalent disorders that can have serious health repercussions for people all over the world; thyroid disorder is widespread, easily recognized, and treated (Schellack *et al.*, 2020). Thyroid disorders are divided according to the endocrine gland that causes the disease, classified as primary or secondary. Primary thyroid disease means a problem with the thyroid gland itself. While thyroid disease is the second type denotes anterior pituitary disorders that have an indirect effect on thyroid activity (Beynon & Pinneri, 2016).

MidKine is a soluble protein that is found in large amounts in a variety of disorders, including cancer, and might be used as a disease biomarker. MK has been found to be overexpressed in various cancers, mainly when tumors develop to more advanced stages (Filippou *et al.*, 2020). Midkine was initially recognized in embryonic cancer cells, and its levels were high during the early stages of differentiation (PÜRNAK *et al.*, 2020). MK promotes migration, survival, growth, repair, and reproduction. In many disorders, it also has a pathogenic role (Cai *et al.*, 2020).

Most of the circulating MK gene expression has been identified in a number of tissue locations in healthy persons; the kidneys appear to produce MK protein. MK is expressed in normal kidneys in distal and proximal tubular epithelial cells, as well as at a lesser quantity in endothelial cells (Gowhari Shabgah *et al.*, 2021). MK is released by the cells that make it and enters the bloodstream. Because MK synthesis continues in healthy patients, there is a healthy 'background' amount of MK in the peripheral blood. It is critical to define healthy normal MK ranges as a first step in evaluating the efficacy of assessing MK as a disease biomarker (Zhang *et al.*, 2017). The aim of this study is to detection of various thyroid diseases in the early stages and their impact on the metabolic state of the body through the estimation of the Midkine by using ELISA method.

Methodology

Patients and Control Group

Case-control study design 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

Before sample taking from patient and after the agreement of him, the questionnaire was filled and including patient age, gender and Fertility. 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 MidKine 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.

Results and Discussion

1. Basic Demographic of hyperthyroidism, hypothyroidism and Control Subjects

The results of demographic characteristics in Table (1) show that the highest percentage for age categories were 34.78% (31-40) and 50% (31-40) for Hyperthyroidism and Hypothyroidism respectively, while the highest percentage for age category was 31.11% (41 Up) for control group.

On the other hand, most participants are females; 86.96% ($n = 20$), 95.45% ($n=21$), and 77.78% ($n=35$) of Hyperthyroidism, Hypothyroidism, and control groups respectively. Most Participants were married; 82.61% ($n = 19$), 77.72% ($n=17$), and 68.89% ($n=31$) of Hyperthyroidism, Hypothyroidism, and control groups respectively.

Table (1): Demographics of Thyroid disease Patients and Control

Demographic Data		Hyperthyroidism		Hypothyroidism		Control	
		Freq.	%	Freq.	%	Freq.	%
Age groups (Years)	≤ 20	3	13.04	1	4.55	10	22.22
	21 - 30	7	30.43	6	27.27	12	26.67
	31 - 40	8	34.78	11	50.00	9	20.00
	41 Up	5	21.74	4	18.18	14	31.11
	Mean±SD (Range)	32.6±11 (9-55)		34.9±10.6 (20-55)		32.1±11.5 (11-50)	
Gender	Males	3	13.04	1	4.55	10	22.22

	Females	20	86.96	21	95.45	35	77.78
Married	Yes	19	82.61	17	77.27	31	68.89
	No	4	17.39	5	22.73	14	31.11
Total		23	100%	22	100%	45	100%

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

The results in Table (2) 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 reported the total of hypothyroidism patients had higher T3.

Table (2): Hormonal markers in Hyperthyroidism (O), Hypothyroidism (R) in comparison with Control (N).

Hormona 1 Level	Hyperthyroidism n=23	Hypothyroidism 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.

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

The results in Table (3) shows levels of Midkine and Fetuine among the study groups, where the level of Midkine was higher in Hyperthyroidism and Hypothyroidism (499.6 ± 120.2 and 489.1 ± 108.1 respectively) compared to and the control group (372.9 ± 92.5). This result was statistically high significant with p-value = <0.001.

Midkine is a pleiotropic growth factor that is significantly expressed during embryogenesis and regulates cell growth, survival, migration, angiogenesis, and anti-apoptotic activities, but usually has low expression levels in adulthood. Some studies have shown that midkine expression in thyroid dysfunction is strong, and it is related to the clinicopathological characteristics and metastasis of thyroid cancer (wang *et al.*, 2020).

Meng *et al.*, 2015 has successively confirmed midkine's potential as a marker of thyroid cancer. Sheriba *et al.*, 2019 mentioned that Midkine was significantly higher in patients with thyroid nodules compared to control group and significantly higher in those with malignant than with benign nodules, which might be the indicator of malignant thyroid cytopathy, suggesting that Midkine might serve as a novel biomarker in the assessment of thyroid nodules.

Table (3): Biochemical markers in Hyperthyroidism (O), Hypothyroidism (R) in comparison with Control (N).

Biochemical Markers	Hyperthyroidism n=23	Hypothyroidism n=22	Control n=45	Statistics	p value	Post-hoc analysis
	Mean ± SD	Mean ± SD	Mean ± SD			
Midkine (MK)	499.6 ± 120.2	489.1 ± 108.1	372.9 ± 92.5	F = 15.442	<0.001	O, R > N ^c

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.

4. Effects of gender on Biomarker Midkine protein

Figure 1 shows the level of Midkine among the study groups according to Gender. The level of Midkine was higher in both the hyper and control group in males compared to females, while in the hypo group was lower in males compared to females. However, these results were not statistically significant (p-value was > 0.05).

The American Thyroid Association (ATA) claims that women are much more exposed than men to proliferative thyroid diseases. Risk increases when therapeutic estrogens are used and decreases after menopause. Thyroid disorders are more common in women, probably due to the roles of hormones, which are different in females than in males (Krassas and Markou, 2019).

The results of the present study agree with results of Meng said females had higher incidence of thyroid dysfunction than males (Meng *et al.*, 2015). Also compatible with another study reported that females show higher incidence of hyperthyroidism than males (Castello and Caputo, 2019).

Carlé *et al.*, 2015 reported that Hypothyroidism is a very common condition and affects more women than men. And the most common cause of hypothyroidism is Hashimotos thyroiditis, an autoimmune condition. Also Stahlman and Oh, 2018 reported, the thyroid disorders more common in women than in men. And thus our results was compatible with results of these studies.

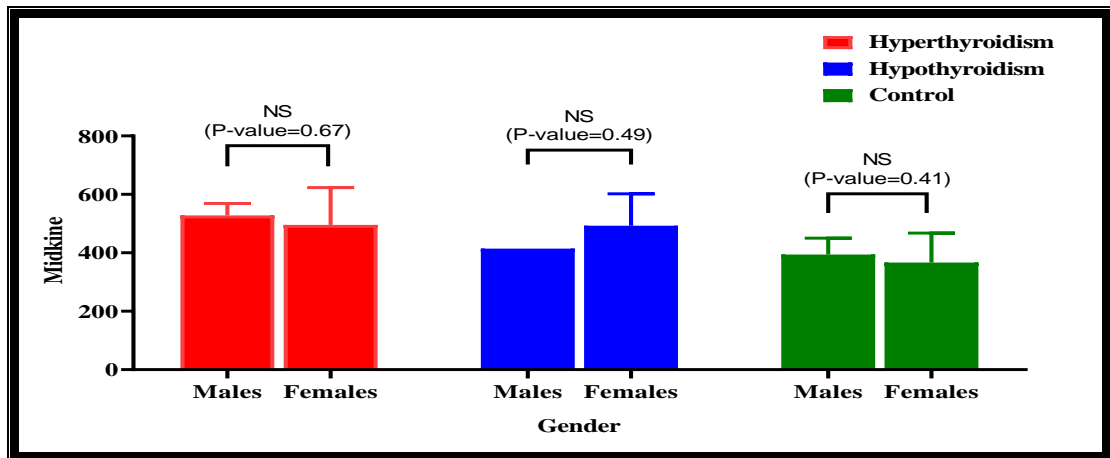


Figure (1): showing the effects of gender on Biomarker protein (MK).

5. Effects of fertility on Biomarker Midkine protein

Figure (2) shows the level of Midkine among the study groups according to married (fertility). The level of Midkine was higher in the hyper group with No fertility with abortion category compared to others, this result was not statistically significant (p -value was > 0.05).

The functions of the thyroid gland have much to do with a woman's reproductive system, particularly if the thyroid is overactive or underactive (Khizroeva *et al.*, 2019). This imbalance in hormone levels may have the following effects on a woman's body:

Thyroid disorders can cause puberty and menstruation to occur abnormally early or late, very light or very heavy menstrual periods, very irregular menstrual periods, or absent menstrual periods this condition called amenorrhea (Kabra and Fisher, 2022).

An overactive or underactive thyroid may also affect ovulation, may prevent ovulation from occurring at all. Severe hypothyroidism can actually cause milk production in the breast, while preventing ovulation (Anwar, S. and Anwar, A. 2016).

Also Thyroid disorders during pregnancy can harm the fetus and may lead to thyroid problems in the mother after birth, such as postpartum thyroiditis. A deficiency of thyroid hormone can cause miscarriages, preterm delivery, stillbirth, and postpartum hemorrhage (Nazarpour *et al.*, 2015). Thyroid disorders may cause the early onset of menopause (before age 40 or in the early 40s). Some symptoms of overactive thyroid (hyperthyroidism) may also be mistaken for early menopause. These include lack of menstruation, hot flashes, inability to sleep (insomnia), and mood swings (Corio, 2013).

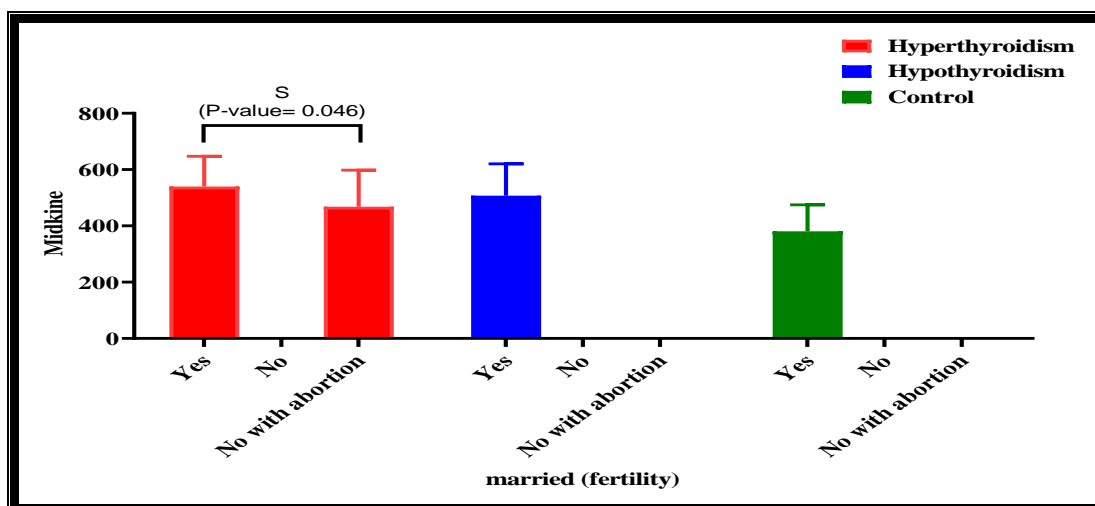
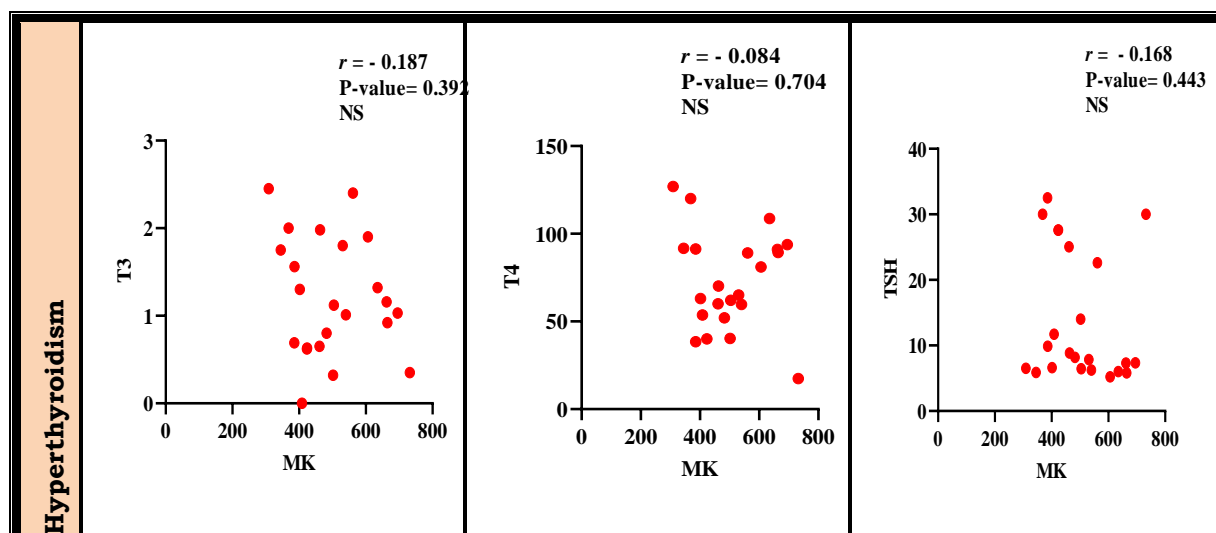


Figure (2): showing the effects of married (fertility) on Biomarker protein (MK).

6. Correlation between Midkine and thyroid hormones in hyperthyroidism, hypothyroidism, and control groups

Figure (3) shows that there are no correlations among Midkine and thyroid hormones in each of the studied groups; (hyper, hypo, and control groups), except there is a statistically high significant positive correlation between level of Midkine and TSH in control group; ($r=0.39$ and p -value was 0.008).



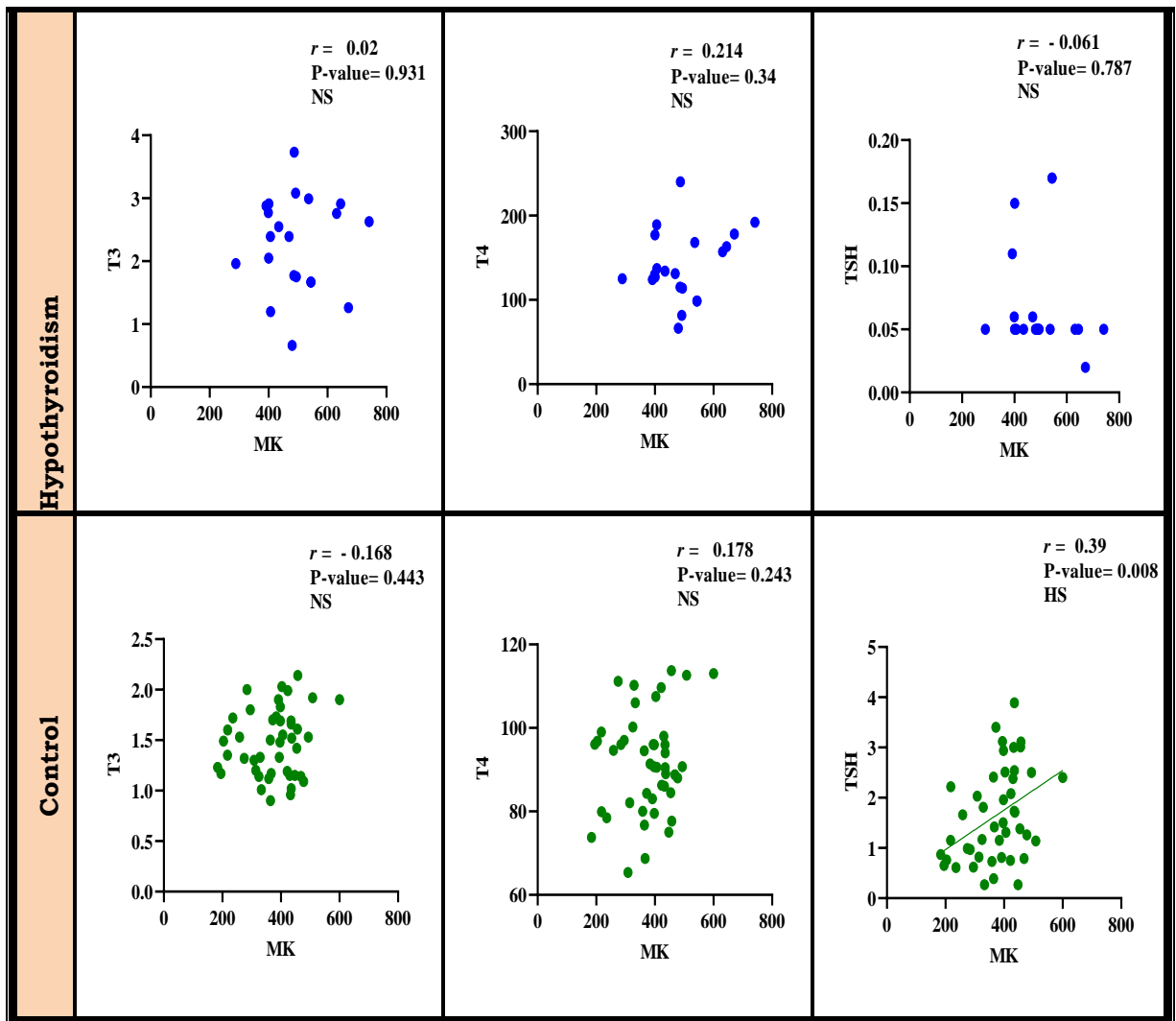


Figure (3): showing Correlation between MK and T3, T4, TSH.

Conclusions

- 1- TSH was higher in Hyperthyroidism compared to Hypothyroidism and the control group.
- 2- The level of Midkine was higher in Hyperthyroidism and Hypothyroidism in comparison with control group.
- 3- Midkine can be used as potential marker of thyroid disease.

Recommendations

It was recommended to study the immunological effect for MidKine on development of thyroid diseases.

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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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