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RESEARCH PAPER

Association of kisspeptin with thyroid hormone levels in patients with hyperthyroidism in Erbil city

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A B S T R A C T:

Background: Hyperthyroidism is a pathological disorder in which excess thyroid hormone is synthesized and secreted by the thyroid gland. There is a few studies ndicate a correlation between the levels of T4 and blood pressure with Kisspeptin hormone levels ,therefore the present study is focusing on the evaluation of kisspeptin in hyperthyroid patients and its association with systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, and body mass index (BMI)
. **Materials and methods:** The study included 80 participants from both sexes who were divided into two groups Group 1 Including healthy subjects and Group 2 Including 40 hyperthyroid subjects. **Results:** The results showed significant differences in the concentration of the T4 and kissiptin hormones between control and patients with hyperthyroidism On the other hand, concentration of Kisspeptin significantly and negatively correlated with DBP .While negative non-significant correlation existed between , SBP, MAP level and kisspeptin concentration . The cut-off value for Kisspeptin was found to be >20.2 with good sensitivity and specificity, indicating its potential use as a diagnostic marker for hyperthyroidism in females. **Conclusions:** These findings highlight the potential role of kisspeptin as a diagnostic marker for hyperthyroidism, particularly in female patients

KEY WORDS: Hyperthyroidism, Kisspeptin, Thyroxin, SBP, DBP, BMI
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1. INTRODUCTION:

The thyroid hormone is well known for controlling a variety of bodily functions, including growth, metabolism, and many more. The primary hormone produced by the thyroid gland is thyroxine, also known as tetraiodothyronine (T4) and triiodothyronine (T3). To maintain an optimal feedback mechanism and homeostasis, TSH from the anterior pituitary gland, thyroid-stimulating hormone (T4) from the hypothalamus, and T4 all function in synchrony. (Núñez et al., 2017).Hyperthyroidism elevated thyroid gland activity manifests as tremor, mild heat sensitivity, diarrhea, and muscle weakness (Schweizer and Köhrle, 2013, Manousaki and Van Vliet, 2022).

Kisspeptins are a family of peptides that promote the release of the gonadotropin releasing hormone (GnRH), which is necessary for normal ovarian function and the onset of puberty (d'Anglemont de Tassigny and Colledge, 2010).

Kisspeptin-54 (Kp-54) and shorter peptides of 14, 13, or 10 amino acids are produced from the 145-amino acid protein that the Kiss1 gene encodes (Dungan et al., 2006).

Additionally, it has been found that kisspeptin is linked to measures of insulin resistance and body mass index (Panidis et al., 2006).The mechanism by which kisspeptin affects body weight is unknown . Studies on mice lacking the kisspeptin receptor (Kiss1r KO mice) have interestingly revealed that mature Kiss1r KO females kept on a conventional chow diet demonstrated a noticeable increase in body weight in comparison with wild type littermates. (Tolson et al., 2016). Further research has revealed that defective kisspeptin signaling lowers metabolism and energy expenditure, which in turn leads to an increase in adiposity. Female mice with the Kiss1r mutation (Tolson et al., 2016) .Whether increased kisspeptin levels increase energy expenditure in humans is still undefined (Andreozzi et al., 2017).

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Kisspeptin has potential as a therapeutic agent in disorders of reproduction it is important to determine whether kisspeptin administration to humans alters blood pressure or heart rate in vivo (Nijher et al., 2010). Early pregnancy kisspeptin concentration is a possible indicator of pre-eclampsia.. More importantly, (Han et al., 2010) concluded that central administration of kisspeptin-10 inhibited volume expansion - induced natriuresis and diuresis. despite plasma atrial natriuretic peptide content and renal sympathetic nerve activity, this impact was probably mediated by an increase in vasopressin release.

However, emerging evidence suggests that kisspeptin might also influence cardiovascular function, including blood pressure regulation previous study aimed to investigate the potential effects of kisspeptin on blood pressure. Study investigated the vascular effects of kisspeptin in humans. Researchers conducted experiments on healthy male volunteers, where they administered kisspeptin-10, a specific isoform of kisspeptin, and measured its impact on the forearm microcirculation. The results revealed that kisspeptin-10 caused significant vasodilation, resulting in increased blood flow in the forearm microvasculature. This vasodilatory effect was mediated through nitric oxide (NO)-dependent mechanisms, as it was abolished when NO synthesis was inhibited (Clarke et al., 2015).However,another study indicated that intravenous administration of kisspeptin led to a significant increase in systolic blood pressure and diastolic blood pressure in a dose-dependent manner. Additionally, administration of a kisspeptin receptor antagonist attenuated this blood pressure response, indicating that the effects are mediated through kisspeptin receptorsvasoconstriction in large human arteries (Sawyer et al., 2011b).

To our knowledge, this is the first study to show the evaluation of kisspeptin in hyperthyroid patients and its association with alteration in systolic blood pressure , diastolic blood pressure , and heart rate compared to healthy subjects. The role of kisspeptin in the regulation of blood pressure and weight loss remains to be elucidated.

Subjects and study design

The design of the present investigations is composed of 80 males and females divided into two groups, Group 1: Included 40 subjects who were clinically healthy and their age range 20-65 years, who served as a control group of the study. Group 2: Included 40 patients with hyperthyroid disease, elevated serum level of T4 with decreased level of TSH their age range 20-65 years and having a definite symptoms of hyperthyroidism like (palpitation, sweating, weight loss, goiter, hand tremor, nervousness and ophthalmopathy).

Inclusion and Exclusion Criteria

Participants in the study were found to be nonsmokers, and there was no clinical evidence of diabetes or infections. This was determined by carefully recording their histories, performing physical exams, and running regular laboratory tests for other diseases (pregnancy, hepatic diseases, diabetes, cardiac diseases and their age above 60 years old).Inclusion criteria for hyperthyroidism with elevated thyroid hormone and decreased TSH level, having hyperthyroid disease with their age between (20-65) years old.

Hormonal analysis

Determination of thyroid hormone and kisspeptin

Five milliliters of peripheral venous blood sample were taken from each subject in Erbil clinical laboratories using disposable plastic syringe and transferred into gel and clot activator test tube 5ml, then the blood was allowed to clot and centrifuged at 1000g for 15 minutes and the serum was obtained. The serum level of T4 was determined by fully automated immunoanalyzer (Cobas e 411 Roche Diagnostics, HITACHI, Japan).Serum kisspeptin was determined according to Sandwich-ELISA method using human kisspeptin ELISA kit(Sunlong Biotech Co.,Ltd).

Anthropometric measurements

Measurement of blood pressure and heart rate

Systolic blood pressure, diastolic blood pressure and heart rate was measured in the sitting position after 10min of rest min from the left arm of each subject using an electronic sphygmomanometer.

Measurement of Body mass index

The height and weight of the participant were measured using a stadiometer , were a coat and shoes are absent. Body mass index was calculated as weight (kg) divided by square of height (m^2), as shown in the following formula:

BMI=Weight in kg / (Height in m)^2

BMIs falls into four major categories: Underweight: less than 18.5, Normal weight : 18.5 to 24.9, Overweight : 25 to 29.9 and obesity: 30or more (Boron and Boulpaep, 2012).

Statistical analysis

Normality test using Kolmogrov-Smirov and Shapiro_willk test were used to confirm Gausian normal data distribution .For non-parametric data ,Mann-Whitney test was applied to compare the control and cases here, the data are expressed as median (interquartile range).For parametric data, independent t-test was used by using Graphpad prism(version 8).The data are expressed as mean±SEM. Med calc software was used for finding out cut-off of measured variables and sensitivity(%), specificity(%)PPV,NPP and AUC(95% CI)are also calculated. Spearman correlation was also applied between kisspeptin as dependent variable with the studied independent variables (SBP, DBP, HR, Weight, Height and BMI) P<0.05 was considered statistically significant.

Results

Statistical analysis using Mann-Whitney test for obtained non-parametric data revealed that serum Kisspeptin levels was significantly (p<0.001)increased in female hyperthyroidism as compared with control subjects, whereas its level did not change in male hyperthyroidism as compared with the male control people (table 1 ,figure 1).The results shown in table (2)illustrated that the cut-point ,sensitivity (%),specificity (%),PPV(%) ,NPP(%) and AUC (95%) were >20.2,72.5,87.5,85.3,76.1,0.8(0.69-0-88)(p<0.0001),respectively.

On the other hand and according to kisspeptin cut-off value (>20.2 and<20.2, the levels of serum T4 were 189(183_201) in cut-off >20.2 and 129(92_182) in cut-off <20.2and the changes was significantly (p<0.001) differences between their cut-off values.

Serum T4 levels in hyperthyroid patients were significantly (P<0.001**)** higher compared with the control subjects, it's clear that in Hyperthyroid patients the level of T4 increases so this test done for confirmation of the incidence of Hyperthyroidism condition.

On the other hand DBP were significantly lower in all subjects ((71.03±1.288 mmHg),male (74.92 ± 1.759 mmHg)and females (77.56 ± 1.320 mmHg), respectively in comparison to control subjects (77.45 ± 1.173 mmHg, 77.23 ± 2.434 mmHg, 77.56 ± 1.320 mmHg respectively). while SBP was slightly higher in male hyperthyroid subjects as compared with control subjects (Table 1).Furthermore ,the cutpoint of DBP was <76 mmHg with sensitivity(77.5),specifity (62.5), PPV(67.4),NPP(73.5) and AUC(95%CI 0.73(0.62-0.82) as presented in (Table 2).

Body mass index of hyperthyroid subjects were slightly non significantly lower than control subject (P Value 0.0406) for both subject, Males P value 0.3191 and Females (P Value 0.042) (Table 1).Furthermore, the cut-point of BMI was ≤25.8 with sensitivity(37.5),specificity (87.5), PPV(75),NPV(58.3) and AUC(95%CI 0.64(0.52-0.74) (Table 2).However, weight of female hyperthyroid subjects was lowered but not statistically significant.

The results in table (4) revealed a positive correlation between kisspeptin concentration and T_4 (r= –0.1477, p=0.3632).Furthermore, T_4 range was 189(183_201) at kisspeptin cutoff (>20.2) and 129(92-182) at kisspptin cutoff (<20.2) (Table 3).

On the other hand, concentration of Kisspeptin significantly and negatively correlated with DBP (r= -0.3140, p=0.0549). While negative non-significant correlation was existed between, SBP, MAP level and kisspeptin concentration (r= – 0.1657, p=0.3202), (r= – 0.2776, p=0.0916) (Table 4).

Discussion

In hyperthyroidism, there is an excess of thyroid hormone in the body, which can lead to an increase in kisspeptin expression. Although the mechanism underlying the increase in kisspeptin levels is not fully understood, it is believed to be related to the effects of thyroid hormones on the hypothalamic-pituitary-gonadal axis (Sun et al., 2018). Serum kisspeptin levels were significantly higher in patients with hyperthyroidism compared to healthy controls (Sun et al., 2018).Another study found that kisspeptin mRNAexpression was increased in the hypothalamus of rats with experimentally –induced hyperthyroidism (Novaira et al., 2009).

Moreover, serum levels of kisspeptin, a neuropeptide that stimulates GnRH secretion and regulates the reproductive axis, are significantly increased in hyperthyroidism. The study found that treatment with antithyroid drugs reduced kisspeptin levels in hyperthyroid patients (Jayasena et al., 2011).The exact mechanism by which thyroid hormones regulate kisspeptin expression is not fully understood but is thought to involve the interaction between thyroid hormone receptors and specific DNA sequences in

the kisspeptin gene promoter (Jayasena et al., 2014).

In the present study, the diastolic blood pressure of hyperthyroid patients was lower than healthy subjects. Number of studies have investigated the potential role of kisspeptin in the regulation of blood pressure. Astudy published in the Journal of Hypertension found that intravenous administration of kisspeptin led to a significant reduction in blood pressure in rats (Sawyer et al., 2011a). Similarly, another study found that infusion of kisspeptin into healthy male volunteers led to a significant decrease in systolic and diastolic blood pressure. Furthermore, central administration of kisspeptin-10 could inhibit sodium excretion and urine flow in anesthetized male rats, which probably mediated by increasing the plasma vasopressin concentration and is independent of plasma arterial natriuretic peptide concentration and renal sympathetic nerve activity (Ten et al., 2010).

The mechanisms underlying the blood pressure lowering effects of kisspeptin are not fully understood. However, it has been suggested that kisspeptin may act through a variety of pathways, including the activation of nitric oxide signaling and the modulation of the renin-angiotensin system, the Nitric Oxide (NO) system has been implicated in the regulation of cardiovascular function, and kisspeptin has been shown to have beneficial effect on health through its interaction with NO (Carlström, 2021) .

According to Rafique and Latif (2015), there may not be a significant difference in serum kisspeptin levels between young women who are overweight or obese and those who are of normal weight. Additionally, there may not be a relationship between serum kisspeptin and anthropometric measurements(Latif and Rafique, 2015). According to Zhu et al. (2016), kisspeptin and obesity-related indicators were positively correlated in a different study with both male and female participants(Zhou et al., 2014).According to animal studies,kisspeptin treatment can help rats eat less and lose weight (Wen et al., 2020).

Conclusions

In conclusion, this study found that serum Kisspeptin levels were significantly in female hyperthyroidism patients compared to control subjects. The cut-off value for Kisspeptin was found to be >20.2 with good sensitivity and specificity, indicating its potential use as a diagnostic marker for hyperthyroidism in females. Finally, there was a positive correlation between the levels of T4 and Kisspeptin and a negative correlation between the levels of DBP and Kisspeptin.These findings highlight the potential role of kisspeptin as a diagnostic marker for hyperthyroidism, particularly in female patients.

Table 1: Mean ± S.E of serum Kisspeptin, T4, SBP,DBP,Weight,Height and BMI in control and male and female patients with Hyperthyroidism.

	Males			Females			Both		
	Control	Hyperthyroidism	P values	Control	Hyperthyroidism	P values	Control	Hyperthyroidism	P values
Kisspeptin	16.98±0.5929	18.32±0.864	**0.1953**	18.05(15.95-19.65	24.94(21.05-27.90)	<**0.001**	17.50(16.23_19.20)	22.21(18.86_26.78)	<**0.0001**
T4	102.3±8.176	191.2±2.778	<**0.0001**	122.3±6.615	193.9±7.203	<**0.001**	115.8±5.349	197.5±2.211	<**0.0001**
SBP	122.2±1.553	135.5±4.635	0.0160	124.9±1.549	121.7±4.924	**0.5430**	**124.5(121-129)**	**129(116-140)**	**0.2292**
DBP	77.23±2.434	74.92±1.759	0.4498	77.56±1.320	67.18±2.506	**0.0007**	**77.45 ±1.173**	**71.03±1.288**	**0.0004**
Weight	73(68_78)	75(70-81)	0.273	71.3±1.43	69.5±1.35	0.228	72.28±1.054	70.18±1.23	0.197
Height	159(158-162)	166(162-173)	<0.001	159(158_162)	160(156_162	0.7135	159(158-163)	160(157_165)	0.5018
BMI	27.70 ±0.3989	26.94±0.6325	0.3191	28.21±0.5208	26.37±0.7047	0.0424	28.04±0.3734	26.87±0.4226	0.0406

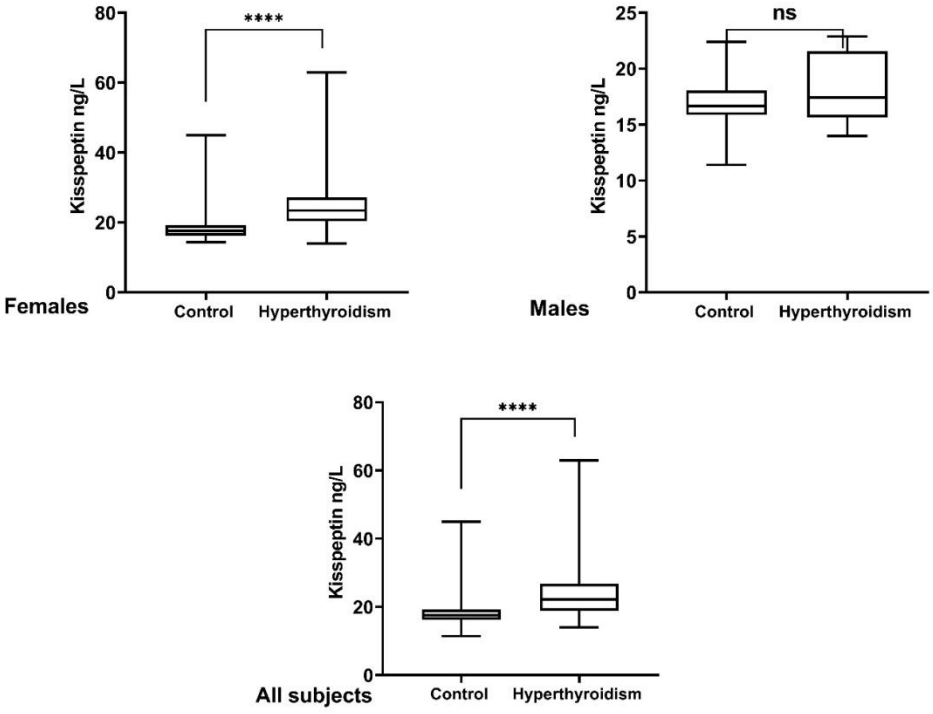

Figure 1. Box plot of serum Kisspeptin of hyperthyroid subjects compared to control subjects In Male ,Female and both sexes respectively is expressed with star sign (*** P<0.001) Data are given as mean ± S.E.

Table 2: Optimal cut-offs for Kisspeptin and anthropometric variables and the associated sensitivities and specificities.

	Cut-point	**Sensitivity (%)**	**Specificity (%)**	**PPV**	**NPP**	**AUC (95% CI)**	**P values**
Kispeptin	>20.2	72.50	87.50	85.3	76.1	0.80(0.69- 0.88)	0.0001
T4	>179	100	100	100	100	1	0.0001
SBP	>138	32.50	100	100	59.7	0.57 (0.46 - 0.68)	0.251
DBP	≤76	77.50	62.50	67.4	73.5	0.73 (0.623 -0.82)	0.0001
MAP	≤90	52.50	70.00	63.6	59.6	0.58(0.47- 0.69)	0.172
HR	>85	45.00	80.00	69.2	59.3	0.62(0.50- 0.72)	0.052
Height	>160	47.50	67.50	59.4	56.2	0.54(0.42- 0.65)	0.507
Weight	≤62	22.50	95.00	81.8	55.1	0.59 (0.47-0.70)	0.146
BMI	≤25.8	37.50	87.50	75.0	58.3	0.64(0.52- 0.74)	0.025

Table 3: Optimal cut-offs for anthropometric variables and the associated sensitivities and specificities.

Variables	Kisspeptin cut off		
	>20.2	<20.2	P values
T4	189(183-201)	129(92-182)	0.0001
SBP	127.0(114-140)	125(121-131)	0.7703
DBP	73 (66-78)	78(70-82)	0.0038
MAP	89(84-98)	94(88-99)	0.7703
Height	159(157-162)	160(158-165)	0.0847
Weight	70(66-73)	75(68-78)	0.0110
BMI	27(25-28)	27(25-29)	0.3808

Table 4: Simple regression analysis for the association between kisspeptin as dependent variables with SBP, DBP, MAP, height, weight and BMI as independent variables in patients with Hyperthyroidism.

	r	CI 95 %	p
T4	0.1477	0.1718 to 0.4390	0.3632
SBP	-0.1657	-0.24610 to 0.1626	0.3202
DBP	-0.3140	-0.5758 to 0.0063	0.0549
MAP	-0.2776	-0.5486 to – 0.0462	0.0916
Height	-0.4108	-0.6457 to -0.1049	0.0104
Weight	-0.2749	- 0.5465 to -0.04912	0.0949
BMI	0.0036	-0.3164 to 0.3229	0.9827

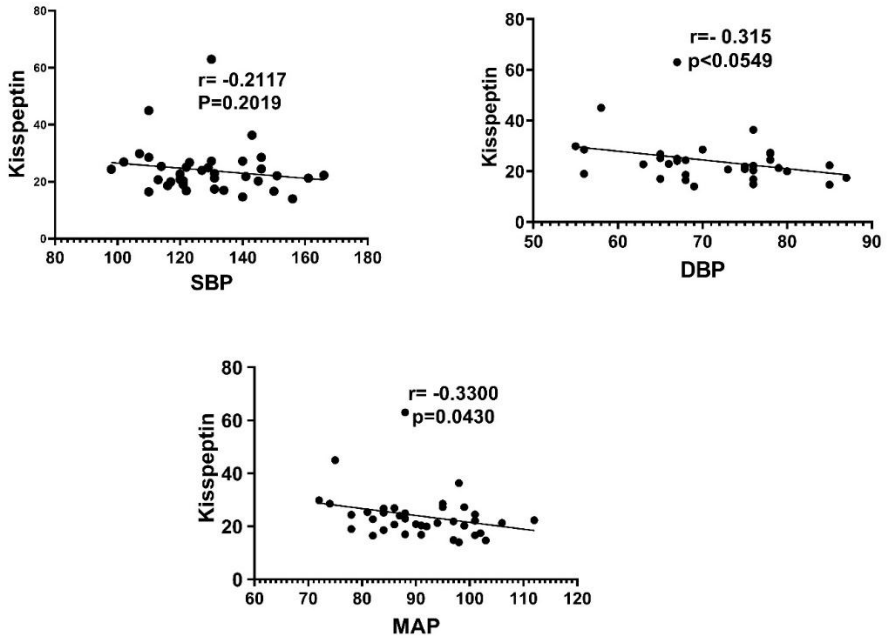

Figure 2: Simple regression analysis for the association between kisspeptin as dependent variables with SBP, DB and MAP as independent variables in patients with Hyperthyroidism.

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