ORIGINAL ARTICLE

Neuropeptide Y-Agouti related peptide ratio (NAR) in patients with idiopathic primary hypothyroidism: Nudge and Risk

Hayder Mutter Al-Kuraishy, May Hassan Al-bdulhadi, Ali Ismail Al-Gareeb

Abstract

Objectives: To illustrate the role of neuropeptide Y (NPY), agouti-related-peptide (AgRp) and their ratio in patients with primary hypothyroidism (PHT) regarding the effect of levothyroxine (LT4) replacement therapy.

Methods: The case-control study was conducted at the Department of Pharmacology, College of Medicine, Mustansiriya University, Baghdad, Iraq, from March to June, 2020 and involved 40 patients with primary hypothyroidism (PHT), including 20 newly diagnosed patients and 20 patients on levothyroxine (LT4) replacement therapy compared to 20 healthy controls. Anthropometric, lipid and pressure profiles were evaluated. Also T3, T4, TSH, NPY and AgRp serum level were estimated in different treated groups. SPSS version 20.00 was used for data analysis.

Results: Body mass index (BMI) was higher in the newly diagnosed patients without thyroxine therapy as compared to patients on thyroxine therapy (P=0.03). Blood pressure profile was higher in patients with PHT compared to the controls (P=0.0001). NPY serum level was lower in patients with PHT without thyroxine therapy (27.32±10.30ng/dL) as compared to patients with PHT on thyroxine therapy (61.10±22.78ng/dL), (P=0.04). AgRP serum level was lower in patients with PHT (9.81±4.86ng/dL) as compared to the patients on the thyroxine therapy (28.99±2.16ng/dL), (P=0.03). Besides, NPY-AgRP ratio (NAR) was higher in patients with PHT (2.78±0.14) as compared to patients with PHT on thyroxine therapy (2.10±0.19), (P=0.0001).

Conclusion: Both of NPY and AgRP serum levels are reduced in the newly diagnosed patients with PHT and ameliorated following LT4 replacement therapy. Also NPY/AgRP ratio is linked with early PHT and regarded as a prognostic value for the outcomes of patients with PHT.

Keywords: Primary hypothyroidism, Neuropeptide Y, Agouti-related-peptide. (JPMA 71: S-27 [Suppl. 8]; 2021)

Introduction

Hypothyroidism is defined as a reduction of thyroid function, due to insufficient production of thyroid hormone initiating diverse dysfunction of body organs due to reduction of basal metabolic rate (BMR).[1] Primary hypothyroidism (PHT) is due to deficiency of thyroid hormones (THs) due to the abnormalities in the thyroid gland such as surgical removal, radioactive iodine therapy and autoimmune thyroiditis.[2]

PHT is mainly affected by dietary iodine supplementation, which is an important component of thyroid hormones (THs).[6] PHT is more common in women compared to men, and the frequency increases with advancing of age.[4,5]

PHT is concomitant with diverse metabolic and neurological disorders including; hypertension, dyslipidaemia, depression, cold intolerance, anorexia, weight gain and hypo-metabolism.[6]

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PHT induced-neurological disorders are due to disturbances of brain neurotransmitters and neuromodulators such as neuropeptide Y (NPY) and Agouti-related-peptide (AgRp).[7]

NPY is a neurotransmitter of the brain and autonomic nervous system, commonly expressed throughout the central nervous system (CNS) and co-secreted with other neurotransmitters like glutamate and gamma amino-butyric acid (GABA). There are four G-protein receptors of NPY, which are: Y1, Y2, and Y4 and Y5, which inhibit cyclic-adenosine monophosphate (cAMP).[8] NPY signaling plays an essential role in neuronal development, thermogenesis, blood pressure, emotional behaviour, feeding and energy expenditure.[9]

It has been reported that experimental hypothyroidism inhibits neuropeptide Y and increases pro-opiomelanocortine (POMC) leading to anorexia and hypophagia, since NPY regulates the activity of thyrotrophic releasing hormone (TRH) neurons.[10]

On the other hand, AgRp which is an orexigenic neuropeptide released by AgRp/NPY neurons of ventromedial part of arcuate nucleus of hypothalamus,

increases appetite through regulation of energy expenditure and body metabolism.[11]

Both of AgRp and NPY neurons inhibit the expression of TRH mRNA leading to central hypothyroidism, and so administration of AgRp improves food intake and body weight through regulation of THs and TRH mRNA.[12]

Therefore, objective of the present study was to illustrate the role of NPY, AgRp and their ratio in patients with PHT regarding the effect of levothyroxine (LT4) replacement therapy.

Patients and Methods

This case-control study was done in the Department of Clinical Pharmacology, College of Medicine, AL-Mustansiriyah University in cooperation with the Endocrinology and Diabetic Center, Baghdad-Iraq from September 2019 till January 2020. This study was approved by Scientific and Ethical Committee and Editorial Board, College of Medicine, AL-Mustansiriyah University.

A total number of 60 patients with idiopathic primary hypothyroidism (PHT) were recruited and compared with 28 healthy controls. The sample size was calculated according to the population size regarding 95% confidence interval and 5% marginal error

The recruited patients in conjugation with healthy controls were allocated into three groups following detailed medical history and physical examination:

Group A: Newly diagnosed patients with PHT before starting thyroxine replacement therapy (n=20). Group B: Patients previously diagnosis with primary hypothyroidism on thyroxine therapy (n=20). Group C: Healthy individual as controls (n=20).

The inclusion criteria of recruited patients were; patients with primary hypothyroidism on thyroxine therapy and patients who were newly diagnosed with primary hypothyroidism before starting thyroxine replacement therapy.

All patients with any chronic disease such as kidney, liver, heart and respiratory diseases, thyroidectomy, secondary or tertiary hypothyroidism, pregnancy and lactation, psychiatric and mental disorder were excluded.

Measurement of biochemical parameters: Five milliliters of blood sample was obtained from each patient, and centrifuged at 3000/rpm for 15 minutes to separate the serum samples. One ml of the serum was utilized to determine TSH, thyroid hormones, and lipid profile by utilizing enzyme-linked-fluorescent immune-assay (ELFA). The remaining part, was stored at (-20°C) for estimation of NPY and AgRp serum levels by ELISA kit methods.

Measurement of anthropometric parameters: Weight and height were calculated by digital stadiometer and tape measure respectively. Body mass index (BMI), BMI= weight (Kg)/height (cm^2). In addition, systolic and diastolic blood pressure were measured by using blood pressure monitoring device, as well mean arterial pressure (MAP) which was measured by specific equation, MAP (mmHg) = diastolic blood pressure + 1/3 pulse pressure (PP) and pulse pressure = systolic blood pressure (SBP) — diastolic blood pressure (DBP).[13]

Data of the present study was analyzed by using Statistical Package for Social Science, version 22.00 (SPSS-22). The data were presented as mean± SD, percentage and number. Unpaired t-test was used to test the significance differences of dissimilar quantitative data (different means). One-way ANOVA test was used for differences between more than 2 independent means. Statistical significance was considered when p value less than 0.05.

Results

The average age of patients with PHT was (43.32±8.09) years, the duration of hypothyroidism was 2.11±1.12 months, with a lower percentage of males 9(10%) compared to females, 81(90%). Most of patients were non-smokers 54(87.09%). The associated diseases were dyslipidaemia and hypertension. The current medication that were used in addition to thyroxine therapy included,

Table-1: Demographic characteristics of the present study.

Variables	n, mean ±SD, %
n	60
Age (years)	43.32±8.09
Gender (Male:Female ratio)	9(10):81(90)
Smoking	8(8.89)
Non-smoker	54(87.09)
Duration of hypothyroidism (months)	2.11±1.12
Associated diseases	
Dyslipidaemia	17(27.41)
Hypertension	26(41.94)
Medications	
Thyroxine	35(56.45)
Amlodipine	17(27.41)
ACEIs	8(12.90)
ARBs	5(8.06)
Statins	4(6.45)
Omega-3-fatty acid	7(11.11)

Data are expressed as n, mean±SD, %, M:F: male:female, ACEIs: angiotensin converting enzyme inhibitors, ARBs: angiotensin receptor blockers.

Table-2: Metabolic profile in patients with primary hypothyroidism.

Variables	Control (n=20)	Newly diagnosed PHT (n=20)	PHT on thyroxine (n=20)	P
BMI(kg/m^2)	31.61±3.72	34.81±5.70	31.75±5.45*	0.03
SBP(mmHg)	111.64±12.85	145.61±15.80#	128.42±13.91*¶	0.0001
DBP(mmHg)	75.93±9.75	97.42±11.73#	128.42±13.91*¶	0.0001
PP(mmHg)	35.71±6.94	48.19±7.56#	46.70±8.52¶	0.0001
MAP(mmHg)	87.83±8.63	113.48±12.96#	46.70±8.52¶	0.0001
TC (mg/dL)	143.67±13.27	176.43±14.88#	150.62±14.14*	0.0001
TG(mg/dL)	132.25±16.54	184.00±13.85#	139.47±16.73*	0.0001
HDL-c(mg/dL)	39.29±12.59	32.62±3.81	35.17±12.04	0.06
LDL(mg/dL)	77.90±8.52	107.00±13.85#	115.45±11.73*¶	0.0001
VLDL(mg/dL)	26.45±12.61	36.85±9.44#	27.89±9.73*	0.0001
AIP	0.161±0.01	0.391±0.04#	0.238±0.03*¶	0.0001
CRR	3.65±1.89	5.40±1.88#	4.28±1.07*	0.03
CVRI	3.36±1.08	5.64±2.31*	3.96±1.67*	0.04
T4(µg/dL)	91.58±0.38	66.19±10.57	97.62±19.54*	0.001
T3 (ng/dL)	0.53±12.00	1.34±0.30#	1.61±0.32*	0.005
TSH (mIU/L)	2.27±0.76	10.17±6.02#	2.53±1.62*	0.001

Data are presented as mean ±SD, ANOVA testandTukey HSD Post-hoc Test, BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; AI: atherogenic index, CRR: Cardiac Risk Ratio, CVRI: cardiovascular risk index, PHT: primary hypothyroidism; T4: thyroxine ; T3: triiodothyronin ; TSH: thyroid stimulating hormone

* P <0.05 compared to untreated group.

# P<0.05compared to the control.

¶ P<0.05compared to the control.

angiotensin converting enzyme inhibitors (ACEIs) 8(12.90%), angiotensin receptor blockers (ARBs) 5(8.06%), omega 3 fatty acid 7(11.11%) and statins 4(6.45%) as shown in Table-1.

Body mass index (BMI) was higher in newly diagnosed patients without thyroxine therapy compared to patients on thyroxine therapy (P=0.03). Blood pressure profile was higher in patients with PHT compared to the controls (P=0.0001), and it was higher in the newly diagnosed patients as compared to PHT on thyroxine therapy.

Besides, lipid profile showed higher values in patients with PHT who were newly diagnosed or on thyroxine therapy compared to the controls (P=0.0001) with exception to HDL-c level which was not significantly different among groups (P=0.06). In addition, atherogenic index (AIP) was higher in patients with PHT compared with the controls (P=0.0001), however, it was higher in patients with PHT (0.391±0.04) as compared to patients with PHT on thyroxine (0.238±0.03), (P=0.0001).

Furthermore, cardiac risk ratio (CRR) and cardiovascular risk index (CVRI) were higher in patients with PHT compared with the controls (P=0.03) and those on Thyroxine therapy (P=0.04) respectively. Both CRR and CVRI were lower in patients with PHT on thyroxine therapy compared with newly diagnosed patients with PHT.

Regarding thyroid hormones, there were significant differences in thyroid function parameters, the differences in T4 value between newly diagnosed and thyroxine treated group was significant (P=0.001), it was higher in patients on thyroxine therapy (97.62±19.54) as compared to newly diagnosed patients (66.19±10.57). The difference of T3 values between newly diagnosed and those on thyroxine was significant. Similar difference was noted between the controls and newly diagnosed cases (P=0.005). TSH value was higher in newly diagnosed patients (10.17±6.02) as compared to patients on thyroxine therapy (2.53±1.62), (Table-2).

Neuropeptide Y (NPY) serum level was lower in patients with PHT without thyroxine therapy (27.32±10.30 ng/dL) as compared to patients with PHT on thyroxine therapy (61.10±22.78 ng/dL), (P=0.04), and controls (66.65±13.77 ng/dL), (P=0.001). Agouti related peptide (AgRP) was lower in patients with PHT compared with controls (P=0.001), it was lower in the newly diagnosed patients not on thyroxine therapy (9.81±4.86 ng/dL) as compared to the patients on thyroxine therapy (28.99±2.16ng/dL), (P=0.03), (Figure-1).

On the other hand, NPY-AgRP ratio (NAR) was higher in patients with PHT (2.78±0.14) as compared with healthy controls (1.83±0.80), (95%CI=0.6493 to 1.2507, P=0.0000), and low as compared with patients with PHT on thyroxine therapy (2.10±0.19), (95%CI=-0.9656 to -0.3944,

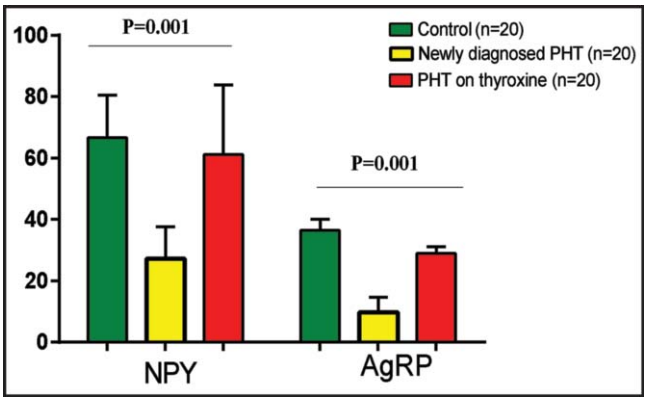
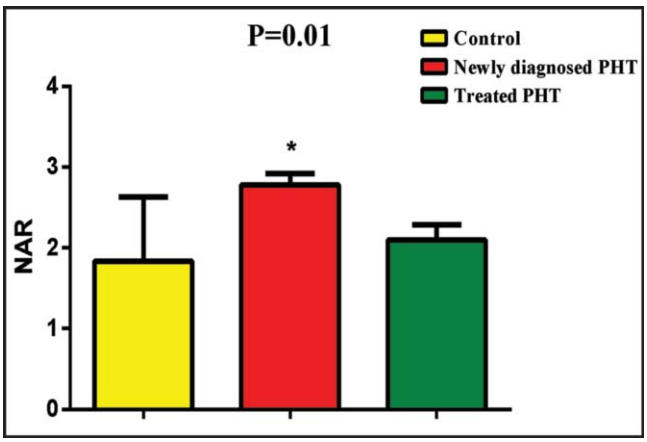


Figure-1: Neuropeptide Y (NPY) and Agouti related peptide (AgRP) in patients with primary hypothyroidism.



*** P<0.01 compared to the treated group or control.**
Treated versus control not significant P=0.06.
NAR: Neuropeptide Y - Agouti related peptide ratio.

Figure-2: (3.2): Neuropeptide Y-Agouti related peptide ratio (NAR) in patients with primary hypothyroidism compared to the controls.

P=0.0000). However, there was no significant difference between control and PHT on thyroxine therapy (95%CI=-0.0127 to 0.5527, P=0.0643), (Figure-2).

Discussion

Human appetite and food intake are induced and stimulated by orexinergic neuropeptides (NPY and AgRP), and inhibited by pro-opiomelanocortine (POMC) neuropeptide. These neurons are found mainly in the arcuate nucleus (ARC) of the hypothalamus.[14] In the present study, both NPY and AgRP were reduced in newly diagnosed patients with PHT as confirmed by Baltaci et al's study, which illustrates that low appetite in PHT is linked with low levels of both NPY and AgRP.[15] It has been noticed, that both of NPY and AgRP are reduced significantly in PHT.[16] Moreover, high TRH and TSH in PHT cause a paradoxical elevation of AgRP, since AgRP has potent inhibitory action on TRH in PHT. Added to this, different studies disclosed that anorexia in PHT is related to reduction of NPY and AgRP and augmentation of POMC pathway.[17]

Following thyroxine replacement therapy, both NPY and AgRP serum levels were increased in patients with PHT. It has been shown, that thyroxine replacement therapy improves body metabolism and metabolic profile not only through activation of BMR, but also centrally through activation of NPY and AgRP at arcuate nucleus (ARC) of hypothalamus.[18] T4 is converted centrally to the biologically active form T3, which induces uncoupling protein 1(UCP-1). UCP-1 improves body energy expenditure and appetite through stimulation of NPY/AgRP axis.[19] Therefore, NPY/AgRP axis is enhanced by thyroxine replacement therapy as reflected through improvement of BMR and BMI. Several studies demonstrated that T3 acts on the ARC to increase the synthesis of AgRP and NPY. Also, an animal model study reported that T3 has a direct effect on the expression of AgRP in ARC.[20]

Interestingly, no previous studies determine the NPY/AgRP ratio (NAR), however, the present study demonstrated that NAR was higher in the newly diagnosed PHT patient as compared to controls and PHT on thyroxine therapy, as well there was no significant differences among controls and patients on thyroxine replacement therapy.

In the present study, higher NAR in patients with PHT was due to a relatively higher reduction of AgRP in relation to NPY serum levels, since NPY mediates its action through up-regulation of AgRP gene in the lateral hypothalamus. Indeed, AgRP is more affected than NPY in PHT and responds better to the effect of thyroid hormones.[21]

Therefore, a higher NAR is linked with early PHT and regarded as a prognostic value for the outcomes of patients with PHT.

Conclusion

Both of NPY and AgRP serum levels are reduced in the newly diagnosed patients with PHT and ameliorated following thyroxine replacement therapy. Also, NAR is linked with early PHT and regarded as a prognostic value for the outcomes of patients with PHT.

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Conflicts of Interest: None.

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