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STUDY OF GHRELIN LEVELS IN HYPOTHYROID PATIENTS BEFORE AND AFTER TREATMENT

Biochemistry

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ABSTRACT

Objective: Aim of the present study was to evaluate the levels of ghrelin in hypothyroid patients before and after treatment with L-thyroxine and to find a possible relationship between ghrelin and thyroid hormones.

Material & Methods: The present study was conducted on 100 hypothyroid patients (44 Males & 56 Females) before treatment (Group A) and after treatment (Group B) attending the outpatient clinics or admitted in wards of J.L.N. Hospitals, Ajmer. 100 healthy control subjects (Group C) of same age group of either gender were selected for the study. Blood samples were drawn from patients and controls, after overnight fast of at least 8 hours. Estimation of Serum Ghrelin, free T3, free T4, and TSH was done by using Enzyme- Linked Immunosorbant Assay (ELISA) technique. Total Cholesterol, Triglyceride, HDL – Cholesterol were measured by automated analyser (Beckman & Coulter's AU680). VLDL – Cholesterol, LDL – Cholesterol were calculated by Friedwald's formula. Differences in the parameters among the groups were analyzed by ANOVA test followed by its Tukey HSD post hoc analysis. Correlations between variables were tested using the Pearson rho (r: Correlation coefficient) correlation test.

Results: Findings of the present study shows that the levels of serum fT3 (1.79 ± 0.29 pg/mL) and serum fT4 (0.34 ± 0.11 ng/dL) were significantly lower in Group A compared to Group B (fT3 = 3.00 ± 0.32 pg/mL & fT4 = 0.81 ± 0.15 ng/dL) and Group C (fT3 = 3.12 ± 0.31 pg/mL & fT4 = 0.85 ± 0.11ng/dL) whereas serum TSH levels were significantly higher in Group A (40.59 ± 13.55 µIU/mL) compared to Group B (5.34 ± 1.47 µIU/mL) and Group C (3.23 ± 1.04 µIU/mL). Levels of serum Ghrelin were significantly higher in Group A (918.19 ± 48.47 pg/mL) compared to Group B (700.34 ± 46.35 pg/mL) and Group C (681.49 ± 35.80 pg/mL). A non significant correlation of Ghrelin with S.fT4 and TSH was found in both Group A and Group B whereas S.fT3 and BMI shows a non significant correlation in Group A in comparison to a significant correlation in Group B.

Conclusion: There is a reversible increase in the levels of serum ghrelin which became normalized after L-thyroxine substitution in hypothyroid patients. Alteration in the levels of serum ghrelin in thyroid disorders indicates a compensatory role of ghrelin in metabolic disturbances and also suggests a possible association between thyroid hormones and serum ghrelin levels.

KEYWORDS

Ghrelin, Hypothyroidism, L-thyroxine

INTRODUCTION

Thyroid diseases are the commonest endocrine disorders in India and throughout the world. Various studies on thyroid diseases suggested that about 42 million people suffer from thyroid diseases in India[1]. In developed countries the prevalence of hypothyroidism is about 4%–5%, whereas in India, it is reported to be around 10.95%. Hypothyroidism is one of the most common disorders which occur from hormone deficiency[13].

Ghrelin is an acylated 28 amino acid gut derived peptide. It is an endogenous ligand of the growth hormone secretagogue type 1a receptor[12]. Ghrelin was discovered as the first endogenous ligand for growth hormone secretagogue receptor (GHS-R) and proved to be a strong dose dependent stimulator of growth hormone release and also plays a significant role in the regulation of metabolic processes[20].

Ghrelin exists mainly in two forms, unacylated and acylated. Earlier unacylated ghrelin was considered to be the inactive form but recent studies shows that it has some biological activity[21]. Ghrelin is also known as hunger hormone as it increases apetite and food intake apart from its effect on growth hormone[16]. Ghrelin stimulates the release of growth hormone at the arcuate nucleus of hypothalamus and regulates thermogenesis, appetitive behavior and energy balance[15].

Majority of the studies have reported that hypothyroidism is associated with increased levels of serum ghrelin that decreases after treatment with levothyroxine. On the other hand, some authors did not found any alteration in serum ghrelin levels or actually observed low levels of serum ghrelin[17]. Contradictory results have been observed regarding the levels of serum ghrelin in hypothyroidism and association of serum ghrelin levels with thyroid hormones. Therefore, aim of this study was to evaluate the levels of serum ghrelin in hypothyroid patients and to assess their levels before and after treatment.

MATERIAL & METHODS

Subjects

The present study was conducted on 100 hypothyroid patients (44 Males & 56 Females) before treatment (Group A) and after treatment (Group B) attending the outpatient clinics or admitted in wards of Medicine Department, J.L.N. Hospital, Ajmer. 100 healthy control subjects (Group C) of same age group of either gender were selected for the study. Patients with diabetes mellitus, chronic renal failure, congestive heart failure, pregnant women and smokers were excluded from the study.

Sample Collection & Measurement :

Blood samples were drawn from patients and controls, after overnight fast of at least 8 hours. Five ml blood was collected in plain vial (without any anticoagulant) and was allowed to clot for 30 minutes at room temperature and then centrifuged at 3000 rotations per minute (rpm) for 10 minutes to obtain clear non-haemolysed serum (Haemolysed serum samples were excluded from analysis). Estimation of Serum Ghrelin, free T_3, free T_4, and TSH was done by using Enzyme- Linked Immunosorbant Assay (ELISA) technique. Total Cholesterol, Triglyceride, HDL – Cholesterol were measured by automated analyser (Beckman & Coulter's AU680). VLDL – Cholesterol, LDL – Cholesterol were calculated by Friedwald's formula. Hypothyroid patients were given a daily dose of L-thyroxine according to their TSH results and the patients were studied before and after L-thyroxine replacement, when thyroid hormones had been normalized for at least 2 months.

Statistical Analysis:

Variables were presented as Mean ± Standard deviation (S.D.). Differences in the parameters among the groups were analyzed by ANOVA test followed by its Tukey HSD post hoc analysis. Correlations between variables were tested using the Pearson rho (r: Correlation coefficient) correlation test. The accepted level of significance for all statistical analyses used in the study was $P \leq 0.05$ (two tailed P value).

RESULT

Findings of the present study shows that the levels of serum fT_3 (1.79 ± 0.29 pg/mL) and serum fT_4 (0.34 ± 0.11 ng/dL) were significantly lower in Group A compared to Group B (fT_3 = 3.00 ± 0.32 pg/mL & fT_4 = 0.81 ± 0.15 ng/dL) and Group C (fT_3 = 3.12 ± 0.31 pg/mL & fT_4 = 0.85 ± 0.11ng/dL) whereas serum TSH levels were significantly higher in Group A (40.59 ± 13.55 µIU/mL) compared to Group B (5.34 ± 1.47 µIU/mL) and Group C (3.23 ± 1.04 µIU/mL). [Table1;Figure1]

Table No. 1 General And Biochemical Parameters

S.No.	Parameter	Control subjects [Mean ±S.D.]	Group A [Mean ±S.D.]	Group B [Mean± S.D.]
1	**Age** (Years)	40.41 ± 5.81	43.89 ± 5.30	46.04 ± 5.71
2	**BMI** (Kg/m^2)	23.88 ± 1.54	26.84 ± 1.41	24.45 ± 1.01
3	**Total Cholesterol** (mg/dL)	170.43 ± 18.50	236.73 ± 23.71	196.37 ± 16.57
4	**Triglyceride** (mg/dL)	141.55 ± 13.03	198.06 ± 16.26	159.54 ± 13.94
5	**HDL** (mg/dL)	39.22 ± 6.89	27.8 ± 4.05	29.57 ± 4.83
6	**VLDL** (mg/dL)	28.31 ± 2.60	39.61 ± 3.25	31.90 ± 2.78
7	**LDL** (mg/dL)	102.9 ± 19.33	169.31 ± 23.84	134.89 ± 17.71
8	**fT_3** (pg/mL)	3.12 ± 0.31	1.79 ± 0.29	3.00 ± 0.32
9	**fT_4** (ng/dL)	0.85 ± 0.11	0.34 ± 0.11	0.81 ± 0.15
10	**TSH** (µIU/mL)	3.23 ± 1.04	40.59 ± 13.55	5.34 ± 1.47
11	**Ghrelin** (pg/mL)	681.49 ± 35.80	918.19 ± 48.47	700.34 ± 46.35

Levels of serum total cholesterol (236.73 ± 23.71 mg/dL), triglycerides (198.06 ± 16.26 mg/dL), VLDL (39.61 ± 3.25 mg/dL) and LDL (169.31 ± 23.84 mg/dL) were significantly higher in Group A compared to Group B (T. Chol =196.37 ± 16.57 mg/dL, T.G.= 159.54 ± 13.94 mg/dL, VLDL=31.90 ± 2.78 mg/dL & LDL=134.89 ± 17.71 mg/dL) and Group C (T. Chol =170.43 ± 18.50 mg/dL, T.G.= 141.55 ± 13.03 mg/dL, VLDL=28.31 ± 2.60 mg/dL, & LDL=102.9 ± 19.33 mg/dL) [Table 1] whereas serum HDL levels were significantly lower in Group A (27.8 ± 4.05 mg/dL) compared to Group B (29.57 ± 4.83 mg/dL) and Group C (39.22 ± 6.89 mg/dL) [Table 1].

Table No. 2 Correlation Of S. Ghrelin With fT_3, fT_4 & TSH in Group A

Parameter	r value	p value	Significance
fT_3	-0.028	0.782	NS
fT_4	0.015	0.882	NS
TSH	0.111	0.271	NS
BMI	0.032	0.751	NS

NS – Non-SignificantLevels of serum Ghrelin were significantly higher in Group A (918.19 ± 48.47 pg/mL) compared to Group B (700.34 ± 46.35 pg/mL) and Group C (681.49 ± 35.80 pg/mL) [Table 2,3; Figure 2,3]. A non significant correlation of Ghrelin with S.fT4 and TSH was found in both Group A and Group B whereas S.fT3 and BMI shows a non significant correlation in Group A in comparison to a significant correlation in Group B.

Table No. 3 Correlation Of S. Ghrelin With fT_3, fT_4, & TSH in Group B

Parameter	r value	p value	Significance
fT_3	-0.224	0.025	S
fT_4	-0.142	0.158	NS
TSH	0.149	0.138	NS
BMI	0.259	0.009	S

S-Significant NS-Non-Significant

DISCUSSION

Ghrelin is a polypeptide hormone which is present in acylated and unacylated form. It also act as a ligand of growth hormone secretagogue receptor (GSH-R) which helps in the regulation of thermogenesis, appetitive behavior, energy balance and stimulation of growth hormone release[18]. Ghrelin is considered as an important factor which compensates for disturbance in energy balance in hypothyroidism where thyroid gland is unable to respond to metabolic changes properly[19].

Thyroid status is known to affect the clearance of various metabolic substances and increase in the levels of circulating ghrelin might be caused by the reduced metabolic clearance rate[10]. The exact pharmacokinetics of ghrelin as a function of thyroid disorders is not known but higher BMI and HDL-cholesterol both are the characteristic features of hypothyroidism are considered to show an increase in the mean residence time of ghrelin in the body[8]. Thyroid hormones affect the activity of various esterases including butrylcholinesterase which are known to catalyze the degradation of ghrelin[9]. In fact, various studies on rodents have reported a delayed activity of butrylcholine-sterase in hypothyroid rats and animals treated with thyroxine shows an increase in the activity of butrylcholinesterase[11].

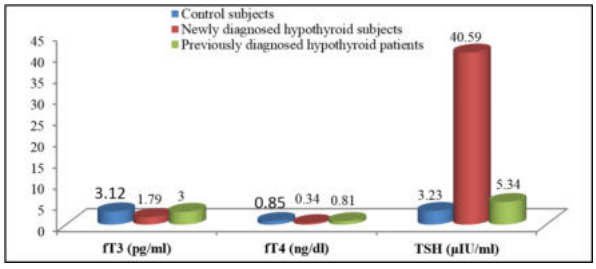

Figure 1 : Comparison of Thyroid Profile in various group subjects.

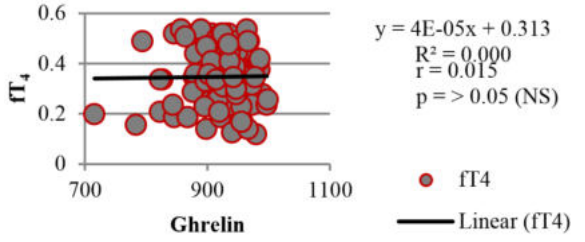

Figure 2 : Correlation of S. Ghrelin with fT_4 in Group A.

The most likely explanation of the above results requires a detailed study of the metabolic changes which occurs in patients with thyroid dysfunction. It is interesting to know that in hypothyroidism even though the body weight is increased it is not considered as a state of positive energy balance. In the deficiency of thyroid hormones appropriate use of energy resources is affected[15]. This condition is similar to starvation where deficiency of authentic energy substrates is observed. Therefore like cachexia, anorexia nervosa and bulimia, hypothyroidism is also considered as a state of negative energy balance[14].

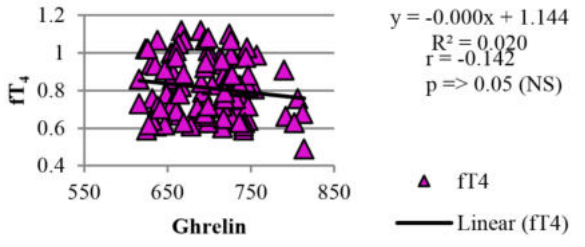

Figure 3 : Correlation of S. Ghrelin with fT_4 in Group B.

Results of the present study are consistent with most of the previous studies where increased levels of plasma ghrelin are observed in hypothyroid patients[1,2,7] and decrease in the levels of plasma ghrelin was observed in hyperthyroid patients[3,4,5,6].

Findings of our study suggests a close reciprocal association between circulating levels of ghrelin and T_4 in hypothyroid patients either due to a direct effect of iodothyronines on gut-derived ghrelin secretion or due to a compensatory mechanism to balance the consequences of primary thyroid dysfunction on substrate metabolism and energy balance. There is a reversible increase in the levels of serum ghrelin which became normalized after L-thyroxine substitution in hypothyroid patients.

CONCLUSION

Alteration in the levels of serum ghrelin in thyroid disorders indicates a compensatory role of ghrelin in metabolic disturbances and also suggests a possible association between thyroid hormones and serum ghrelin levels. Further our study also supports a close reciprocal association between circulating levels of ghrelin and T_4 in hypothyroid

patients.

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