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A Comparative Study of the Amylin Hormone Levels in the Sera of Hypothyroidism Patients with and Without Type 2 Diabetes Mellitus

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Abstract

The study was designed to compare amylin hormone levels and some biochemical parameters in the serum of Iraqi patient with hypothyroidism (hypo) with and without Type 2 diabetes mellitus (T2DM). This study included ninety subjects and was divided into two patient groups: group I, which included 30 hypo without T2DM, and group II, which included 30 hypo with T2DM. Group III included 30 apparently healthy controls. The age range is 40-60 years. A significant increase in body mass index (BMI), fasting blood glucose (FBG), total cholesterol (TC), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), triglyceride (TG), amylin hormone, and thyroid-stimulating hormone (TSH) in patients with hypo and hypo with T2DM (groups I and II) when compared to healthy controls (group III), and a significant decrease in the level of HDL in patient groups (I and II) when compared to healthy control groups. The amylin hormone receiver operating characteristic (ROC) curve showed a clear cut-off value (45.304 and 2) when calculated in hypo with and without T2DM compared with control groups, respectively. While the ROC curve showed a clear cut-off value (50.661) when compared in hypo with and without T2DM groups. Amylin is a hormone marker of glucose homeostasis, so it may be surrogate novel biomarker for all other traditional biomarkers for the prediction of diabetes and thyroid dysfunction.

Keywords: Amylin, Hypothyroidism, Type 2 diabetes mellitus, Thyroid hormone.

دراسة مقارنة لمستويات هرمون الأميلين في مصل مرضى قصور الغدة الدرقية المصابين بداء السكري من النوع الثاني وبدونه

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الخلاصة

صممت الدراسة لإيجاد مقارنة بين مستويات هرمون الأميلين وبعض المتغيرات الكيميائية الحيوية في امصال مرضى قصور الغدة الدرقية (hypo) مع و بدون داء السكري من النوع 2 (T2DM)، و المجموعة الاصحاء. تضمنت هذه الدراسة تسعين عينة تم تقسيمها إلى مجموعتين من المرضى: المجموعة الأولى، وتشمل 30 hypo بدون T2DM، المجموعة الثانية و تشمل 30 hypo مع T2DM. و شملت مجموعة

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الاصحاء المجموعة الثالثة 30 مشاركًا. العمر (40–60 سنة). أظهرت نتائج الدراسة زيادة ملحوظة في مؤشر كتلة الجسم (BMI)، الجلوكوز في الدم أثناء الصوم (FBG)، الكوليسترول الكلي (TC)، البروتين الدهني منخفض الكثافة (LDL)، البروتين الدهني منخفض الكثافة جدا (VLDL)، الدهون الثلاثية (TG)، هرمون الأميلين، والهرمون المنبه للغدة الدرقية (TSH) في المرضى الذين يعانون من hypo بدون T2DM، hypo مع T2DM (المجموعة الاولى و الثانية) عند مقارنتهم بمجموعة الاصحاء (المجموعة الثالثة) و انخفاض كبير في مستوى HDL في مجموعات المرضى (المجموعة الاولى و الثانية) مقارنة بمجموعة الاصحاء. أظهر منحنى خاصية تشغيل مستقبل هرمون الأميلين (ROC) قيمة فاصلة واضحة (45.304 و 2) عند حسابها في حالة نقص السكر مع و بدون T2DM مقارنة بمجموعة السيطرة من الاصحاء على التوالي. بينما أظهر منحنى ROC قيمة قطع واضحة (50.661) عند مقارنته في hypo مع و بدون مجموعات T2DM. الأميلين هو علامه هرمونيه يدل على توازن الجلوكوز، لذلك قد يكون مؤشرات حيوية بديلة للمؤشرات الحيوية التقليدية الأخرى للتنبؤ بعوامل الخطر لمرض السكري واختلال الغدة الدرقية.

1. Introduction

The thyroid hormone TH governs the metabolism of almost all human cells and organs throughout life and is crucial for the appropriate development of many human tissues [1]. A prevalent illness in the general population is hypothyroidism (hypo), the clinical state of thyroid hormone insufficiency [2]. Most adult hypo patients have acquired hypo, which may be thyroid in origin (primary hypothyroidism), pituitary, or hypothalamic in origin (central hypothyroidism) [3]. Diabetes mellitus (DM) has been the most frequent endocrine disorder throughout the previous century. The rising prevalence of DM is directly related to the rise in obesity on a worldwide scale, which is a major type 2 diabetes mellitus (T2DM) risk factor [4]. Recently, T2DM has become more common [5]. When the body is unable to properly respond to insulin, it develops T2DM, a prolonged condition of glucose intolerance and hyperglycemia that is followed by an increase in insulin production and an insulin deficiency [6]. Thyroid dysfunction and DM frequently coexist in people. Patients with T2DM are more likely to experience both hyperthyroidism and hypothyroidism than their non-diabetic counterparts [7]. The interference of hypothyroidism with the action and metabolism of insulin results in the development of insulin resistance. Several studies have found altered TH in patients with T2DM, particularly those with poor glycemic control [8]. Amylin is a hormone that insulin co-releases from pancreatic beta cells. The hormones are kept in the same islet secretory vesicles that hold other secretions at a ratio of around 1:100 (amylin to insulin), and their expression levels are regulated by shared promoter elements [9]. Amylin is a peptide of 37 amino acids. It has a significant impact on stomach emptying, glycemic control, and appetite [10]. Numerous studies have shown that amylin reduces human body weight and food consumption [11]. Histopathologically, T2DM is distinguished by the buildup of fibrillary amyloid deposits in the pancreas, which are predominantly made up of amylin, also known as human islet amyloid polypeptide [12,13]. The development of T2DM is accomplished by three determining elements: the inability of pancreatic beta cells to release insulin, decreased insulin sensitivity of the peripheral tissues, and deposition of human pancreatic islet amyloid polypeptide (hIAPP) or amyloid [8,14]. The most extensively researched of amylin's many physiological actions is its function as a short-term satiation hormone. Amylin serves as a signal to terminate meals in order to reduce meal size. Amylin also has a crucial role in regulating blood sugar levels after meals. It does this by inhibiting the production of glucagon from pancreatic beta cells and working in conjunction with insulin [15,16]. The study aims to compare amylin hormone levels in hypo with and without T2DM with those in the control group.

2. Subjects, materials and methods

2.1. Subjects

A randomized case-control study was done on a sample of patients to determine the level of amylin in T2DM with hypo and hypo patients between September 2022 and December 2022. Ninety subjects attended the national diabetes center in Baghdad at Mustansiriyah University. The patients were divided into two groups: group I (hypo without T2DM) patients and group II (hypo with T2DM) patients. 30 Apparently healthy control subjects are included in group III. The age ranges from 40 to 60 years.

2.2. Samples

Blood samples were collected between 8:30 and 11:30 a.m., after 8-12 hours of fasting, using a 10-mL disposable syringe. The 10 mL was transferred into a gel tube and allowed to clot at room temperature. Then centrifugation for 15 minutes at 3000 rpm separated the sample. One mL of the serum was used to measure FBG, TC, TG, HDL, and LDL, and 2 mL of the sample was used for further investigation of the thyroid function test: Triiodothyronine (T3), Thyroxine (T4), and TSH. The remaining serum was transferred to a tube and saved in a deep freezer (-20 ºC) to be used for assaying amylin levels. All biochemistry parameters (FBG, TC, HDL, LDL, and TG) were measured by the Cobas c111 instrument. Using Vidas Instruments and a Biomerieux kit, the Thyroid hormones (TSH, T4, and T3) were assayed. The amylin kit was tested using an enzyme-linked immunoassay (ELISA) kit (Al-Shkairate establishments from Jordan). Body Mass Index (BMI) = Wt. (kg)/Ht. m^2 is taken as an anthropometric measurement of obesity and is described as a BMI [17].

2.3. Inclusion criteria

The age range is between 40 and 60 for hypothyroidism patients with type 2 diabetes mellitus and hypothyroidism.

2.4. Exclusion criteria

Type 1 diabetes mellitus, thyroid cancer, patients with pregnancy, and hyperthyroidism patients.

2.5. Statistical analysis

The data was interpreted as the median (25^{th} and 75^{th} percentiles) and non-normally distributed numerically. To examine the normal distribution of data, a Shapiro-Wilk test was utilized. To determine if there was a significant difference between the typically numerical variables, an ANOVA test was utilized. The Mann-Whitney tests were employed to describe numerical variables that were not regularly distributed. In a non-parametric analysis, $P \leq 0.05$ is considered significant. The Spearman's rank coefficient was used to assess the significance of correlation for the link between the two numerical variables. The amylin cut-off value was determined using ROC curve analysis.

3. Results

The results of this study found a highly significant increase in BMI, TC, TG, VLDL, LDL, and amylin levels in patients with hypo with and without T2DM when compared with the control group. A significant decrease in HDL levels in patients with hypo with and without T2DM when compared with the control group, as shown in Table 1.

Table 1: Anthropometric and biochemical parameters (Age, BMI, FBG, TC, TG, HDL, LDL, VLDL, and Amylin) in the study group

Variables	Hypo without T2DM	Hypo with T2DM	Control	P value
Age (year)	47.00(39.00-54.25)	44.00(37.75-50.00)	49.50(45.00-55.00)	0.08
BMI Kg/m^2	32.81(29.75-34.89)a,c	29.96(27.77-32.00)b,c	24.36(23.42-25.00)	0.00
FBG (mg/dL)	90.40(88.23-93.80)a,c	222.00(130.35230.00)b	87.65(81.75-91.75)	0.00
TC(mg/dL)	276.00(265.00-210.00)a	282.80(263.182)b	135.50(113.00-150.00)	0.00
TG(mg/dL)	189.70(176.00158.50)a,c	207.50(178.72186.80)b	84.00(76.75-90.00)	0.01
HDL(mg/dL)	33.00(29.75-40.00)a,c	31.00(27.63-33.60)b,c	47.00(45.00-49.00)	0.00
VLDL(mg/dL)	29.80(19.20-34.30)a	31.50(25.73-37.36)b	17.00(15.50-18.00)	0.00
LDL(mg/dL)	123.20(94.70-138.80)a,c	121.55(91.10-129.00)b	71.50(49.00-87.00)	0.00
Amylin(ng/mL)	49.98(37.19-56.74)a,c	56.42(55.07-57.89)b,c	35.15(32.52-40.48)	0.00

The median (25thand 75th percentiles) wereused to determine whether there was a significant difference between three independent means using the Mann-Whitny test at the 0.05 level.
a) Indicates whether there is a significant difference between the control groups and the hypo without T2DM group.
b) Determines whether the difference between the control and hypo with T2DM group is statistically significant.
c) Determines whether the hypo without T2DM group and the Hypo with T2DM group have a statistically significant difference.

Hypo: hypothyroidism BMI: body mass index T2DM, hypo Type 2 diabetes mellitus, and hypothyroidism FBG: Fasting blood glucose, TC: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein VLDL: Very Low Density Lipoprotein P-value<0.05 is significant.

The median (25th and 75th percentiles) of Table 2 found that the serum levels of T4 in hypo and T2DM patients were significantly increased when compared with control 89.00 (88.00-101.00), 89.65 (83.49-100.35) and 83.50 (78.50-89.00), respectively. On the other hand, there is a highly significant increase in TSH levels in patients with hypo and (T2DM and hypo) when compared with the control groups of 12.00 (6.68-19.80), 13.40 (5.50-36.50), and 1.40 (1.00-1.80), respectively.

Table 2: The median (25th and 75th percentiles) of T3, T4, and TSH between study groups

Variables	Hypo without T2DM	Hypo with T2DM	Control	P value
T3(ng/mL)	1.40(1.23-1.78)a	1.5(1.25-1.73)	1.50(1.351.90)	0.721
T4(ng/mL)	89.00(88.00-101.00)	89.65(83.49-100.35)b	83.50(78.50-89.00)	0.002
TSH(mLU/mL)	12.00(6.68-19.80)a	13.40(5.50-36.50)b	1.40(1.00-1.80)	0.000

The median (25thand 75th percentiles)were used to determine whether there was a significant difference between three independent means using the Mann-Whitney test at the 0.05 level.
a) Indicates whether there is a significant difference between the control group and the hypo without T2DM groups.
b) Determines whether the difference between the control and the hypo with T2DM group is statistically significant.
c) Determines whether the hypo without T2DM and the hypo with T2DM have a statistically significant difference.

Table 3 shows the correlation coefficient between amylin and the other variables in hypo (T2DM and hypo) and the control groups. Amylin had a negative correlation with T4, TC, VLDL, and FBG in the hypo group, and amylin had a negative correlation with BMI, T4, T3, and TC in the T2DM and hypo groups.

Table 3: The correlation analysis between amylin and variables in the hypo (T2DM and hypo) and control groups

Variables	Correlation coefficient (r)	Amylin(ng/mL)		
		Hypo	T2DM and hypo	Control
Age (year)	Pearson Correlation	.311	.301	.088
	Sig. (2-tailed)	.094	.106	.645
BMI	Pearson Correlation	.123	-.177	-.113
	Sig. (2-tailed)	.518	.349	.552
T3(ng/mL)	Pearson Correlation	.289	-.048	-.082
	Sig. (2-tailed)	.122	.802	.668
T4(ng/mL)	Pearson Correlation	-.241	-.270	-.144
	Sig. (2-tailed)	.200	.149	.449
TSH(ulU/mL)	Pearson Correlation	.113	.360	-.186
	Sig. (2-tailed)	.553	.051	.326
TC(mg/dL)	Pearson Correlation	-.208	-.025	-.062
	Sig. (2-tailed)	.269	.894	.744
TG(mg/dL)	Pearson Correlation	.094	.316	.191
	Sig. (2-tailed)	.620	.089	.312
HDL(mg/dL)	Pearson Correlation	.189	.173	-.032
	Sig. (2-tailed)	.318	.362	.866
VLDL(mg/dL)	Pearson Correlation	-.014	.315	-.089
	Sig. (2-tailed)	.943	.090	.640
VLDL(mg/dL)	Pearson Correlation	.155	.360	.198
	Sig. (2-tailed)	.415	.050	.294
FBG(mg/dl)	Pearson Correlation	-.035	.280	-.359
	Sig. (2-tailed)	.853	.134	.052
*The Correlation is significant at the 0.05 *The Correlation is a highly significant at the 0.01				

4. ROC curve analysis

Receiver operator characteristics (ROC) used in the study to evaluate the area under the curve (AUC), cutoff value (CV), specificity, and sensitivity were calculated. In this study, three curves were made. The first was the Roc curve for amylin between hypo without T2DM and control. The second was the Roc curve for amylin between hypo with T2DM patients versus control, and the third was the roc curve for amylin between hypo without T2DM and hypo with T2DM, as shown in Table 4 and Figures 1, 2, and 3, respectively.

Table 4: Amylin hormone AUC and validity in distinguishing study groups

Variables	(Hypo without T2DM) and control	(Hypo with T2DM)and control	Hypo with T2DM and hypo without T2DM)
AUC	1	0.950	0.676
cut off value	2	45.304	50.661
Sensitivity	100	90.00	90
Specificity	100	90.00	60
Accuracy	1	0.80	0.500
NPV	100	90	85.7
PPV	100	90	69.2
P-Value	0.001	0.001	0.128
AUC: Area under the curve, NPV: Negative predictive value , PPV: Positive predictive value			

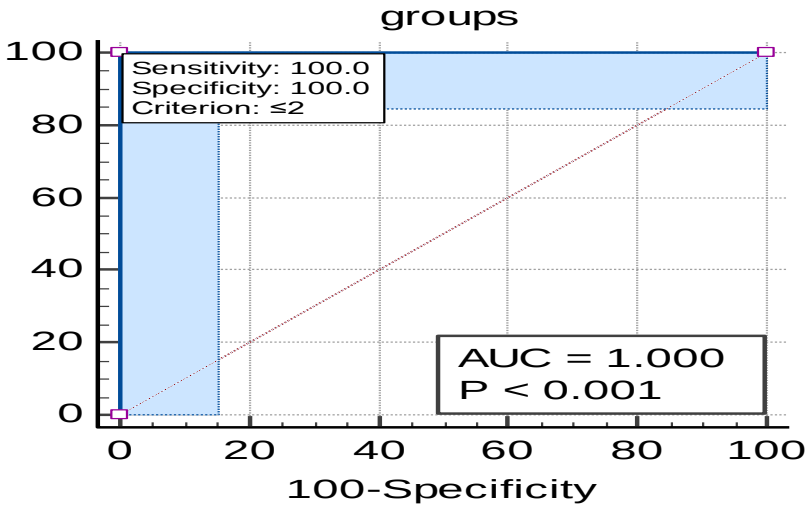

Figure 1: The ROC curve analysisto examine the predictive value of amylin serum levels in hypo versus control

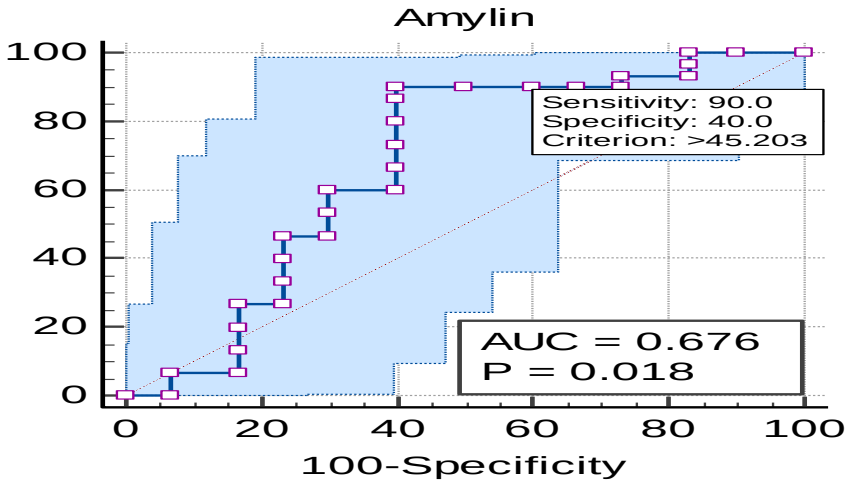

Figure 2: The ROC curve analysis to examine the predictive value of amylin serum levels in T2DM and hypo versus control

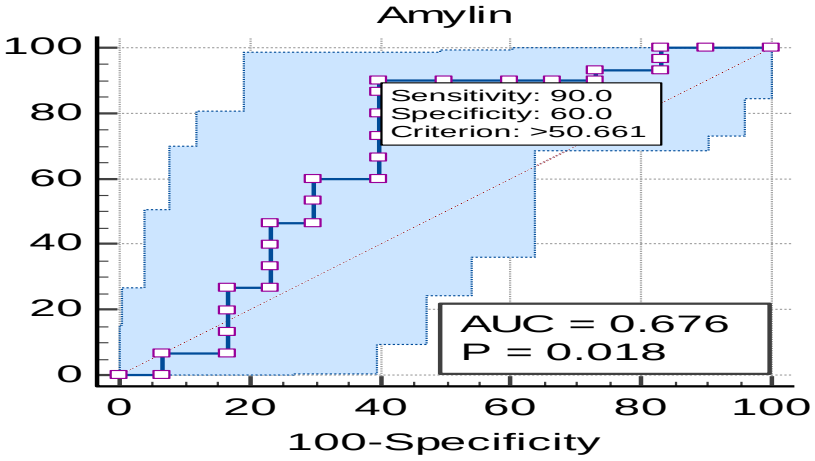

Figure 3: The ROC curve analysis to examine the predictive value of amylin serum levels in hypo versus T2DM and hypo

5. Discussion

Many Studies have found a link between thyroid dysfunction and T2DM, suggesting that thyroid hormones, which are essential regulators of metabolism, particularly protein and glucose metabolism, may also affect both conditions . Reduced metabolic rate, obesity, numerous cardiovascular risk factors, and insulin resistance are all potential effects of hypothyroidism that may make type 2 diabetes more common[18]. The current study found a high significance of the BMI increase in the hypo and T2DM groups, which is consistent with previous studies. The increase in BMI in patients with T2DM is explained by individuals with a genetic predisposition to T2DM having a higher risk of developing obesity because their pancreatic islet cells and skeletal muscle are more vulnerable to insulin resistance. Insulin resistance causes the liver to produce more glucose, which raises blood glucose levels and causes obesity. Adipose tissue macrophages release pro-inflammatory cytokines that affect beta cells and tissues that are dependent on insulin. Also, the study found a significantly higher lipid profile (TC, T G, and LDL)and lower HDL levels in the hypo and (T2DM and hypo) groups. This result is in agreement with the previous study changes in the plasma lipoprotein that occur in diabetic patients during fasting and post-prandial settings, controlled by errors in insulin action and hyperglycemia, can be used to explain the dyslipidemia phenomenon. Certain regions of the brain receive information from amylin, a pancreatic hormone that controls homeostatic energy balance. During a meal, insulin and amylin are jointly released, and amylin's binding to the amylin receptor (AMY) causes a feeling of satiety [14]. Amylin's purpose is to limit increases in blood glucose after meals, where it works in conjunction with insulin by inhibiting the release of glucagon [19]. The glucose regulatory hormones, including amylin, gastric inhibitory polypeptide, cortisol, glucagon-like peptide-1, growth hormone, and epinephrine, also influence glucose homeostasis. In response to nutritional signals, pancreatic beta cells co-secrete insulin and amylin, which are separated from pancreatic amyloid plaques [20]. The present study found a high-significance increase in amylin hormone levels in the hypo and (T2DM and hypo) groups. Amylin hormones are higher in people with T2DM, obese people who are insulin resistant, and people who have impaired glucose tolerance. This homeostasis was lost in patients with insulin resistance, reduced glucose tolerance, and T2DM, and excessive amounts of amylin were lost by apoptosis as a result of the amyloidogenic toxicity linked to the unfolded hormone polypeptide amylin [21].

6. Conclusion

Amylin is a hormone marker of glucose homeostasis; therefore, it may be surrogate novel biomarker for all other traditional biomarkers for the prediction of diabetes and thyroid dysfunction. Amylin hormone is the most specific and sensitive marker in T2DM and hypothyroidism patients in terms of defining and excluding the disease. Thyroid hormone levels may change with type 2 diabetes mellitus.

Ethics clearance

The research ethical committee at scientific research has the ethical approval of environmental, health, higher education, and scientific research ministries in Iraq.

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