

# Human Amylin as a Novel Diagnostic Marker for Hypothyroidism in Iraqi Patients

Duha Khalil Ibrahim<sup>id</sup>✉, Layla Othman Farhan\*<sup>id</sup>✉

Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq.

\*Corresponding Author.

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## Abstract

Amylin is a peptide hormone that the pancreas cell secretes along with insulin. As a neuroendocrine hormone that sends a specific signal to the brain, amylin is essential for maintaining homeostasis in the metabolism. The goal of the current study is to determine the role of Amylin as a diagnostic marker of hypothyroidism. The 120 samples were divided into two groups (80 patients with hypothyroidism) and the second group (40 healthy people). Amylin levels significantly increased compared to the control group in the group with hypothyroidism, according to the data ( $p < 0.001$ ). Amylin levels raised in Iraqi patients with Hypothyroidism. Amylin a good diagnostic marker for Hypothyroidism with most accurately, sensitively cut off value 51.

**Keywords:** Amylin, hypothyroidism, Thyroid Stimulating Hormone, Tetraiodothyronin, Tri iodothyronine.

## Introduction

One of the most significant endocrine glands in the body and one that plays a function in control is the thyroid gland<sup>1</sup>. Thyroid disorders are a major public health concern with far-reaching effects. Hypothyroidism and hyperthyroidism both impact large portions of the global population and can have potentially fatal health effects<sup>2,3</sup>. Together, hyperthyroidism and hypothyroidism affect nearly every aspect of health. Because they are sometimes misdiagnosed and go unnoticed, the biochemical test can be performed to confirm the diagnosis<sup>4</sup>. An abnormal thyroid function test result, comprising elevated or decreased thyroid-stimulating hormone Thyroid Stimulating Hormone (TSH), triiodothyronine(T3), and thyroxine (T4) values, is an easy way to define thyroid illness<sup>5</sup>.

Hyperthyroidism is a less common type of thyroid illness that causes the thyroid gland's activity and the production of both T3 and T4 hormones to increase. This condition causes a rise in body metabolism, which causes rapid heartbeats and unexpected weight loss<sup>6,7</sup>. Hypothyroidism is a frequent clinical disease with a varying prevalence<sup>8</sup>. A primary care physician frequently deals with the clinical condition hypothyroidism. This condition, if left untreated, may result in hypertension, dyslipidemia, infertility, cognitive decline, and neuromuscular dysfunction<sup>9,10</sup>. Amylin a peptide of 37 amino acids is secreted by the pancreas alongside insulin beta cells' reaction to the consumption of nutrients such glucose, amino acids, and lipids<sup>11</sup>. Amylin, insulin, and C- peptide are all made by pancreatic cells. In

smaller amounts, it is also produced in the stomach and the posterior spinal cord ganglia<sup>12</sup>. The amylin and insulin are a pair of synergistic partner genes that are co-expressed by a shared promoter and regulate glucose levels through intricate endocrine and neural pathways in a healthy state, the islet -cells' parallel pattern in response to glucose stimulation is caused by the simultaneous release of amylin and insulin from the secretory granules<sup>13</sup>. Amylin and insulin are co-secreted from and stored in the same islet secretory vesicles at a ratio of around 1:100, and their expression levels are regulated by shared promoter elements<sup>14</sup>. A study indicates that amylin has a relationship with thyroid cancer<sup>12,15</sup>. The hormones that interact with receptors based on the calcitonin receptor (CTR) or the calcitonin receptor-like receptor, including amylin and calcitonin, are known as the peptide superfamily (CLR)<sup>16</sup>. Amylin

decreases body weight and body fat through particular metabolic processes while largely retaining lean tissue. By lowering food intake or boosting energy expenditure, amylin might affect energy balance. Amylin can dramatically boost energy. When given to the third cerebral ventricle, it can prevent the weight-reduced animals' predicted decrease in energy expenditure<sup>17,18</sup>. It is regarded as a neuroendocrine hormone that plays a significant part in controlling the rate of glucose inflow into circulation following meals. Amylin's natural form has a Cys2 to Cys7 disulfide bridge and is amidated at the C-terminus<sup>19,20</sup>. The goal of the current study was the determine the role of Amylin as adiagnostic marker of hypothyroidism. To our knowledge, there hasn't been any published research that look on relationship between Amylin and hypothyroidism patients.

## Materials and Methods

### Study subjects:

The researchers worked in cooperation with Al-Amal National Hospital of the Medical City Department in the Republic of Iraq to investigate .80 patients with hypothyroidism and 40 healthy people, aged 30-60 years, participated in the study.

### Blood Sample Collection:

Blood samples (10 ml) were collected ten to twelve hours after fasting from 80 patients with hypothyroidism and 40 healthy subjects. In gel tubes, blood samples were taken from hypothyroidism patients and the control group under aseptic conditions and centrifuged for 15 minutes at 1000xg. Next, serum samples were divided into small aliquots for storage at -20 °C until a portion of the acquired serum was used for lipid profile measurement.

### Analysis:

Each person's weight and height were measured in kilogram/m<sup>2</sup> By dividing the weight (in kg) by the

square of the height (in m<sup>2</sup>), the body mass index (BMI) was determined<sup>21</sup>. The kenza (240TX) equipment (Biolabo) and kit (Biolabo) were used to measure biochemistry (TG, TC, HDL). A commercially available ELISA kit was used to measure the level of amylin analytes according to the manufacturer's instructions. T3, T4, and TSH hormones were measured for each patient.

### Statistical Analysis

The median (25th and 75th percentiles) for irregularly distributed numerical variables. The (Mann-Whitney) tests were employed to describe numerical variables that were not regularly distributed. The significance level was set at a P value of 0.05. Pearson's correlation was used to test the significance of correlation for the relationship between two quantitative variables. Through the use of receiver operating characteristic (ROC) curve analysis, the Amylin cut-off value was established.

## Results and Discussion

The Age and BMI of Hypothyroidism patients and, healthy subject are listed in Table 1. By The median (25th and 75th percentiles).

**Table 1. The demographic characteristics of Hypothyroidism and Healthy subject.**

Variables	Hypothyroidism	Healthy subject	p-value
Age (Year)	40 (32 - 47 )	39 (30 - 44 )	N.S
BMI (kg/m <sup>2</sup> )	40.10 (33.21 - 39.48)	25.86 (23.84 - 29.90 )	0.05*

**There was a significant difference between the two independent means when the gathered data were examined by median (25th and 75th percentiles) using the Man-Whitney test at the  $p < 0.05$  level.**

The median (25th and 75th percentiles) of the age distribution of the Hypothyroidism 40.50(32.25 - 47.0 ) years, and the healthy subjects group 39.00 (30.00– 44.25).The data of BMI distribution of the Hypothyroidism is 40.10 (33.21 – 39.48) patients, and the healthy subjects group is 25.86 (23.84 – 29.90) , respectively as listed in Table 1 . Thyroid hormones(TH) and body composition seem to be connected. The basal metabolism, thermogenesis, lipid and glucose metabolism, appetite, and fat oxidation are all significantly influenced by TH. Independent of physical activity, thyroid dysfunction is linked to changes in body weight and composition, core body temperature, and total and resting energy expenditure (REE). A greater body mass index (BMI) and a higher prevalence of obesity have all been linked to hypothyroidism, along with lower thermogenesis and metabolic rate. Clinical data indicates that subclinical hypothyroidism, a minor form of thyroid malfunction, is associated with considerable changes in body weight and is a risk factor for overweight and obesity<sup>22,23</sup>.

Table 2, shows the serum levels of cholesterol, T.G, HDL, VLDL, and, LDL in Hypothyroidism subject and healthy subject. High significant difference appeared in cholesterol, T.G, HDL, VLDL, and, LDL levels when compared with two groups of patient Hypothyroidism and control group with  $p \leq 0.05$  as shown as in Table 2. The cholesterol levels increased significantly in Hypothyroidism patients group 299.80(269.25-355.50) when compared with control group 155.50(150.50-160.00), as shown as in Table 2. The T.G, LDL and, VLDL levels increased significantly in Hypothyroidism patients group 199.00(169.25-239.00), 217.50(195.50-267.88), 39.80(33.85-47.80),when compared with control group

120.00(98.50-169.00), 81.35(71.40-88.90), 25.70(20.70-34.45), respectively  $p \leq 0.05$ , while there was a low significant difference in HDL when compared Hypothyroidism patients group 41.25 (40.50-47.80) with control 48.00(46.93-49.00),  $p < 0.001$  as shown as in Table 2.

Exogenous or free fatty acids (FFAs) produced by glycolysis and fat are the source of TG. The synthesis of VLDL-TG in the liver may be decreased by TH. When lipolysis continues at its current rate, hypothyroidism will result in lower lipid oxidation rates and higher TG levels<sup>24</sup>. Lipid levels rise when TSH levels rise because TH has an impact on how lipids are metabolized, according to multiple studies<sup>25,26,27</sup>. TH affects the production, movement, and breakdown of fat.

Apolipoprotein carrying capacity to TG and LDL was impacted, and lipoprotein activity decreased, and the level of cholesterol transport in hypothyroidism patients was lower than in the normal population. As a result, less cholesterol was cleared and degraded, leading to an increase in the concentration of TG and LDL in the blood. By encouraging the occurrence of oxidative stress disorder in vivo and controlling the lipoprotein lipase activity and LDL receptor expression on the surface of liver cells, the process by which cholesterol is transported backward in liver epithelial cells can be hampered by hypothyroidism. and cause a decrease in LDL receptor sensitivity the amount of LDL receptors and their activation on the surface of liver cells, and a decrease in LDL cholesterol. Large amounts of free fatty acids will enter the liver as a result of hypothyroidism, increasing the production of LDL there and ultimately raising TG and LDL levels<sup>28</sup>.

**Table 2. The Cholesterol, T.G, HDL, VLDL, and LDL of Hypothyroidism and Healthy subject.**

Variables	Hypothyroidism	Healthy subject	p-value
<b>Cholesterol(mg/dl)</b>	299.80(269.25-355.50)	155.50(150.50-160.00)	0.00
<b>T.G(mg/dl)</b>	199.00(169.25-239.00)	120.00(98.50-169.00)	0.00
<b>HDL (mg/dl)</b>	41.25 (40.50-47.80)	48.00(46.93-49.00)	0.00
<b>LDL (mg/dl)</b>	217.50(195.50-267.88)	81.35(71.40-88.90)	0.00
<b>VLDL (mg/dl)</b>	39.80(33.85-47.80)	25.70(20.70-34.45)	0.00

**The collected data were analyzed by median ( 25<sup>th</sup> and 75<sup>th</sup> percentiles ) via the man whitney test at the 0.05 level, there was a significant difference between the two indepent mean.**

Table 3, shows the serum levels of T3,T4,TSH, and Amylin of Hypothyroidism and Healthy subject groups.

The median of T3 show a significant difference in the Hypothyroidism group 1.57(1.30-1.80), compared to that of the healthy subject groups 1.72(1.32-2.12) P<0.05 , T4 revealed no significant decrease in the Hypothyroidism group 93.59(88.12-104.85) compared to that of the healthy subject groups ), 99.00(89.00-110.45) P=0.128 and TSH revealed a significant increase in the Hypothyroidism group 16.75(8.10-35.50) compared to that of the healthy subject groups 1.67 (1.23-2.18) P<0.05.

The result showed high significantly increase in Amylin level when we compared Hypothyroidism group 56.91(55.58-58.13) with Healthy subject 36.46(33.99-42.52) p<0.001. The TSH and TH are important regulators of food intake and energy storage. They have regulatory functions in adipogenesis, the balance of lipogenesis and lipolysis, and thermogenesis. A higher TSH could

result in low-grade chronic inflammation Adipose tissue can influence thyroid function through a variety of methods, and it is well recognized that small differences in thyroid function are strongly tied to changes in weight. Adipocytokine release, persistent inflammation, and lipotoxicity are involved. Even a mild thyroid dysfunction in humans may contribute to the accumulation of body fat, and subclinical hypothyroidism may be the cause of patients' slightly raised TSH levels in obese individuals. The interpretation of TSH tests depends heavily on determining the reason of hypothyroidism. However, it is possible that hyperthyrotropinemia is brought on by the hypothalamic-pituitary-thyroid axis becoming active. It may be possible to distinguish between different sources of the increased TSH levels by measuring the FT3 concentration. It is hypothesized that obesity, an inflammatory state, and lipotoxicity may result in thyroid gland malfunction<sup>29</sup>.

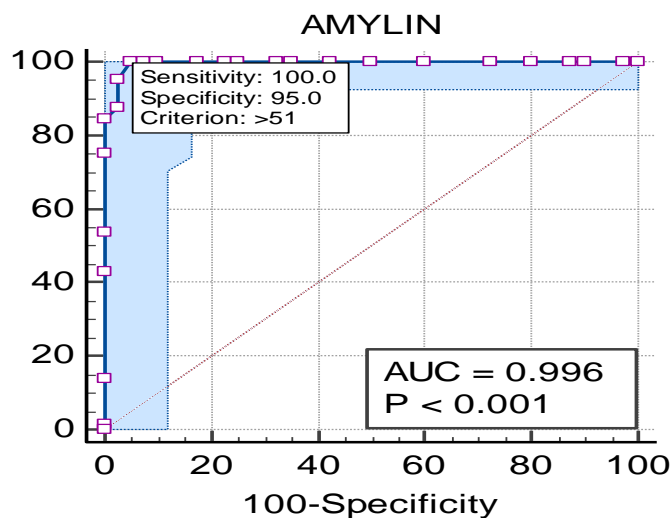
**Table 3. The median (25<sup>th</sup> and 75<sup>th</sup> percentiles ) of T3,T4,TSH, FBG and Amylin of Hypothyroidism and Healthy subject groups.**

Variables	Hypothyroidism	Healthy subject	P value
<b>T3(ng/ml)</b>	1.57(1.30-1.80)	1.72(1.32-2.12)	0.050
<b>T4(ng/ml)</b>	93.59(88.12-104.85)	99.00(89.00-110.45)	0.128
<b>TSH(mIU/ML)</b>	16.75(8.10-35.50)	1.67(1.23-2.18)	0.000
<b>Amylin(ng/ml)</b>	56.91(55.58-58.13)	36.46(33.99-42.52)	0.000

**The collected data were analyzed by the median ( 25<sup>th</sup> and 75<sup>th</sup> percentiles ) via the manwhitney test at the p<0.05 level, there was a significant difference between the two independent mean.**

The capacity of serum Amylin levels to distinguish patients with Hypothyroidism from healthy

individuals was evaluated using the ROC curve analysis Table 4 and Fig. 1.



**Figure 1.** The ROC curve analysis of serum Amylin concentration in Hypothyroidism patients (n =80) against healthy subjects (n = 40) (AUC is 0.996), p 0.001.

**Table 4.** Amylin AUC and validity in distinguishing between Hypothyroidism and Healthy subject.

Variable	AUC	P-Value	cutoff value	Sensitivity	Specificity	Accuracy	PPV	NPV
Amylin	0.996	0.001	51	100.0	95.00	0.9500	97.0	<b>100.0</b>

**The term AUC refers to the area under the curve. The terms negative predictive value (NPV) and positive predictive value (PPV) are used interchangeably.**

High sensitivity (100.0) and specificity (95.0) of the ROC curve over the Hypothyroidism diagnostic test indicated improved validity. The AUC of the ROC curve for the presence of a Hypothyroidism diagnosis revealed that the best level of accurate Hypothyroidism prediction was 0.996 (p <0.001). In this study, patients with Hypothyroidism had their serum levels of Amylin evaluated. According to the study's findings, serum Amylin levels were higher in Hypothyroidism patients than in healthy people. Amylin's effects on controlling and maintaining blood glucose levels are complimentary to those of insulin. Additionally, a major component of the

protein clusters that build up in the islets of Langerhans of people with type 2 diabetes mellitus is amylin. Moreover, it has been connected to the disruption of  $\beta$ -cells' cellular membrane, because of the unregulated inflow of ions into the cell, which results in stress on the membrane. Previous preclinical research revealed that amylin has the capacity to attach to specific brain regions, including the region known as the Postrema, with significant affinity<sup>19,30</sup>. According to a previous study, Amylin produces oligomers that cause abnormal signals to be sent through their native receptors. It causes widespread nerve damage from abnormal  $Ca^{2+}$  influx. Uncontrolled vesicular discharge, endogenous neurotransmitters, and OS are similar. Malfunction, inflammation and cell death as a result<sup>31</sup>.

## Conclusion

Serum Amylin levels raised in Iraqi patients with Hypothyroidism. Amylin a good diagnostic marker for Hypothyroidism with most accurately,

sensitively with cut off value 51. The lipid profile can be strongly influenced by thyroid dysfunction.

## Acknowledgment

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## Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad and Ministry of health (51413 in 5-12-2022).

## Authors' Contribution Statement

D.K.I.'s role in this research was collecting samples and, analyzing the results. L.O.F. designed,

analyzed, presented ideas of the research, and proofread the manuscript.

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## الأميلين البشري كعلامة تشخيصية جديدة لقصور الغدة الدرقية لدى المرضى العراقيين

ضحى خليل ابراهيم، ليلى عثمان فرحان

قسم الكيمياء، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

### الخلاصة

الأميلين هو هرمون ببتيدي تفرزه خلية البنكرياس مع الأنسولين. بصفته هرموناً عصبيًا صماويًا يرسل إشارة محددة إلى الدماغ، فإن الأميلين ضروري للحفاظ على التوازن في عملية التمثيل الغذائي. كان الهدف من الدراسة الحالية هو تحديد دور الأميلين كعلامة تشخيصية لقصور الغدة الدرقية. تم تقسيم 120 عينة إلى مجموعتين المجموعة الأولى (80 مريض بقصور الغدة الدرقية) والمجموعة الثانية (40 شخصًا سليمًا). زادت مستويات الأميلين بشكل ملحوظ في المجموعة المصابة بقصور الغدة الدرقية مقارنة بمجموعة الأصحاء، وفقًا للبيانات ( $P < 0.001$ )، ارتفعت مستويات الأميلين في الدم في المرضى العراقيين المصابين بقصور الغدة الدرقية. يعد الأميلين علامة تشخيصية جيدة لقصور الغدة الدرقية بأكبر قدر من الدقة والحساسية مع القيمة المقطوعة 51.

**الكلمات المفتاحية:** الأميلين، قصور الغدة الدرقية، هرمون تحفيز الغدة الدرقية، رباعي يودوثيرونين، ثلاثي يودوثيرونين.