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Impact of PCSK9 and acid ceramidase on obese hypothyroidism female patients

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Abstract
Background: Proprotein Convertase subtilisin Kexin type 9 (PCSK9) is a serine protease composed of 692 residues. Acid ceramidase is an enzyme that hydrolyzed ceramide to sphingosine and fatty acids. **Objectives:** The present study aimed to measure levels of PCSK9 and acid ceramidase, Thyroid hormones in women suffering from hypothyroidism obese. **Materials and methods:** This study included 90 participants 30 control and 60 patients (30 patient hypothyroidism normal weight and 30 patient hypothyroidisms obese) aged 35–45 years. 5–7 ml of blood was drawn, and serum was obtained. PCSK9, acid ceramidase and Thyroid hormones tests were performed on all women. **Results:** Through investigation the date statically the results showed there are a significant increase at (P<0.01) in the levels of PCSK9, whereas showed there are a significant decrease at (P< 0.05) in the levels of acid ceramidase. **Conclusion:** Hypothyroidism and obesity have a cause increasing in the levels of PCSK9 and decreasing in the levels of acid ceramidase. Indeed, both have an effect on the concentrations of the PCSK9 and acid ceramidase.

Keywords: Hypothyroidism, obesity, PCSK9 and acid ceramidase

Introduction

Hypothyroidism is caused by deficiency of thyroid hormones levels and has a variety of etiologies and symptoms, Hypothyroidism, if left untreated, increases morbidity and mortality [1]. The low levels of iodine and thyroid autoimmunity are that the common causes hypothyroidism disease [2]. Hypothyroidism is diagnosed when the level of serum (TSH) concentration raised above normal range reference [3]. Obesity is now so prevalent in the world's population that it is beginning to supplant malnutrition and infectious diseases as the leading cause of illness, Obesity is defined by a body-mass index (weight divided by square of the height) of 30 kg m−2 or greater [4]. Proprotein Convertase subtilisin Kexin type 9 (PCSK9) is a serine protease composed of 692 residues. It contains a prodomain, catalytic domain, and a histidine rich C-terminal domain; the PCSK9 zymogen undergoes auto catalytic cleavage between the pro and catalytic domain before secretion into the extracellular matrix [5]. A variety of PCSK9 inhibitors have been developed to inhibit PCSK9 at different stages in its life cycle. Inhibition mechanisms can be classified under 3 different groups: (1) LDLR binding inhibition, (2) PCSK9 synthesis inhibition, and (3) inhibition of auto-catalytic processing [6]. The relation between PCSK9 and levels LDL-C was found firstly by the

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discovery of missense mutations in PCSK9 in patients with an autosomal dominant form of familial hypercholesterolemia (FH) [7]. Acid ceramidase (N-acylsphingosine deacylase, EC 3.5.1.23; AC) that discovered by *Gatt* in rat brain homogenates in 1963 [8]. Acid ceramidase (aCDase) is a N-acylsphingosine deacylase with an acidic pH optimum. Ac is found in lysosomes and preferentially hydrolyzes medium-chain length ceramides (C12-C16)[9]. Ceramide is hydrolyzed to sphingosine by ceramidases, which is then phosphorylated by sphingosine kinases to create sphingosine-1-phosphate (S1P). So far, five distinct human ceramidases have been identified based on their optimal pH: acid ceramidase (Ac, N-acylsphingosine amidohydrolase 1, ASAH1), neutral ceramidase (Nc, ASAH2), and alkaline ceramidases 1-3 (Acer1-3, ACER1-3) [10].

Materials & methods

This study was conducted on 90 women in reproductive age group (30–45 years) who attended Tikrit Teaching Hospital and the Al-Shirqat Hospital in Salah Alden /Iraq from December 2022 to June 2023. The women were divided into four groups and each group comprised 22 women as follows: first group (control thin), second group (control obese), third group (patient thin with hypothyroidism) and fourth group (patient obese with hypothyroidism). 5–7 ml of whole blood was obtained by a medical syringe of each participant (patients and controls). The blood sample was put in a gel tube for 20 min at room temperature for clotting. Then, it was centrifuged at 3000 rpm for 15 min to collect the serum; a part of the serum was used for the purpose of test of thyroid hormones (TSH, T3, and T4) by using ELIFA) method by auto-chemistry analyzer Minivans, Biomerieux, France, and another part of the serum was used for the test (PCSK9 and acid ceramidase) by using ELIZA methods.

Ethical considerations

Permission to conduct this study was issued by the Health Institutional Committee at Tikrit Teaching Hospital and the Al-Shirqat Hospital in Salah Alden /Iraq province, and the samples were taken from patients under the supervision of professional health-care workers**.**

Statistical analysis

The data obtained during the current study were analyzed statistically to determine the significance of the different parameters by ANOVA. The comparisons between means were made using least significant differences, and the data were presented as mean ± standard deviation.

Results

The result in the table (1) and fig (1) indicated that the levels of T3 in (A1 / A2) there are no significant differences at ($P > 0.05$), while between the groups (A1 / A3, A1 / A4, A2 / A3and A2 / A4) showed there are significant increase at ($P < 0.01$) respectively and there are significant increase at ($P < 0.05$) between the groups (A3/A4). While in T4 the results showed there are no significant difference at ($P > 0.05$) between the groups (A1 / A2 and A3 / A4), while between groups (A1/A3 and A2 / A3) there are significant decrease at ($P < 0.05$), and between the groups (A1/A4 and A2/A4) there are significant decrease at ($P < 0.01$). In the TSH the results showed there are no significant differences at ($P > 0.05$) between the groups (A1 / A2), while between the groups (A1 / A3, A1 / A4, A2 / A3 and A2 / A4) showed there are significant increase at ($P < 0.01$) respectively and there is a significant increase at ($P < 0.05$) between the groups (A3/A4). The result in the table (2) and fig (2) indicated that the levels of PCSK9 in the serum of the patients and the control groups there are a

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significant increase at (P<0.01) When compared control to the hypothyroidism patients, while within the groups the results showed there are significant increase at (P < 0.01) between (A1 / A3, A1/A4, A2/A3 and A2/A4), while between groups (A1/A2, A3/A4) showed there are no significant differences at (P > 0.05). The result in the table (3) and fig (3) indicated that the levels of Acid ceramidase in the serum of the patients and the control groups that a significant decrease at (P< 0.05) when comparison the patients' groups and control groups. While within the groups the results showed there are significant increase at (P < 0.05) between the groups (A1/A3), while showed there are no significant differences at (P > 0.05) between the groups (A1/A2, A1/A4, A2/A3, A2/A4 and A3/A4).

Discussions

The current study confirms the findings of which indicated that clinical hypothyroidism females had significantly lower T4 and higher in T3, TSH levels compared to controls [11]. The current study findings are consistent with those of *Elzobir et al.* who discovered that serum TSH concentrations increased considerably in the primary hypothyroidism group when compared to the control group [12].

Obesity is frequently associated with thyroid function issues, with a high prevalence of subclinical hypothyroidism [13]. A study that conducted in a cohort selected for euthyroid state, mean serum T4 levels were well within the normal range and very similar among lean and obese subjects [14]. The main rule of PCSK9 is to mature proteins such as released hormones, growth factors, cytokines and cell surface receptors through proteolysis [15]. Obesity was linked to higher PCSK9 levels, *Kwakernaak et al.* found that obese patients did not have a favorable association between serum TSH and PCSK9 levels, as increased adipose tissue disturbed the link, *Gagnon et al* found that PCSK9 levels did not impact TSH stimulation in obese individuals. The link between serum TSH and PCSK9 levels decreased in obese patients, supporting the findings of previous investigations [16- 18]. In contrast, *Gagnon et al* did not find a significant relation between PCSK9 levels and TSH after acute administration of TSH in euthyroid subjects suggesting that chronic TSH stimulation is needed to affect hepatocyte PCSK9 secretion and elevation in human serum, previous studies have been indicated that ceramides play a specific role in controlling insulin sensitivity and glucose homeostasis [19]. The development of obesity is accompanied by an increase in the creation of ceramides in some organs, and transgenic expression of six (dihydro) ceramide synthases in primary hepatocytes is sufficient to prevent insulin stimulated phosphorylation of AKT [20]. No data on the involvement of thyroid hormones in the regulation of the content of ceramide in other tissues are available. Hypothyroidism may reduce requirements for insulin and cause a tendency to hypoglycemia in diabetic patients [21]. *Gorska, M. et al.* did not observe any significant change in the content of ceramide in hypothyroidism and thus our data are different in this respect [22].

Conclusion

The results concluded that there is a significant increase in the levels of proprotein convertase subtilisin kexin type 9 (PCSK9), also the results concluded there are a significant decrease in the levels of acid ceramidase. Indeed, both have an effect on the concentrations of the PCSK9 and acid ceramidase

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Conflicts of interest
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Table (1): Serum levels of thyroid hormones TSH, T4, T3

Groups	Mean ±SD			
	Control (n=30)		Hypothyroidism (n=60)	
	BMI (24-27) A1 (n= 15)	BMI (29-34) A2 (n= 15)	BMI (24-27) A3 (n= 30)	BMI (29-34) A4 (n= 30)
T3 nmoL/L	1.7 ± 0.08	1.8 ± 0.06	2.3±0.5	3.4±0.7
T4 nmoL/mL	103.3±11.7	116.3±14.9	88.2±10.1	80.2±10.9
TSH µlU/mL	2.8±0.32	2.6±0.31	6.38±0.04	7.95±0.02

P Value						
Parameters	A1 / A2	A1 / A3	A1 / A4	A2 / A3	A2 / A4	A3 / A4
T3	> 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05
T4	> 0.05	< 0.05	< 0.01	< 0.05	< 0.01	> 0.05
TSH	> 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05

Table (2) Serum levels of PCSK9 hormone

Groups	Mean ±SD of PCSK9 (ng/dl)			
	Control (n=30)		Hypothyroidism (n=60)	
Total	1708.2±323.5		2235.01±347.9	
BMI (24-27)	A1 (n= 15)	1662.4±306.0	A3 (n= 30)	2347.2±289.9
BMI (29-34)	A2 (n= 15)	1744.5±337.5	A4 (n= 30)	2122.8±373.7

P Value						
Control / Patient	A1 / A2	A1 / A3	A1 / A4	A2 / A3	A2 / A4	A3 / A4
< 0.01	> 0.05	< 0.01	< 0.01	< 0.01	< 0.01	> 0.05

Table (3) Serum levels of ACDase (ng/ml)

Groups	Mean ±SD of ACDase (ng/dl)			
	Control (n=30)		Hypothyroidism (n=60)	
Total	223.1±58.6		182.5±32.8	
BMI (24-27)	A1 (n= 15)	231.9±56.8	A3 (n= 30)	179.9±29.7
BMI (29-34)	A2 (n= 15)	214.3±55.9	A4 (n= 30)	184.6±36.9

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P Value						
Control / Patient	A1 / A2	A1 / A3	A1 / A4	A2 / A3	A2 / A4	A3 / A4
< 0.05	> 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05

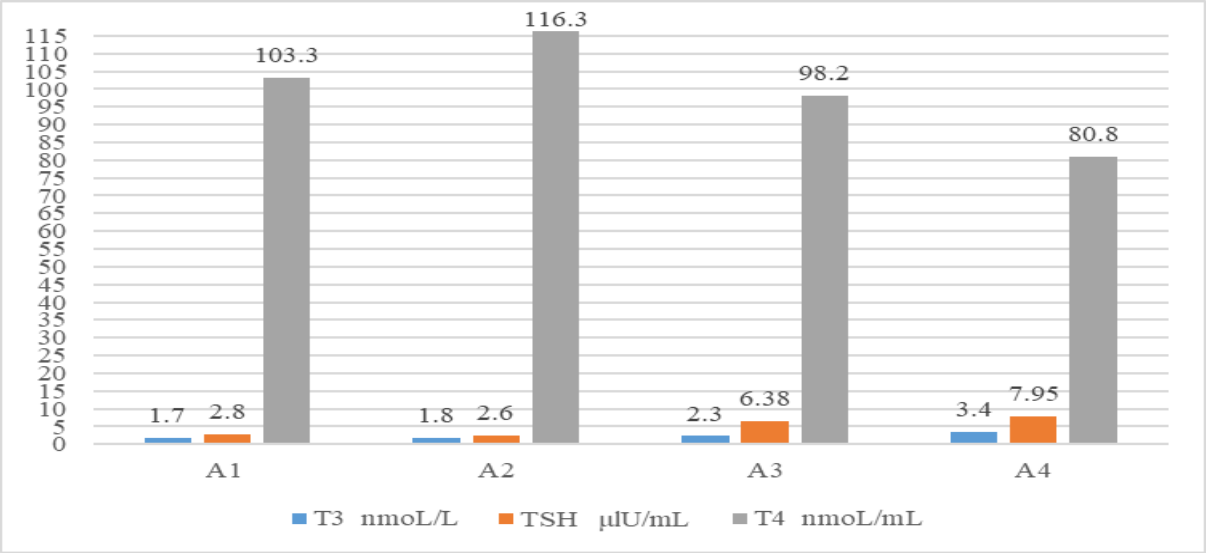

Fig (1): Serum levels of thyroid hormones TSH, T4, T3

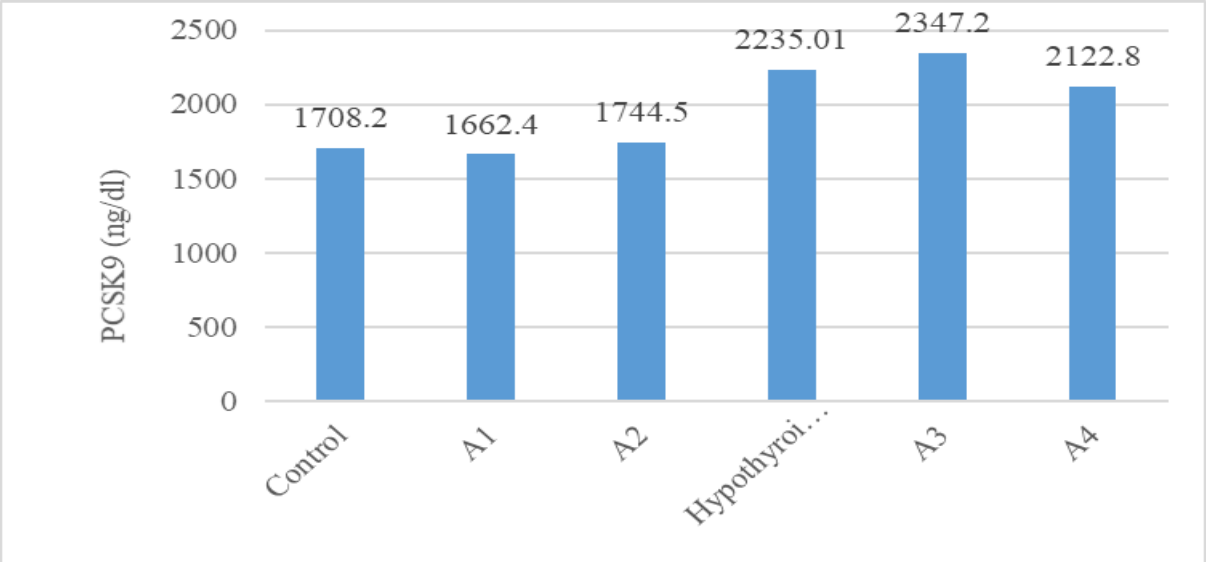

Fig (2): Serum levels of PCSK9 (ng/dl)

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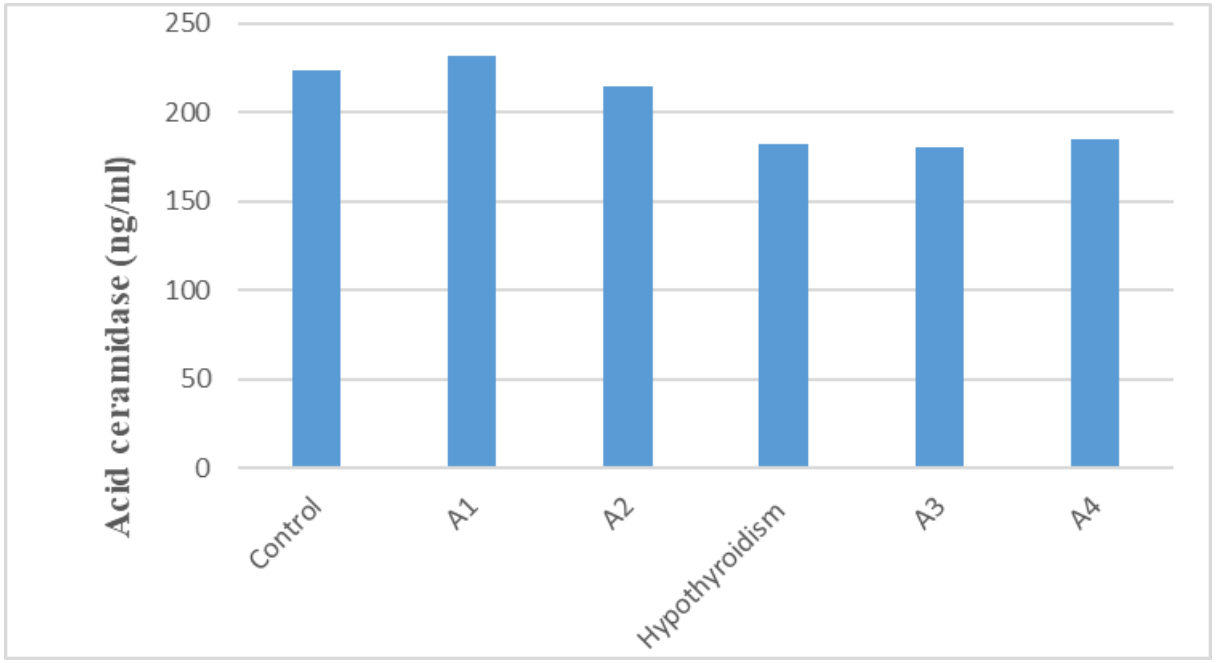

Fig (3): Serum levels ACDase

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