Evaluation of Growth Differentiation Factor-15 and Ampk Level in Hypothyroidism with Hyperlipidemia Patients

Baneen Chasib Jabal Al-Ganabi[1], Israa ketab husein alyasiri[2], Zainab Shnewer Mahdi Al-turfi[3]

[1]The Islamic university/college of medical technology /medical laboratory/Iraq
[2]Medical College- Jabber Ibn Hyyan Medical University/Iraq
[3]University of Kufa/Faculty of Education for Girls/Department of Biology/Iraq
Email: Baneen.Jasib@iunajaf.edu.iq

Abstract

The study was conducted at the Specialized Center for Endocrinology and Diabetes in Al-Sadder Medical City in Najaf Governorate, Iraq throughout the period from first of April to first of September 2021 to determine the relation between physiological, immunological and biochemical marker in hypothyroidism with hyperlipidemia in both sexes. The study was included 90 blood samples, 60 blood sample from male and female affected with hypothyroidism with hyperlipidemia where their age ranged between 20 to 60 years and 30 blood samples as a control group in the same age range. Body mass was measured for both groups. Blood samples of male and female with hypothyroidism and hyperlipidemia and control were divided into Three groups depending on patients age, the first age group (20-34) years (27 samples), the second group (35-49) years (13 samples), the third group (>=50) years (20 samples), and were divided into Three categories depending on BMI the normal weight in patients (7 sample) and control (13 sample), overweight patients (22 sample) control (11 sample) and obesity patients (31 sample) control (6 sample) respectively. The study compared the age, gender, BMI, lipid profile, thyroid hormone and AMPK, and GDF-15 biomarkers between patients with hypothyroidism and hyperlipidemia and control group. The results revealed that there were no significant differences in age ($P<0.05$) between patients and controls, while there were significant differences ($P<0.05$) in gender, BMI, lipid profile, thyroid hormone and AMPK GDF-15 biomarkers between patients and controls. The study compare the level of AMPK and GDF-15 biomarkers between male and female patients, the results showed that AMPK was not-significant ($P<0.05$) between male and female patients, and result show significant differences in GDF-15 ($P<0.05$) between male and female patients, also the results showed that AMPK and GDF-15 non-significant ($P>0.05$) between age categories in patients, and results showed significant differences ($P<0.05$) between BMI categories and AMPK and GDF-15 in patients and control. The study showed there was positive correlation of levels AMPK and GDF-15 biomarkers with age, lipid profile and thyroid hormone in patients, the results showed that negative correlation of levels AMPK and GDF-15 and age, Total Ch., TG, LDL, VLDL and TSH, but there was positive correlation with HDL, T3 and T4 except GDF-15 there was no significant correlation with T3. In conclusions AMPK and GDF-15 levels biomarkers are decreased in patients with hypothyroidism with hyperlipidemia.

Keywords: AMPK, GDF-15, hypothyroidism, hyperlipidemia, thyroid hormone

.

1. Introduction

Hypothyroidism is defined as a decrease in the secretion of triiodothyronine (T3) and thyroxine (T4), as well as diminished thyroid gland activity [1]. Reduced T3 and T4 secretion may be linked with increased Pituitary TSH secretion, which is a significant laboratory finding, especially in the early diagnosis of thyroid dysfunction [2]. Hyperlipidemia is one of the symptoms of metabolic syndrome [3]. A rise in total cholesterol and low-density lipoprotein (LDL) in hypothyroidism could be related to a variety of alterations in fat synthesis, metabolism, and mobilization [3]. Thyroid hormones lower cholesterol levels through increasing the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) reductase in the liver, Thyroid hormones also boost cholesterol absorption from the intestine by increasing LDL receptors on fibroblasts, liver, and other organs. These hormones also affect the amounts of high-density lipoprotein (HDL) cholesterol and hepatic lipase synthesis, as well as the excretion of cholesterol from the gut by bile acids While some studies have identified a link between subclinical hypothyroidism and an increased risk of atherosclerosis-related cardiovascular disease [4].

AMPK (monophosphate-activated protein kinase) is an important enzyme in energy metabolism that regulates glucose levels and lipid uptake, Adipose tissue, the liver, skeletal muscle, the heart, pancreatic beta cells, and brain cells are among the tissues that express AMPK [5]. When AMPK is phosphorylated, it inactivates metabolic enzymes that are involved de the synthesis of fatty acids and cholesterol. also acts as an upstream signal for PPAR/CEBP, suppressing preadipocyte development into adipocytes, AMPK activation lowers cellular cholesterol and fatty acids, as well as SREBP-1 and HMGCR [6].

The transforming growth factor (TGF) cytokine superfamily includes growth differentiation factor 15 (GDF15), also known as macrophage inhibitory factor-1, GDF15 is a stress-response cytokine that is only detected in the placenta and prostate in significant amounts. It is

doi.org/10.31838/hiv22.02.217 Received: 24.06.22, Revised: 25.06.22, Accepted: 24.08.22

infrequently expressed in cardiac tissues under normal physiological settings, according to studies, Cardiovascular injury, such as pressure overload, myocardial infarction, heart failure, and atherosclerosis, can drastically increase GDF15 expression and release [7]. GDF15 levels in the blood are higher in obese and type 2 diabetes patients, and it has a strong relationship with BMI, body fat, glucose, and C reactive proteins, implying that it could have a role in the development of metabolic diseases [8].

2. Materials and Methods

Detection of a Lipid Profile: Full Automatic Biochemistry Analyzer Spin200 was used to check the Lipid Profile levels.

Detection of thyroid hormone (Thyrotropin (TSH) Test system, Total Triiodothyronine (tT3) test system and Total Thyroxine (T4) test system, monobind Inc, IUS) were used to determine the levels of thyroid hormone in both control and hypothyroidism with hyperlipidemia serum sample, The Quantitative Determination of Thyroid Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric per the manufacturer's protocol.

Detection of AMPK and GDF-15 (Human Phosphorylated Adenosine Monophosphate Activated Protein Kinase ELISA Kit and Human Growth Differentiation Factor 15 ELISA Kit) were used to determine the levels of AMPK and GDF-15 in both control and hypothyroidism with hyperlipidemia serum sample, this kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human AMPK antibody. AMPK present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human AMPK Antibody is added and binds to AMPK in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated AMPKantibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human AMPK. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

3. Statistical Analysis

All data were analyzed by the SPSS software (V.28 Inc., Chicago, USA) and Microsoft Excel 2019. Kolmogorov-Smirnov test for variables distribution. Normally distributed Numerical Variables were compared between two groups by independent t-test, all data expressed as (mean ± SD) standard deviation. Nominal variables were presented as frequency and percentage (%) were compared between studied groups using the Chi-square test. Correlation coefficient analysis was completed with Pearson's test and graphic with scatter blots. Whereas the point-biserial correlation coefficient is used in correlation analysis between continuous and binary variables,Significance of differences was detected at $p<0.05$.

4. Results & Discussion

Table (1): Demographic and clinical characteristic in hypothyroidism with hyperlipidemia patients and controls groups

Variables	Mean ± SD		p-value
	Patients n=60	Controls n=30	
Age	39.9 ± 12.17	35.47 ± 11.81	0.104
BMI	30.69±4.35	27.31±4.87	0.0001***
Total Ch (mg/dL)	224.1±43.43	166.97±35.78	0.0001***
TG (mg/dL)	287.72±116.63	107.43±45.16	0.0001***
HDL (mg/dL)	45.54±11.15	50.71±9.56	0.033**
LDL (mg/dL)	156.94±29.57	103.85±41.01	0.0001***
VLDL (mg/dL)	58.24±23	25.83±11.47	0.0001***
TSH (mIU/L)	21.75±8.47	4.24±1.66	0.0001***
T3 (mIU/L)	0.67±0.25	0.85±0.2	0.001**
T4 (mIU/L)	5.49±1.46	6.56±1.29	0.001**

*Significant differences at p-value <0.05. Independent t-test.

According to age the results in Table (1) demonstrated that there were no Significant differences between pateints (39.9 ± 12.17) and control (35.47 ± 11.81) p-value =0.104. This finding differs from that of [9], who found that age has a significant impact on the connection between thyroid status and lipoprotein profile.

The results demonstrated a significantly higher BMI difference between patients (30.694.35) and control (27.314.87) (p-value =0.0001). The findings of this study are consistent with those of [10], who found a link between hypothyroidism with hyperlipidemia and BMI. Hypothyroidism causes a reduction in basal metabolism and thermogenesis, as well as an accumulation of hyaluronic acid and a reduction in renal flow, all of which contribute to water retention. Myxoedema is a clinical picture of severe hypothyroidism in which hyperkeratosis of the skin and facial edema can give the patient the appearance of being overweight. Slow peristalsis is another symptom of hypothyroidism, which can lead to persistent constipation and weight gain. This weight gain is primarily due to water retention and has little to do with fat mass gain. Obese people also have a higher prevalence of subclinical hypothyroidism, according to certain research. Overall, these factors are likely to have contributed to the widespread assumption that hypothyroidism causes obesity [11]. Kelderman-Bolk et al. discovered that among hypothyroidism patients, a greater BMI was linked to a lower quality of life [12].

T3 and T4 levels are often lower in hypothyroidism, while TSH levels are higher, as thyroid hormones decline, fats

are retained in the body rather than being oxidized for energy, resulting in increased body weight and lipid profile changes.

Our results revealed that serum HDL levels in patients (45.54±11.15) were significant lower (p-value 0.033) than in controls (50.71±9.56). Serum LDL, VLDL, triglyceride, and total cholesterol levels were also found to be significant higher (p-value 0.0001) in cases than controls. Other studies have found higher serum total and LDL cholesterol, as well as serum triglycerides in groups of patients, and these findings are consistent with [13]. In addition, the data show that the patients group had lower HDL cholesterol than the control group. This results compatible with a prior study [14].

this finding may be due to Thyroid hormone (TH) regulates energy metabolism by stimulating glucose, lipid, and protein oxidation in tissues and is crucial for metabolic balance throughout life. The liver and thyroid are known to be intricately related, with TH being involved in de novo lipogenesis, beta-oxidation, cholesterol metabolism, and glucose metabolism, among other things. In hypothyroidism, T3 and T4 levels are lower, but TSH levels are higher. Lipids are stored in the body rather than being metabolized for energy when thyroid hormones are low. Body weight rises as a result, and the lipid profile varies.

According to our findings there is significant rise in TSH between patients (21.75+8.17) and control (4.241.66) (p-value = 0.0001). In addition, the data in the same table revealed significant variations in thyroid hormone levels, which were greater in the control group (p-value =0.001). These finding because the thyroid hormones thyroxine (T4) and tri-iodothyronine (T3) have a complex inverse relationship with the pituitary hormone thyrotropin (TSH). TSH and thyroid hormones have a negative feedback system, hence TSH levels are the most sensitive indicator of a person's thyroid function. this results were agreement with [13]. Who found that a consistently increased serum TSH level, either in association with a low serum FT4 (over hypothyroidism) or within the reference range (primary hypothyroidism), is the biochemical hallmark of primary hypothyroidism (subclinical hypothyroidism). A lack of thyroid hormones in the blood causes hypothyroidism, Because of the ultrasensitive negative feedback link between the hypothalamic-pituitary-thyroid axes, serum thyroid hormone levels are low and pituitary thyrotropin (TSH) levels are increased in primary hypothyroidism due to thyroid gland failure.

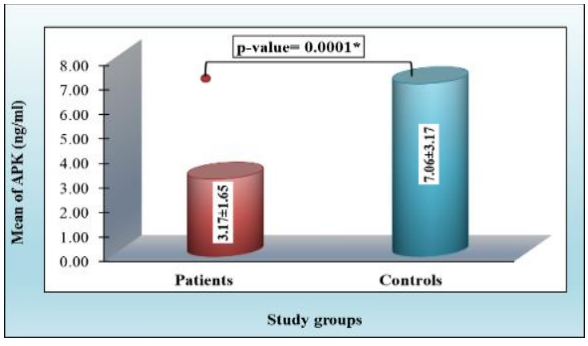

Figure (1) Mean of serum AMPK levels in patients compared with controls groups.

* Significant differences at p-value <0.05. patients n=60, controls n=30. Independent t-test.

The results revealed that there was highly Significant decrease in AMPK level between patients (3.38±1.74) and control (7.06±3.17) (p-value = 0.0001) The findings of this study are consistent with Shudong Wang et al. [13] who reported that TSH suppresses AMPK phosphorylation/activation in the thyroid gland and liver. On the other hand, Parket et al., (21) found T3 has been shown to upregulate the expression of muscle AMPK; leading to AMPK activation via phosphorylation. And Yamauchi et al. [15] suggesting that low T3 could be the source of low AMPK levels.

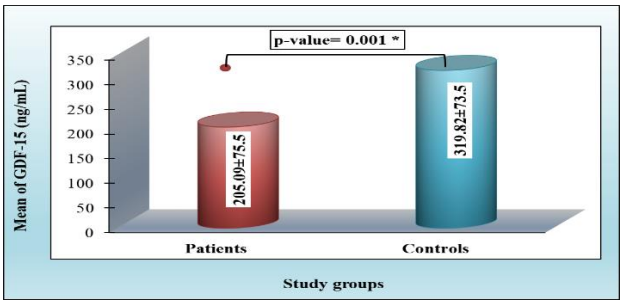

Figure (2) Mean of serum GDF-15 levels in patients compared with controls groups.

*Significant differences at p-value <0.05. patients n=60, controls n=30. Independent t-test.

The results revealed that that there was highly Significant decrease in GDF-15 between patients and control (p-value = 0.001) as shown in figure (2). our results are not similar to Salam et al. [16] Who were found GDF-15 elevated in

hypothyroid patients with elevation in TSH, But our results were similar to the result reported by Reznick et al. [17], Ruchala et al. [18] who found that Due to some similarities in metabolic actions of thyroid hormone and GDF15, especially in preventing obesity and increasing thermogenesis, recent studies suggest that some cytokines, including Growth differentiation factor 15 (GDF15), may mediate the action of thyroid hormone, suggesting the importance of indirect mechanisms of thyroid hormone signaling in the regulation of energy homeostasis.

Therefore, Thyroid hormone plays a crucial function in the control of GDF15 expression.

Table (2): Correlation of levels of APK and GDF-15 biomarkers with lipid profile in hypothyroidism with hyperlipidemia patients

		APK (ng/mL)	GDF-15 (ng/mL)
Age (year)	R	-0.154	-0.122
	P-value	0.241	0.352
Total Ch (mg/dL)	R	-0.462**	-0.474**
	P-value	0.0001	0.0001
TG (mg/dL)	R	-0.008	-0.047
	P-value	0.950	0.719
HDL (mg/dL)	R	0.340**	0.342**
	P-value	0.008	0.008
LDL (mg/dL)	R	-0.520**	-0.437**
	P-value	0.0001	0.0001
VLDL (mg/dL)	R	-0.081	-0.123
	P-value	0.541	0.348

*. Correlation is significant at p-value < 0.05 level **. Correlation is significant at p-value < 0.01 level N=60

Our result showed there was negative correlations between age and other variables in the hypothyroidism with hyperlipidemia group (Table 2). Age were negatively associated with AMPK (r = -0.154, P = 0.241), and GDF-15 (r = -0.122, P < 0.352). Our findings were agrrement with the results by Reznick et al. [17] who indicating that overall AMPKα phosphorylation and in vitro activity of AMPKα2 is attenuated with age in response to endurance-type muscle contraction.

As well as this result was in agreement with [19] who found that GDF-15 is strongly correlated with age in both healthy and unhealthy adults [13] who showed that GDF-15 is significantly higher in healthy elderlies than younger adults.

Table (3): Correlation of levels of APK and GDF-15 biomarkers with thyroid hormones in hypothyroidism with hyperlipidemia patients

		APK (ng/mL)	GDF-15 (ng/mL)
TSH (mIU/L)	R	-0.638**	-0.522**
	P-value	0.0001	0.0001
T3 (mIU/L)	R	0.443**	0.237
	P-value	0.0001	0.069
T4 (mIU/L)	R	0.440**	0.368**
	P-value	0.0001	0.004

*. Correlation is significant at p-value < 0.05 level
**. Correlation is significant at p-value < 0.01 level
N=60

Our result showed there was negative correlation were found between TSH with AMPK and GDF-15 in the hypothyroidism with hyperlipidemia patients. While there was positive correlation between T4 with AMPK and GDF-15 levels as shown in Table(3).as well as there was positive correlation between T3 with AMPK, but there was no significant correlation between T3 and GDF-15 levels (p-value = 0.069) as shown in table (3).

According to the data in the table above, there is a negative correlation between TSH and AMPK levels. This result was accepted with (29) who reported that TSH reduces AMPK phosphorylation/activation in the thyroid gland and liver, according to other researchers.

As well as There was a negative correlation between TSH levels and GDF15 levels, according to the results provided in the table above? Thyroid hormone appears to play a role in GDF15 regulation, according to one study (30) who reveald that GDF15 is linked to hyperthyroidism and hypothyroidism in humans and animals.

On the other hand there was a positive correlation between T3 levels and AMPK levels, according to the results provided in the table above.these results was accepted with (31) who reported that In studies under different experimental conditions, T3 was reported to upregulate the expression of muscle AMPK leading to AMPK activation by phosphorylation with concomitant phosphorylation of the AMPK target protein acetyl-CoA carboxylase (ACC).

5. Conclusions

Our findings demonstrated that patients with hypothyroidism and hyperlipidemia have decreased levels of AMPK and GDF-15. The correlation between AMPK, GDF-15, thyroid hormones, and lipid profile suggested that they may interact to regulate energy metabolism.

References

1.Rowe M, Hoermann R, Warmingham P. The Diagnosis and Treatment of Hypothyroidism: A Patient" s Perspective. Thyroiduk UK. 2016:1-38. Available from: https://thyroiduk.org/wp-content/uploads/2019/07/The-Diagnosis-and-Treatment-of-Hypothyroidism-August-2017-Update-V2.pdf

2.Prakash A, Lal AK. Serum lipids in hypothyroidism: our experience. Indian Journal of Clinical Biochemistry. 2006;21(2):153-5. https://doi.org/10.1007/BF02912932

3.Alloubani A, Nimer R, Samara R. Relationship between hyperlipidemia, cardiovascular disease and stroke: a systematic review. Current Cardiology Reviews. 2021;17(6):52-66. https://doi.org/10.2174/1573403X16999201210200342

4.Duntas LH, Brenta G. The effect of thyroid disorders on lipid levels and metabolism. Medical Clinics. 2012;96(2):269-81. https://doi.org/10.1016/j.mcna.2012.01.012

5.Carling D, Mayer FV, Sanders MJ, Gamblin SJ. AMP-activated protein kinase: nature's energy sensor. Nature chemical biology. 2011;7(8):512-8. https://doi.org/10.1038/nchembio.610

6.Zibrova D, Vandermoere F, Göransson O, Peggie M, Mariño KV, Knierim A, Spengler K, Weigert C, Viollet B, Morrice NA. GFAT1 phosphorylation by AMPK promotes VEGF-induced angiogenesis. Biochemical journal. 2017;474(6):983-1001. Available from: https://t.co/EaBp1AzcWI?ref_src=twsrc%5Etfw%7Ctwcamp%5Eembeddedtimeline%7Ctwterm%5Escreen-name%3ABiochem_Journal%7Ctwcon%5Es1_c14

7.Tsai VW, Husaini Y, Sainsbury A, Brown DA, Breit SN. The MIC-1/GDF15-GFRAL pathway in energy homeostasis: implications for obesity, cachexia, and other associated diseases. Cell metabolism. 2018;28(3):353-68. https://doi.org/10.1016/j.cmet.2018.07.018

8.Atici E, Menevse E, Baltaci A, Mogulkoc R. Both experimental hypothyroidism and hyperthyroidism increase cardiac irisin levels in rats. Bratislavske Lekarske Listy. 2018;119(1):32-5. https://doi.org/10.4149/bll_2018_007

9.Tognini S, Polini A, Pasqualetti G, Ursino S, Caraccio N, Ferdeghini M, Monzani F. Age and gender substantially influence the relationship between thyroid status and the lipoprotein profile: results from a large cross-sectional study. Thyroid. 2012;22(11):1096-103. https://doi.org/10.1089/thy.2012.0013

10.Garg OP, Singh S, Verma R. Correlation Between TSH and Body Mass Index in Male and Female Patients with Subclinical Hypothyroidism. Era's Journal of Medical Research. 2018;5(2):94-8. https://doi.org/10.24041/ejmr2018.72

11.Karmisholt J, Andersen S, Laurberg P. Weight loss after

therapy of hypothyroidism is mainly caused by excretion of excess body water associated with myxoedema. The Journal of Clinical Endocrinology & Metabolism. 2011;96(1):E99-E103. https://doi.org/10.1210/jc.2010-1521

12.Kelderman-Bolk N, Visser TJ, Tijssen JP, Berghout A. Quality of life in patients with primary hypothyroidism related to BMI. Eur J Endocrinol. 2015;173(4):507-15.

13.Wang T, Xu J, Bo T, Zhou X, Jiang X, Gao L, Zhao J. Decreased fasting blood glucose is associated with impaired hepatic glucose production in thyroid-stimulating hormone receptor knockout mice. Endocrine journal. 2013:EJ12-0462. https://doi.org/10.1507/endocrj.EJ12-0462

14.Saif A, Mousa S, Assem M, Tharwat N, Abdelhamid A. Endothelial dysfunction and the risk of atherosclerosis in overt and subclinical hypothyroidism. Endocrine connections. 2018;7(10):1075-80. Available from: https://ec.bioscientifica.com/view/journals/ec/7/10/EC-18-0194.xml

15.Yamauchi M, Kambe F, Cao X, Lu X, Kozaki Y, Oiso Y, Seo H. Thyroid hormone activates adenosine 5′-monophosphate-activated protein kinase via intracellular calcium mobilization and activation of calcium/calmodulin-dependent protein kinase kinase-β. Molecular Endocrinology. 2008;22(4):893-903. https://doi.org/10.1210/me.2007-0249

16.Salam S, Abbas M. Evaluation of Growth Differentiation Factor-15 in Non-Diabetic Hypothyroid Patients with Normal Insulin Sensitivity and Maintained on Oral Thyroxin Therapy.

17.Reznick RM, Zong H, Li J, Morino K, Moore IK, Hannah JY, Liu Z-X, Dong J, Mustard KJ, Hawley SA. Aging-associated reductions in AMP-activated protein kinase activity and mitochondrial biogenesis. Cell metabolism. 2007;5(2):151-6. https://doi.org/10.1016/j.cmet.2007.01.008

18.Ruchala M, Zybek A, Szczepanek-Parulska E. Serum irisin levels and thyroid function—Newly discovered association. Peptides. 2014;60:51-5. https://doi.org/10.1016/j.peptides.2014.07.021

19.Ho JE, Mahajan A, Chen M-H, Larson MG, McCabe EL, Ghorbani A, Cheng S, Johnson AD, Lindgren CM, Kempf T. Clinical and genetic correlates of growth differentiation factor 15 in the community. Clinical chemistry. 2012;58(11):1582-91. https://doi.org/10.1373/clinchem.2012.190322