International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Evaluation of Growth Differentiation Factor-15 in Non-Diabetic Hypothyroid Patients with Normal Insulin Sensitivity and Maintained on Oral Thyroxin Therapy

Sarmad Salam[1], Muhammed Abbas[2]

[1]Analytical Biochemistry Department, College of Pharmacy, Hawler Medical University, Erbil, Iraq

[2] Clinical Laboratory Science Department, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract: ***Background: Hypothyroidism is a common disorder of the endocrine system in which the thyroid gland does not produce enough thyroid hormone. It has been associated with many systemic disorders including insulin resistance due to various mechanisms. In the other hand insulin resistance is associated with Growth differentiation factor – 15, that has a role in regulating inflammatory and apoptotic pathways in injured tissues and during disease processes. Objective: This study was designed to evaluate growth differentiation factor-15 in non-diabetic hypothyroid patients with normal insulin sensitivity. Subjects and Methods: The case-control study comprised of 85 subjects as a total, among them 33 hypothyroid patients aging between 40 to 60 years and 52 apparently healthy subjects of comparable age, weight, height, and sex. Exclusion criteria were cigarettes smoking, pregnancy, cardiovascular, kidney, liver, respiratory diseases, diabetes mellitus, insulin resistance, malignancy, and other autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. Fasting blood specimens were obtained for testing serum levels of growth differentiation factor-15, thyroid stimulating hormone, thyroxine, insulin, glucose. While quantitative insulin sensitivity check index was calculated. Results: The results showed that, growth differentiation factor-15 levels were elevated in patients' group as compared with that of control subjects group. Only in hypothyroid patients group, there was a positive correlation between growth differentiation factor-15 and serum thyroid stimulating hormone. Conclusion: GDF-15 was elevated even with normal insulin sensitivity in non-diabetic hypothyroid patients.***

Keywords: Hypothyroid, growth differentiation factor-15, Thyroid stimulation hormone

1. Introduction

Thyroid gland may be affected by many diseases, hypothyroidism is a state of insufficient thyroid hormone production, in iodine-sufficient regions, the most common cause of hypothyroidism is Hashimoto's thyroiditis which is also an autoimmune disease [1]. This hypothyroidism is controlled with replacement of the thyroxine hormone [2]. Thyroid disorders, including hypothyroidism have been found to be associated with insulin resistance (IR) due to different mechanisms such as altered insulin secretion and lipid levels [3]. Insulin resistance occurs when the insulin-sensitive tissues, mainly skeletal muscle, adipose tissue, and liver, lose the ability to respond properly to the hormone [4][5], Among the conditions associated with insulin resistance is the Growth differentiation factor 15 (GDF15) [6]. GDF-15 was identified as one of the important plasma stress responsive cytokines, which correlates with cardiometabolic syndrome. It is highly expressed in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells in normal and pathological condition [7]. GDF-15 is a member of the transforming growth factor-□ /bone morphogenetic protein super family, and has a role in regulation of inflammatory and apoptotic pathways in injured tissues and during disease processes [8].

Aim of the Study

This study was designed to evaluate growth differentiation factor-15 in non-diabetic hypothyroid patients with normal insulin sensitivity.

2. Literature Survey

This study was carried out at the Specialist Center for Endocrinology and Diabetes, Alkindy Teaching Hospital, Baghdad, Iraq, for the period from June 2016 to January 2017.

3. Subjects and Methods

Subjects:, **85** subjects were included as a total aging between 40 to 60 years old. **33** patients diagnosed by senior physicians as suffering from hypothyroidism, aging between 40 to 60 years old and maintained on oral thyroxin have been included in this study. In another hand, this study included **52** apparently healthy control subjects whom age, weight, height and sex matching that of the patients group. Exclusion criteria were cigarettes smoking, pregnancy, cardiovascular, kidney, liver, respiratory diseases, diabetes mellitus, insulin resistance, malignancy, and other autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis.

Specimen Collection: Venous blood specimen were withdrawn from the arm of patients and healthy controls after an overnight fasting, in order to obtain serum, a part of which was utilized for performance of fasting serum glucose test, thyroxin and thyroid stimulation hormone, while the remaining amount of serum was kept in deep freeze at -25 C^{o} for later measurement of fasting serum insulin and growth differentiation factor-15.

Estimation of Insulin Sensitivity: The estimation of Insulin sensitivity was made by evaluating the Quantitative Insulin Sensitivity Check Index (QUICKI) which is calculated from fasting serum glucose (FSG) and fasting serum Insulin (FSI) [9].
The method of measurement of each parameter was shown in table (1).

Statistical Analysis: Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) program, version 22, by which independent T-test have been used to examine the degree of significance. P-values less than 0.05 were considered as statistically significant. In addition, Pearson's correlation coefficient (r) was used to test for statistical correlations between studied parameters, and the results were expressed as mean ± standard deviation (SD).

Table 1: Chemicals and Their Suppliers.

Chemicals and Suppliers	Parameters
Enzyme – linked immunosorbent assay (ELISA) kit, Shanghai Yehua Bio. Techno. Co., Ltd., China[10]	Growth Differentiation Factor - 15
Enzyme-linked fluorescent immunoassay (ELFA) kit, Biomerieux SA, France. By Vidas® automatic analyzer [11]	Thyroid Stimulation Hormone and Thyroxin Hormone
Chemiluminescence immunoassay (CLIA) kit, DiaSorin S.p.A., Italy. By Liaison® automatic analyzer [10]	Insulin
Enzymatic Colorimetric glucose kit, Biolabo, France. By Kenza automatic analyzer [12]	Glucose

4. Results

Levels of Parameters Among The Study Groups: GDF - 15 levels were significantly elevated in hypothyroid patients (755.46 ± 177.55 pg/ml) (p = 0.0001) as compared to GDF - 15 levels of the control subjects (486.83 ± 60.79 pg/ml). Serum TSH levels were significantly elevated in hypothyroid patients (5.57 ± 2.89 µIU/ml) (p = 0.0001) as compared to GDF - 15 levels of the control subjects (2.22 ± 0.95 µIU/ml). FSI levels were significantly elevated in hypothyroid patients (15.59 ± 3.55 µIU/ml) (p = 0.0001) as compared to that of the control subjects (11.98 ± 4.42 µIU/ml). There were no significant difference between Insulin Sensitivity levels of hypothyroid patients and that of control subjects (p = 0.872). Levels of parameters among the study groups were illustrated in table (2) below.

Correlation Studies: There were statistically significant (p < 0.05) positive correlations between serum GDF-15 and serum TSH in hypothyroid patients group (r = 0.576, p = 0.01) (figure 1), while there were no such correlation in control subjects (r = 0.053, p = 0.707).

There were statistically significant (p < 0.05) positive correlations between serum GDF-15 and FSI in control subjects (r = 0.496, p = 0.011) (figure 2), and hypothyroid patients group (r = 0.735, p = 0.0001)(figure 3).

Statistically significant (p < 0.05) positive correlations have been found between serum TSH level and fasting serum insulin levels in hypothyroid patients group (r = 0.444, p = 0.010)(figure 4). While there were no such correlations in control subjects group(r = - 0.186, p = 0.186).

Table 2: Baseline characteristics for the selected subjects between the Hypothyroid Patients, and Control Subjects groups

Control Subjects	Hypothyroid Patients	Study Groups Variables
52	33	Number
45/7	29/4	Gender (Female/Male)
46.52 ± 3.83	50.15 ± 4.60	Age (years)
71.83 ± 8.17	70.42 ± 7.19	Weight (Kg)
161.83 ± 4.61	162.79 ± 6.19	Height (Cm)
54.34 ± 4.14	55.21 ± 5.57	Lean body weight (kg)
486.83 ± 60.79	486.83 ± 137.55 [a]	Serum Growth Differentiation Factor – 15 (pg/ml)
2.22 ± 0.95	5.57 ± 2.89 [a]	Serum Thyroid Stimulating Hormone (µIU/ml)
87.98 ± 18.84	77.58 ± 13.13	Serum Thyroxine (nmol/L)
11.98 ± 4.42	15.59 ± 3.55 [a]	Fasting Serum Insulin (µIU/ml)
0.35 ± 0.02	0.35 ± 0.02	Quantitative Insulin Sensitivity Check Index
92.71 ± 8.95	79.73 ± 18.61	Fasting Serum Glucose (mg/dl)

Data was presented as Mean ± SD, a = Significant difference from control subjects (p < 0.5).

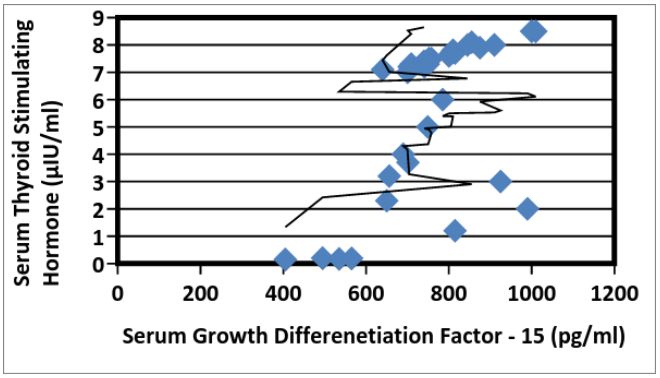

Figure 1: Correlation between Growth Differentiation Factor – 15 (GDF-15) and Serum Thyroid Stimulating Hormone (TSH) in Hypothyroid Patients group (r = 0. 576, p < 0.05).

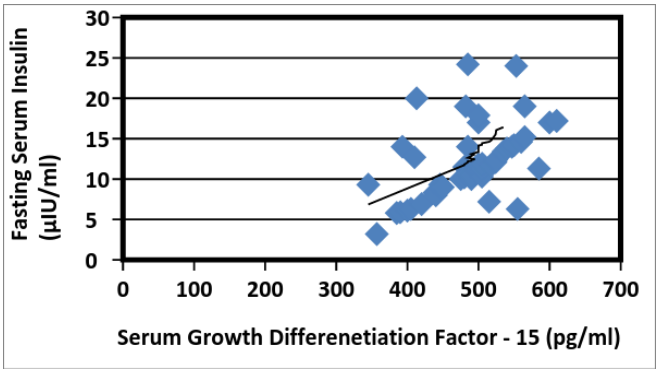

Figure 2: Correlation between Growth Differentiation Factor – 15 (GDF-15) and Fasting Serum Insulin (FSI) in control subjects group (r = 0. 496, p < 0.05).

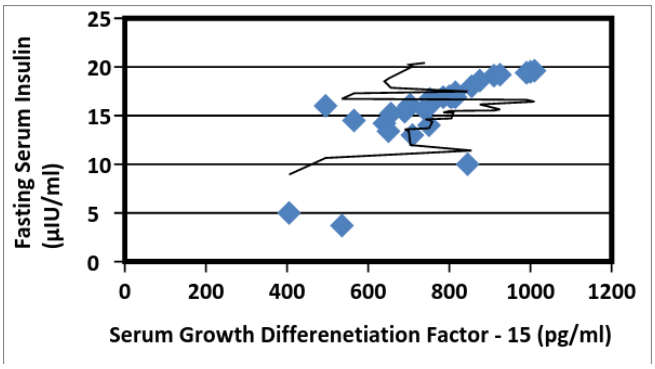

Figure 3: Correlation between Growth Differentiation Factor – 15 (GDF-15) and Fasting Serum Insulin (FSI) in Hypothyroid Patients group (r = 0. 735, p < 0.05).

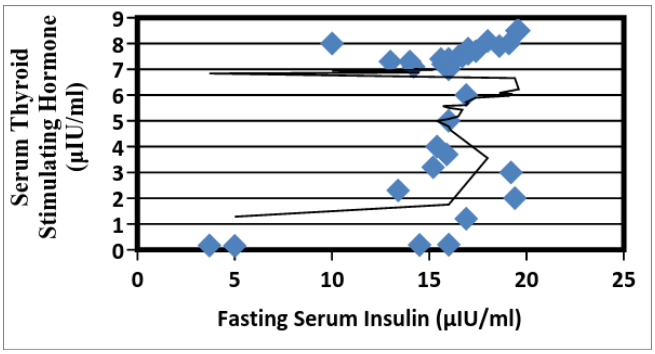

Figure 4: Correlation between Serum Thyroid Stimulating Hormone (TSH) and Fasting Serum Insulin (FSI) in hypothyroid patients group (r = 0.444, p < 0.05).

5. Discussion

The results would be discussed according to the contribution of thyroid function on the growth differentiation factor – 15 in hypothyroid patients with normal insulin sensitivity that compared with apparently healthy control subjects whom age, weight, height and sex matching that of the patients groups. When the laboratory analysis of hypothyroid patients' serum compared with that of healthy subjects, referred as a control group, the result indicated that GDF-15 was significantly elevated ($p < 0.05$) in hypothyroid patients group as compared with control group as previously shown in table (2), but the reason standing behind this elevation is not clear yet. Most of the study patients were classified as primary hypothyroid patients based on thyroid stimulating hormone and serum thyroxine levels [13]. that TSH levels either higher or within the normal range, so it has been found that TSH levels were significantly elevated in hypothyroid patients ($p < 0.05$) as compared to that of the control subjects, as shown in table (2). As mentioned above, thyroid disorders including hypothyroidism have been found to be associated with insulin resistance and elevated insulin levels due to different mechanisms such as altered insulin secretion and lipid levels [3], so, similar to Kunal et al study,[3] the result of recent study indicated that hypothyroid patients group have fasting serum insulin level significantly higher ($p < 0.05$) than that of control group (table 2). In regarding to insulin sensitivity (QIUCKI), dissimilar to Kunal et al study [3], the result of recent study, as shown in table (1), indicated that there was no significant difference ($p \geq 0.05$) in insulin sensitivity between hypothyroid patients group and control subjects. In the current study, statistically significant ($p < 0.05$) positive correlations have been found between serum GDF-15 levels and serum thyroid stimulating hormone (TSH) levels in hypothyroid patients group (figure-1), as mentioned above, GDF-15 elevated in hypothyroid patients whom classified as primary hypothyroid patients with net elevation in TSH (table 2), so this may be the possible reason behind this correlation between GDF-15 and TSH. In addition to that, as will be discussed later there were statistically significant ($p < 0.05$) positive correlations between serum GDF-15 and fasting serum insulin level in both study's groups (figure 2 and 3), at the same time, statistically significant ($p < 0.05$) positive correlation have been found between serum TSH level and fasting serum insulin levels (figure -4). In the current study, similar to Jun Hwa Hong et al. (2014) study [14], there were statistically significant ($p < 0.05$) positive correlations between GDF-15 and fasting serum insulin, as elevated fasting serum insulin and insulin resistance are associated with inflammatory process that accompanied by recruitment of macrophages [15] as well as elevation of GDF-15 level [16]; and this correlation appeared in control subjects (figure 2) and Hypothyroid Patients group (figure 3). In a clinical experiment, Karczewska-Kupczewska et al [17]. reported that there was an inverse correlation between insulin sensitivity (QUICKI) and GDF15 values in obese patients, while such correlation didn't take place in the current study, this difference in the outcomes may be due to the effect of the sample size. As hypothyroidism have been associated with many systemic disorders including insulin resistance[3], and because the large proportion of hypothyroid patients included in the current study were with primary hypothyroidism whom serum TSH levels were normal or higher (net elevation in TSH), in the present study statistically significant ($p < 0.05$) positive correlation have been found between serum TSH level and fasting serum insulin levels in hypothyroid patients group (figure -4), this result was comparable with Kunal et al [3] study that analyzed different thyroid states in regarding to the insulin and insulin resistance levels stated that there were positive correlations between TSH and fasting serum insulin in patients suffering from hypothyroidism.

6. Conclusion

Although, there were statistically significant positive correlations between serum GDF-15 and FSI level in both study groups, GDF-15 was elevated even with normal

insulin sensitivity in non-diabetic hypothyroid patients. In addition, there were statistically significant positive correlations between serum GDF-15 levels and serum TSH levels in hypothyroid patients group, but the reason standing behind this correlation and elevation of GDF-15 is not clear yet. More large scale researches needs to be carried out to determine the actual cause of GDF-15 elevation, and whether it belongs to insulin elevation and resistance or there is another factor backstage as TSH.

7. Acknowledgments

We forward our deepest thanks to Dr.Khalid Ibrahim Al-lehibi (Consultant of Endocrinology in Specialized center for Endocrinology and Diabetes) for his great help and supports, and we are thankful to all patients and volunteers for their participation in this study.

References

[1] Brent GA. Clinical practice. Graves' disease. N Engl J Med 2008; 358: 2594–2605.
[2] Fatourechi V, Aniszewski JP, Fatourechi GZ, Atkinson EJ, Jacobsen SJ. Clinical features and outcome of subacute thyroiditis in an incidence cohort: Olmsted County, Minnesota, study. J Clin Endocrinol Metab. 2003 May; 88(5):2100-5.
[3] Kunal B. Kapadia, Parloop A. Bhatt, and Jigna S. Shah Association between altered thyroid state and insulin resistance. J Pharmacol Pharmacother. 2012; 3(2): 156–160.
[4] M. A. Abdul-Ghani and R. A. Defronzo. (2010) "Pathogenesis of insulin resistance in skeletal muscle," Journal of Biomedicine and Biotechnology, vol. 2010, Article ID 476279, 19 pages.
[5] A. Kontrogianni-Konstantopoulos, G. Benian, and H. Granzier. (2010) "Advances in muscle physiology and pathophysiology," Journal of Biomedicine and Biotechnology, vol. 2010, Article ID 780417, 1 pages.
[6] Vila G, Riedl M, Anderwald C, Resl M, Handisurya A, Clodi M, Prager G, Ludvik B, Krebs M, Luger A. The relationship between insulin resistance and the cardiovascular biomarker growth differentiation factor-15 in obese patients. Clin Chem. 2011 Feb;57(2):309-16.
[7] Ramu Adela and Sanjay K. Banerjee. GDF-15 as a Target and Biomarker for Diabetes and Cardiovascular Diseases: A Translational Prospective (Review Article). Journal of Diabetes Research. Volume 2015, Article ID 490842, 14 pages.
[8] Zimmers T, Jin X, Hsiao E, McGrath S, Esquela A, Koniaris L. "Growth differentiation factor-15/macrophage inhibitory cytokine-1 induction after kidney and lung injury". Shock. 2005; 23 (6): 543–8.
[9] Wayne,PA. National Committee for Clinical Laboratory Standards, 4th ed., (1998). Procedures for the collection of diagnostic blood specimens by venipuncture, Approved standards. NCCLS Document H3-A4.
[10] John Albright. Food and Drug Administration, Department of Health & Human Services, Re: K141133. July 9, 2015. Page 25-27.
[11] Yokoyama H, Emoto M, Fujiwara S. Quantitative insulin sensitivity check index and the reciprocal index of homeostasis model assessment are useful indexes of insulin resistance in type 2 diabetic patients with wide range of fasting plasma glucose. J Clin Endocrinol Metab. 2004; 89: 1481-1484.
[12] Tietz N W. (1995). Clinical Guide to Laboratory Tests, AACC.
[13] I Kostoglou-Athanassiou and K Ntalles. Hypothyroidism - new aspects of an old disease. Hippokratia. 2010 Apr-Jun; 14(2): 82–87.
[14] Jun Hwa Hong, Hyo Kyun Chung, Hye Yoon Park, Kyong-Hye Joung, Ju Hee Lee, Jin Gyu Jung, Koon Soon Kim,1,3 Hyun Jin Kim, Bon Jeong Ku, and Minho Shong. GDF15 Is a Novel Biomarker for Impaired Fasting Glucose. Diabetes Metab J. 2014 Dec; 38(6): 472–479.
[15] Sandro M. Hirabara. Renata Gorj. Marco A. Vinolo. Alice C. Rodrigues. Renato T. Nachbar. and Rui Curi. Molecular Targets Related to Inflammation and Insulin Resistance and Potential Interventions. Journal of Biomedicine and Biotechnology. Volume 2012, Article ID 379024, 16 pages.
[16] Tanrıkulu O, Sarıyıldız MA, Batmaz İ, Yazmalar L, Polat N, Kaplan İ, Çevik R. Serum GDF-15 Level in Rheumatoid Arthritis: Relationship with Disease Activity, and Subclinical Atherosclerosis. Acta Reumatol Port. 2016 Jul 30.
[17] Karczewska-Kupczewska M, Kowalska I, Nikolajuk A, Adamska A, Otziomek E, Gorska M, Straczkowski M. Hyperinsulinemia acutely increases serum macrophage inhibitory cytokine-1 concentration in anorexia nervosa and obesity. Clin Endocrinol (Oxf) 2012;76:46–50.

Author Profile

Sarmad Salam live is Erbil, Iraq. Did MSc in Clinical Biochemistry and presently working as Analytical Biochemistry Department, College of Pharmacy, Hawler Medical University, Erbil, Iraq

Muhammed Abbas lives in Baghdad, Iraq. He is Professor in Clinical Biochemistry. Presently working at Clinical Laboratory Science Department, College of Pharmacy, University of Baghdad, Baghdad, Iraq.