DocID: https://connectjournals.com/03896.2020.20.5027

Biochem. Cell. Arch. Vol. 20, No. 2, pp. 5027-5031, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

COMPARATIVE STUDY OF FETUIN-ALEVES IN IRAQI DIABETIC PATIENTS WITH HYPER AND THYROID DISORDER

Zeinab M. Al-Rubaei[1], Ghid H. Abdulhadi[2], Imad M. M. Alrubaye[3] and Ali H. Alrugaibi[1]

[1]Department of Chemistry, College of Education for Pure Science (Ibn –Al-Haitham), University of Baghdad, Iraq.
[2]Department of Biochemistry, College of Medicine, University of Baghdad, Iraq.
[3]Baghdad College of Medical Sciences, Iraq.

(Received 19 May 2020, Revised 9 September 2020, Accepted 12 September 2020)

ABSTRUCT : Objectives: 1. To determine fetuin-A levels in Iraqi diabetic patients with hyperthyroidism. 2. To determine fetuin-A levels in Iraqi diabetic patients with hypothyroidism. 3. To correlate fetuin -A levels with HbA1c, T3, T4, TSH and insulin levels. Ninety subjects were including in this study. First group (G1) consisted of (30) healthy individuals who have no history of any thyroid disorders or diabetes mellitus as control group. Second group (G2) (n=30) patients with diabetic and hyperthyroidism as association disease and third group (G3) (n=30) include patients with diabetic and hypothyroidism as association disease. The T3, T4, TSH, HbA1c, insulin and fetuin -A were determined in all subjects. Result revealed a significant increase in T3 and T4 levels in G2 when comparing with G1 and G3. While there are significant decrease in these parameters in G3 compared to G1 and G2. Results illustrated a significant decrease in G2 in TSH levels comparing to G1and G3. While there is significant increase in TSH level in G3 compared to G1 and G2. Results showed a significant elevation in patients' groups (G2, G3) comparing to control group in HbA1c. Results showed a significant increase in insulin and HOMA-IR levels in G2and G3 comparing to G1. In addition, there are significant increase in these parameters in G3 comparing to G2. Results showed a significant increase in fetuin-A levels in G2and G3 comparing to G1. In addition, there are significant increase in these parameters in G3 comparing to G2. Results illustrated a correlation between fetuin- A and HbA1C% which showed a significant positive correlation in G1, G2 and G3. A significant negative correlation was found between fetuin- A and TSH in G3 while a significant positive correlation was found in G1 and G2 A significant positive correlation was found in G1 between fetuin -A and insulin while a significant negative correlation was found in G2 and G3 A significant positive correlation was found in G1 between fetuin -A and T3, while a significant negative correlation was found in G2 and G3. A significant negative correlation was found between fetuin- A and T4 in G1, G2 and G3. Conclusion could be drowning from this study that fetuin -A may be helpful in monitoring and early diagnosis of thyroid disorder in these patients based on significant correlation between fetuin –A and studied parameters.

Key words : Fetuin -A, Iraqi diabetic, diabetic patients with thyroid disorder.

INTRODUCTION

The thyroid gland has two important physiological endocrine systems. Among this, the 1st system, which has most of the thyroid, is accountable for the making of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) (Shafer *et al*, 2003). The activity of thyroid gland is controlled by thyroid–stimulating hormone (TSH) from the anterior pituitary gland. The secretion of this is controlled by thyrotrophin releasing hormone (TRH) from the hypothalamus. Thyroid hormones (T4 and T3) suppress TSH secretion, negative feedback (Elbers *et al*, 2018). Hyperthyroidism (thyrotoxicosis) is a hypermetabolic and biochemical state related to excessive synthesis of thyroid hormones. In females the disease is more common and linked to elevation in T3 and T4 levels, in spite of elevation in T3 is higher than T4 levels. Hypothyroidism is the condition resulting from lack of the effects of the thyroid hormone on body tissues. The increased TSH levels are associated with low values of thyroid hormones (Walsh *et al*, 2016).

The most reasonable mechanism for advancement of T2DM in patients with thyroid disorder could be due to disturbed genetic expression of several genes in conjunction with physiological aberrations leading to impaired glucose consumption by the augmented hepatic glucose output, muscles and higher glucose absorption from intestine (Manish *et al*, 2018). These endocrine disorders effect each other in a variety of ways (Hanne *et al*, 2016). Thyroid hormones contribute to the regulation of carbohydrate metabolism and pancreatic function, and on the contrary, diabetes affects thyroid function tests to variable extents (Wang *et al*, 2013).

Fetuin-A is a 49-kDa glycoprotein that originates mainly in liver and which is abundant in plasma and

mineralized bone. Human fetuin-A, secreted by the liver into circulation is a glycoprotein consisting of two subunits, an A chain or heavy chain which is comprised of 282 amino acids and a B chain or light chain made up of 27 amino acids (Shalini *et al*, 2008). Several functions have been described, including bone mineralization and osteogenesis, regulation of the insulin and hepatocyte growth factor receptors and the response to inflammation. Increased levels of Fetuin-A are linked to insulin resistance. Several studies have reported the role of Fetuin-A in the development of type 2 diabetes and its plasma level could predict the incidence of type 2diabetes independent of other established risk factors (Al-Said *et al*, 2018).

Recent epidemiological studies showed that serum fetuin-A was associated with insulin resistance and its comorbidities, such as metabolic syndrome and type 2 diabetes. Hyperthyroidism involves a significant increase in the level of tissue metabolism and is often accompanied by abnormal glucose tolerance and insulin resistance (Samar *et al*, 2017).

Study aimed to determination of fetuin -A levels in Iraqi diabetic patients with thyroid disorder. Also, study aimed to found relation correlation for fetuin-A with T3, T4 , TSH, HbA1c and insulin that may be useful in predict thyroids disorder in early stage in diabetic patients.

SUBJECTS AND METHODS

Subjects

A prospective study was conducted on three groups of subjects during the period from June to August (2018) at the Specialized Center for Endocrinology and Diabetes / Baghdad. The age range of all subjects was (40-65) years. Ninety subjects were including in this study that divided into control group (G1) that consisted of (30) healthy individuals who have no history of any thyroid disorders or diabetes mellitus. Group (G2) (n=30) that consisted of patients with diabetic and hyperthyroidism as associated disease and group (G3) (n=30) include patients with diabetic and hypothyroidism as associated disease.

Blood sample collection

Five ml of peripheral veins blood was taken from all subjects in plain tubes. Blood samples were allowed to clot then centrifuged for 15 min at 3000 rpm to obtained serum that stored at about (-20ºC) until used for analysis of other parameters (T3, T4, TSH, insulin and fetuin -A). Whole blood used in determination of HbA1c.

Triiodothyronine (T3),Thyroxin (T4),and Thyroid Stimulating Hormone (TSH) measurement: The assay principle combines an Enzyme linked Fluorescent Immunoassay (ELFA) competition method with a final detection (Caryon *et al*, 2002). The Solid Phase Receptacle serve as the solid phase as well as the pipetting device for the assay. All of the assay steps were performed automatically by the instrument. The reaction medium was cycled in and out of the SPR several times.

Fetuin -A

Sandwich ELISA technique was used in determination of fetuin-A by ready kit from my bio source, Cat. No.: MBS765849.

Statistical analyses

Statistical Analyses was done by Excel. The results were expressed as mean ±SD. Students t-test was applied to compare the significant of the difference among the groups. P-value ($P \geq 0.05$), ($P \leq 0.05$), ($P \leq 0.01$) were considered statically nonsignificant, significant and highly significant, respectively. The correlation coefficient test used for describing the association between the different studied parameters.

RESULTS AND DISCUSSION

Results of descriptive parameters were presented in Table 1, which display the levels of FSG, T3, T4 and TSH for all studied groups, that G1 represents control group, G2 as diabetic group with hyperthyroidism and G3 diabetic group with hypothyroidism. Table 1 showed a highly significant elevation in patients' groups (G2, G3) comparing to control group in FSG levels. In addition, a significant elevation in G3 compared to G2 was found.

Data in Table 1 showed a significant elevation in patients' groups (G2, G3) comparing to control group in levels of HbA1C. In addition a significant elevation in G3 compared to G2 was seen. Research have been demonstrated that increase in HbA1C levels associated with long-term risk of micro vascular complications and currently. Also, HbA1C assessment used for monitoring effective glycemic control as a keystone of diabetes care (Baswe *et al*, 2016). Study revealed that there was a significant correlation between glycosylated hemoglobin and thyroid hormones (Lee *et al*, 2016).

Results, also, revealed a significant increase in T3 and T4 levels in G2 when comparing with G1 and G3. While, there is significant decrease in these parameters in G3 compared to G1 and G2. Results revealed a highly significant decrease in G2 in TSH levels comparing to G1 and G3. In addition, there is highly significant increase in TSH level in G3 compared to G1 and G2.

Diabetic seem to influence thyroid function in two locations. Firstly, at the level of hypothalamic control of

TSH release and secondly at peripheral tissue by converting T4 to T3 (Wang *et al*, 2013). Hyperglycemia lead to reduction in hepatic concentration of T4-5 deiodinase, low serum concentration of T3, raised levels of reverse T3 and low, normal, or high level of T4 (Fleiner *et al*, 2016). Hyperglycemia causes reduction in hepatic concentration of T4-5 deiodinase, low serum concentration of T3, raised levels of reverse T3 and low, normal, or high level of T4. Thyroid hormones regulate metabolism and diabetes can alter metabolism (Bagcchi *et al*, 2014).

Table 1 display the levels of insulin, HOMA-IR levels for G1, G2 and G3. Results showed a significant increase in insulin and HOMA-IR levels in G2and G3 comparing to G1. In addition, there are significant increase in these parameters in G3 comparing to G2. Results agreement with a recent cross-sectional study, which found a significant correlation between glycated hemoglobin and thyroid volume or the number of nodules. Therefore, as an effective insulin-sensitizing drug, metformin (Unger *et al*, 2014). On the other hand, TSH itself is a major regulator of growth and differentiation of thyroid cells and plays a role in nodule formation. In the presence of insulin in cell cultures, TSH is a well-known mitogen and also suppresses apoptotic cell death in response to various stimuli (Schafer *et al*, 2003). Another study agreement with current result that was observed that expression of insulin receptor was increased in hypo functioning benign thyroid adenomas, which lost differentiated functions such as iodine uptake. Therefore, overexpression or activation of insulin receptor may be an early event in thyroid tumor genesis and nodular formation (Lupoil *et al*, 2014).

Reasonable mechanism for advancement of T2DM in thyroid disorder patients may be related to disturbed genetic expression of genes in conjunction with physiological aberrations leading to impaired glucose consumption by the augmented hepatic glucose output, muscles, and glucose absorption elevation from intestine (Song *et al*, 2011; Mori *et al*, 2006). Results of recent study suggested that metformin exerts an anti-proliferative activity, providing a rationale for an innovative therapy of thyroid proliferative diseases with metformin (Ix *et al*, 2008).

Another randomized aforementioned placebo-controlled clinical trial also indicated that metformin can reduce the size of small solid thyroid nodules and prevent an increase in the thyroid volume (). A preliminary study also found that metformin therapy significantly decreased

Table 1 : Analytical parameters for G1, G2, G3.

Parameters	Mean ±SD (G1)	Mean ±SD (G2)	Mean ±SD (G3)	T-Test G1 vs G2	T-Test G2 vs G3	T-Test G1 vs G3
HbA_{1C} (%)	4.46±0.66	9.745±1.780	9.672±2.133	S	S	S
T3(nmoL/L)	1.66±0.384	2.78±0.170	1.09±0.068	S	S	S
T4(nmoL/L)	82.3±9.450	145.21±27.90	48.71±17.507	S	S	S
TSH(nmoL/L)	2.15±0.411	0.154±0.06	21.43±4.827	HS	HS	HS
Insulin (µIU/mL)	6.71±2.010	14.97±3.617	18.91±5.495	S	HS	HS
HOMA-IR	1.99±0.003	13.97±4.00	16.58±3.99	S	S	S
Fetuin- A (ng/mL)	0.01±0.002	0.96±0.001	4.78±0.44	HS	HS	HS

*N.S. considered ≥ 0.05, S ≤ 0.05, HS≤0.01

*G1 : control group, G2: diabetic group, G3: diabetic neuropathy group.

Table 2 : r value and p-value for G1, G2 and G3 for fetuin –A with studied parameters.

parameters	Fetuin-A (ng/mL) r1	T-Test	Fetuin-A (ng/mL) r2	T-Test	Fetuin-A (ng/mL) r3	T-Test
HbA1C%	2.44	p≤0.05	4.34	p ≤ 0.05	4.66	p≤0.05
TSH (µIU/mL)	-0.042	p≤0.05	-0.36	p ≤ 0.05	0.12	p≤0.05
Insulin (µIU/mL)	7.918	p≤0.05	- 6.96	p ≤ 0.05	6.75	p≤0.05
T3 (µIU/mL)	0.23	p≤0.05	-0.18	p ≤ 0.05	-0.20	p≤0.05
T4 (µIU/mL)	-0.90	p≤0.05	-0.09	p ≤ 0.05	-0.17	p≤0.05

thyroid volume and nodule size in subjects with IR. Therefore, in patients with IR and nodular goiter, metformin may be a useful drug both to decrease IR and the size of solid nodules. By taking the role of TSH and IR in nodule formation into account, metformin may also be effective on prevention or treatment of thyroid nodule (Müge *et al*, 2017).

Furthermore, results showed a significant increase in fetuin-A levels in G2and G3 comparing to G1. In addition, there are significant increase in these parameters in G3 comparing to G2 which agreement with study revealed that fetuin-A is increased to act as an endogenous inhibitor of the insulin receptor tyrosine kinase in liver and skeletal muscle, resulting in insulin resistance in these target tissues (Sardar *et al*, 2016). Many epidemiological studies, agreement with present results that revealed higher serum fetuin-A levels are associated with insulin resistance, metabolic syndrome and T2DM (Mukhopadhyay *et al*, 2014; Pamuk *et al*, 2013; Deng *et al*, 2017). Fetuin-A is known as natural inhibitor of insulin receptor tyrosine kinase. So that prevents Insulin Receptor Substrate-1 production. That have significant role in regulating insulin signaling pathway and several cellular functions including glucose storage transport, protein-fat metabolism, differentiation and growth of cell. Metabolic pathway of the relationship between high fetuin-A levels and developing atherosclerosis including obesity, adipocyte dysfunction and IR, which leading to elevation in fetuin – A levels that agree with current study (Di Rose *et al*, 2016; Elmenshawi *et al*, 2017).

Correlation relation (r) of fetuin-A with the studied parameters are examined which r value and p-value for G1, G2 and G3 shown in Table 2.

The correlation between fetuin- A and HbA1C% showed a significant positive correlation in G1, G2 and G3 ($p \leq 0.05$, r1=2.44, r2= 4.34, r3= 4.66). A significant negative correlation was found between fetuin- A and TSH in G1and G3 ($p \leq 0.05$, r1 = -0.042, r3 = -0.12), while a significant positive. A significant positive correlation was found in G1 and G3.

Between fetuin -A and insulin (p £ 0.05, r1=7.981, r3= 6.57), while a significant negative correlation was found in G2 ($p \leq 0.05$, r2 = -0.36). A significant positive correlation was found between fetuin- A and T3 in G1, G2 and G3 ($p \leq 0.05$, r1=0.23, r2=0.18, r3=0.20). A significant negative correlation was found between fet.uin- A and T4 in G1and G2 ($p \leq 0.05$, r1=-0.90, r2=-

0.09), but there was a significant positive correlation in G3 ($p \leq 0.05$, r3=-0.17).

Conclusion could be drowning from this study that fetuin –A levels were changed in patients' groups that may be used in monitoring and early diagnosis of thyroid disorder in these patients depending on the significant relation for fetuin –A with HbA1c%, T3, T4, TSH and insulin.

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