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CORRELATION OF SERUM ZINC-ALPHA-2-GLYCOPROTEIN WITH THYROID HORMONE IN NEWLY DIAGNOSED HYPERTHYROIDISM

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ABSTRACT

Background: Patients with long-standing untreated hyperthyroidism may develop atrial fibrillation or heart failure. Treatment of hyperthyroidism requires accurate identification of underlying etiology. ZAG is known to be a lipid-mobilizing adipokine .It is involved in lipid metabolism in several ways. The purpose of the study to correlate of ZAG with thyroid hormones (FT3 & FT4) and to illustrate role of ZAG as a predictor for the outcome of treatment in patients newly diagnosed hyperthyroidism. **Patients and methods:** The study included 46 patients divided as 23 in the case group and 23 in the control group. Carbimazole was given to case group for three months and re-evaluated. **All participants in this study were subjected to f**ull history and thorough clinical examination. Laboratory investigation including: liver function tests, thyroid function tests, lipogram and fasting plasma glucose were done. Radiological thyroid scan was investigated. All tested parameters were performed before and after treartment. **Results:** There was negative correlation between ZAG and fasting plasma glucose in hyperthyroidism case group.ZAG was significantly higher in case group than control group and return to normal values after obtaining normal thyroid function.Thyroid hormone up-regulates ZAG production in a dose-dependent manner. This up-regulation of ZAG induced by thyroid hormone in the liver leading to a significant increase in ZAG circulating levels. The decreased ZAG levels were significantly correlated with the decreased FT3 even after adjustment for the independent variables of age, gender and BMI. **Conclusion:** ZAG levels were positively related with serum free T3 levels after adjusting for age, gender and BMI in patients with newly diagnosed hyperthyroidism both before and after treatment with carbimazole. ZAG will return to normal values after obtaining normal thyroid function.

Keywords: ZAG**;** FT3 ; FT4; Carbimazole

INTRODUCTION:

Thyroid hormones are produced by the thyroid gland. This gland is located in the lower part of the neck, below the Adam's apple. The gland enwraps around the trachea and has a shape that is similar to a butterfly - formed by lobes and attached by a middle part called isthmus **(1)**. Clinical suspicion of hyperthyroidism needsquick laboratory testing. Some physicians first order a TSH test, which has the highest sensitivity and specificity for hyperthyroidism, and then subsequently obtain free thyroxine (T4) and total triiodothyronine (T3) levels (free T3 assays are poorly validated if the TSH level is low) **(2)**.

The importance of age as a determinant of the prevalence and severity of hyperthyroid symptoms has recently been confirmed (**3**). Cardiac evaluation may be necessary, especially in the older patient, and may require an echocardiogram, Holter monitor, or myocardial perfusion studies (**4**).

The main ATD are thionamides, such as propylthiouracil (PTU), carbimazole (CBZ), and the active metabolite of the latter, methimazole (MMI). CBZ is not an active substance; it has to be decarboxylated to MMI in the liver, thionamides inhibit the coupling of iodothyronines and hence reduce the biosynthesis of TH **(5)**.

All inhibit the function of thyroid peroxidase, reducing oxidation and the organification of iodide. ATD are indicated as a first-line treatment of GD, particularly in younger subjects, and for short-term treatment of GD before RAI therapy or thyroidectomy **(6)**.

Zinc-α2-glycoprotien (ZAG) as an adipokinehasa close link to lipid metabolism (**7).** ZAG treatment has also been found to increase the levels of adipose triglyceride lipase and hormone-sensitive lipase, which contribute to lipolysis **(8).**

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The purpose of the study to correlate of ZAG with thyroid hormones (FT3 & FT4) and to illustrate role of ZAG as a predictor for the outcome of treatment in patients newly diagnosed hyperthyroidism.

PATIENTS AND METHODS

A case-cohort study included 46 cases divided as 23 case groups with hyperthyroidism and 23 control group. Carbimazole was given to case group for three months and re-evaluated at Internal medicine department, Zagazig University Hospital. Approval of the study protocol by the Institutional Review Board (IRB).

Inclusion criteria:

Patients with typical symptoms of hyperthyroidism. Elevated serum free T3 and free T4 with reduced TSH, thyroid scan and drug-naïve before recruitment.

Exclusion criteria:

Participants aged < 18 years, BMI > 35 kg/m^2. Known cardiovascular disease, neoplasms, smoking, diabetes, hypertension, and renal impairment (serum creatinine >1.3 mg/dl), pregnancy and patient with liver cell failure.

Technical design:

All participants in this study were subjected to full history and thorough clinical examination. Blood sample (10 ml) was collected from peripheral fasting venous from each subject for measurement of liver and thyroid function (TSH, FT3 and FT4). Also, serum samples were kept to assay lipid profile (LDL-C, HDL-C, TC and TG) and ZAG.

Laboratory investigations including: Fasting plasma glucose**,** Liver function tests [serum bilirubin (total and direct), serum alanine transaminase (ALT) and aspartate transferase (AST)], TSH, free T3 and free T4, Lipid profile and Zinc α2 glycoprotein (ZAG)

Estimation of TSH:

Human thyroid stimulating hormone ELISA kit, Inteco Diagnostics UK Ltd, 62 Beechwood Road, E8 3DY England. The TSH enzyme immunoassay test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The intensity of the color formed is directly proportional to the TSH concentration in the sample. Usage the mean absorbance values for each specimen to determine the corresponding concentration of TSH in μIU/mL from the standard curve **(9).**

Estimation of FT3:

Human Free Triiodothyronine (F-T3) ELISA kit, Catalog No. E1004. AUTOBIO Diagnostics Co., LTD, Zhengzhou, China.The free T3 present in the sample originates a coloured complex. The color intensity is inversely related to the concentration of free T3 in the test sample. The concentration was recorded for each control and sample by interpolating on the standard curve.

Estimation of FT4:

Human Free thyroxine (F-T4) ELISA kit, Catalog No. E1005. AUTOBIO Diagnostics Co., LTD, Zhengzhou, China.The free T4 present in the sample originates a colored complex. The color intensity is inversely related to the concentration of free T4 in the test sample. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

Estimation of ZAG:

Human Alpha-2-Glycoprotein 1, Zinc Binding(ZAG) ELISA Kit Catalogue No. (Sheet, P. D. HUMAN ZINC-ALPHA-2-GLYCOPROTEIN ELISA.) The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Alpha-2-Glycoprotein 1, Zinc Binding (ZAG) in samples. Add Alpha-2 Glycoprotein 1, Zinc Binding(ZAG) to monoclonal antibody Enzyme well which is pre-coated with Human Alpha-2- Glycoprotein 1, Zinc Binding(ZAG) monoclonal antibody, incubation; then, add(ZAG) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human (ZAG) of sample were positively correlated.

Statistical Analysis

The collected data were analyzed using Statistical Package for Social Sciences (SPSS 24 Inc. Chicago, IL, USA). Data were tested using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used. Quantitative data were expressed as mean ± SD. Independent T test and paired sample t-test to compare the initial and

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follow up measures.All statistical comparisons were two tailed with significance Level of P-value≤ 0.05 indicates significant, p <0.001 indicates highly significant difference while, P> 0.05 indicates Non-significant difference.

RESULTS

The present study showed a significant among hyperthyroid group **(Figure 1)**. ALT and AST had significant lower levels in case group after treatment with carbimazole, Also SBP and DBP had significant lower levels after the treatment. However, BMI had significant higher levels after treatment **(Table 1)**. There was significant reducion in both FT3 and FT4 levels levels after tratment with carbimazole in case group.While levels of TSH were signficant higher after the treatment in case groups **(Table 2)**. Case group had a significant higher levels of total cholesterol, Triglycerides, LDL cholesterol after treatment. However,there was significant reduction in HDL levels after the treatment **(Table 3)**.The difference in ZAG levels in case group before and after treatment with carbimazol (FU:follow up means after treatment). Significant reduction in ZAG levels after treatment with carbimazole in case group **(Figure 2)**.

There was statistically significant negative correlation between ZAG and Fasting plasma glucose in the clinical hyperthyroid group.There was non-significant correlation between ZAG and neither BMI, WC, FT3,FT4,TSH,TG,Total cholesterol, LDL or HDL cholesterol, ALT, AST, T BIL, SBP or DBP in thein the clinical hyperthyroidgroup before treatment **(Table 4)**. There is significant positive correlation between ZAG and FT3 after treatment with carbimazole. There was non-significant correlation between ZAG and neither BMI, WC, FT4, TSH, TG, Total cholesterol, LDL or HDL cholesterol, ALT, AST, T BIL, SBP or DBP in the in the clinical hyperthyroidgroup After treatment with carbimazole **(Table 5)**.

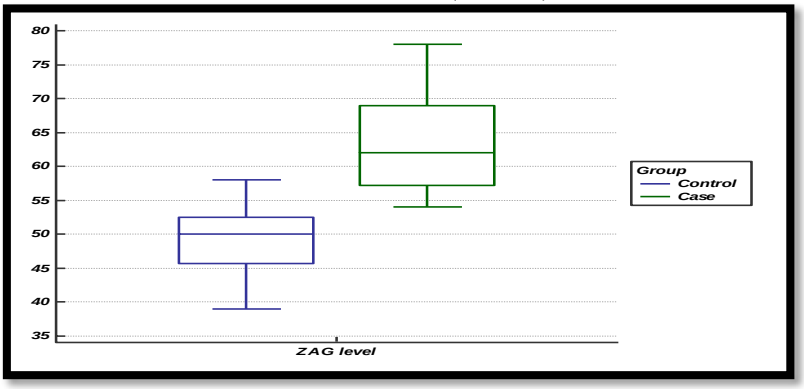

Fig (1): Box-plot diagram represents the range of ZAG levels both groups; the upper & lower line in each box represents the 75th& 25th percentile respectively while the line through each box indicates the median. Whiskers represent the range between the minimum and maximum values excluding outliers (rounded markers).

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Table (1): Comparison of clinico-lab data before and after treatment with carbimazole in the Clinical hyperthyroid case group

	Clinical hyperthyroid group		Paired t test	P
	Baseline	**After therapy**		
SBP(mmHg)	145±5	121±6	-14.7	**<0.001**
DBP (mmHg)	76±4	72±4	-3.3	**0.002**
BMI(kg/m²)	22.00±1.35	24.0±1.4	-0.4	**<0.001**
ALT (IU/L)	26.2±4.8	14±5	-8.3	**<0.001**
AST (IU/L)	25±4	12±4	-11	**<0.001**

Table (2): Comparison of thyroid profile before and after treatment in the clinical hyperthyroid group

	Clinical hyperthyroid group		Paired t test	P
	Baseline	**After therapy**		
Free T3(pmol/L)	13.6±2.7	9.0±2.0	15.4	**<0.001**
Free T4(pmol/L)	39.8±4.1	25.5±2.0	16.7	**<0.001**
TSH(uIU/mL)	0.020±0.015	0.246±0.110	-9.8	**<0.001**

Table (3): Comparison of lipid profile before and after treatment in the clinical hyperthyroid group

	Clinical hyperthyroid group		Paired t test	P
	Baseline	**After therapy**		
Total Cholesterol(mg/dl)	149±21	161±17	-6.2	**<0.001**
Triglycerides(mg/dl)	94±16	111±28	-3.4	**0.003**
LDL(mg/dl)	65±7	73±6	-8.1	**<0.001**
HDL(mg/dl)	61±9	56±8	9.2	**<0.001**

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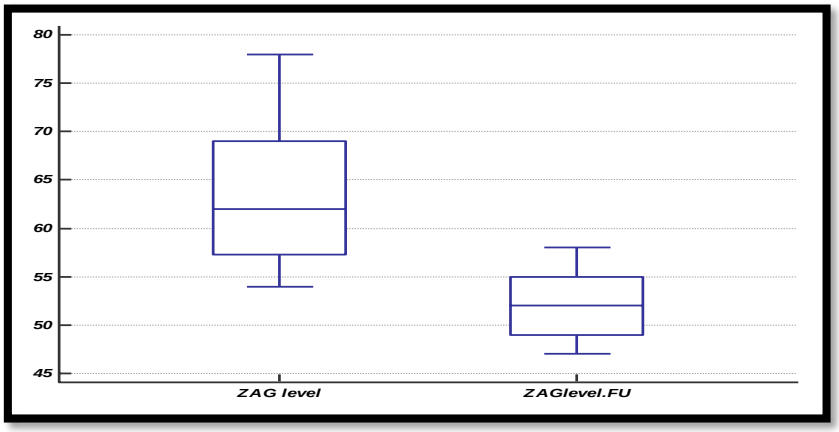

Fig (2): Box-plot diagram represents the range of ZAG level before and after treatment in the clinical hyperthyroidgroup; the upper & lower line in each box represents the 75th& 25th percentile respectively while the line through each box indicates the median. Whiskers represent the range between the minimum and maximum values excluding outliers (rounded markers).

Table (4): Correlations between serum ZAG mg/L level and certain studied parameters in the clinical hyperthyroid group.

	Clinical hyperthyroid group	
	ZAG level	
	Pearson Correlation	Sig. (2-tailed)
BMI(kg/m²)	0.036	0.871
WC (cm)	0.010	0.964
Fasting Plasma Glucose (mg/dl)	-0.461	0.027
Free T3(pmol/L)	-0.091	0.679
Free T4(pmol/L)	0.331	0.123
TSH(uIU/mL)	-0.113	0.608
Total Cholesterol(mg/dl)	-0.225	0.302
Triglycerides(mg/dl)	-0.219	0.316
LDL(mg/dl)	0.043	0.846
HDL(mg/dl)	0.103	0.642
ALT (IU/L)	0.191	0.382
AST (IU/L)	0.233	0.286
T.Bil. (umol/L)	-0.274	0.205
SBP (mmHg)	0.106	0.630
DBP(mmHg))	-0.124	0.574

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Table (5): Correlations between serum ZAG mg/L level and certain studied parameters after treatment in the Clinical hyperthyroid group

	Clinical hyperthyroid group	
	ZAG level. FU (after treatment)	
	Pearson Correlation	Sig. (2-tailed)
BMI.FU(kg/m²)	0.311	0.148
Free T3. FU(pmol/L)	0.414	0.049
Free T4. FU(pmol/L)	0.191	0.382
TSH.FU(uIU/mL)	-0.025	0.909
Total Cholesterol. FU(mg/dl)	-0.045	0.839
Triglycerides. FU(mg/dl)	-0.158	0.473
LDL.FU(mg/dl)	-0.101	0.648
HDL.FU(mg/dl)	-0.164	0.453
ALT.FUIU/L)	0.081	0.715
AST.FU (IU/L)	-0.064	0.770
SBP.FU (mmHg)	0.010	0.963
DBP.FU(mmHg)	0.305	0.156

*p<0.05 is statistically significant.

DISCUSSION

Hyperthyroidism is a clinical situation where there is excess thyroxin in the circulation due to increased synthesis of hormone from a hyperactive thyroid gland. In addition to typical clinical symptoms like increasing resting energy expenditure and weight loss directly related to excess thyroxin, patients with hyperthyroidism are likely to accompanied by changes in lipid metabolism **(10).** Thyroxin and ZAG are involved in regulating energy expenditure and metabolism of lipids. Moreover, in vitro and animal studies suggest that thyroxin up-regulates ZAG production in hepatocytes. Overt hyperthyroidism might alter the production of ZAG **(11).**

The study included 46 patients divided as 23 in the case group and 23 in the control group. Carbimazole was given to case group for three months and re-evaluated to correlate of ZAG with thyroid hormones (FT3 & FT4) and to illustrate role of ZAG as a predictorfor the outcome of treatment in patients newly diagnosed hyperthyroidism in Internal medicine department, Zagazig University Hospital during the year 2020.

Regarding SBP, hyperthyroidism increases cardiac output secondary to increased heart rate with more rapid diastolic depolarization and shorter action potentials in sinoatrial cells, increased myocardial contractility. Peripheral vascular resistance is decreased up to 70% through direct effects of T3 on the vascular smooth muscle **(12)**

Furthermore, T3 directly stimulates renin synthesis and secretion in the liver and high T3 levels also increase arterial stiffness and upregulate erythropoietin synthesis, consequently increasing erythrocyte production leading to increases in intravascular volume. All together, these changes create a hyperdynamic state and isolated systolic hypertension **(13)**

Our results were in agreement with **Xiao et al. (14)** who reported that serum TC, TG and LDL levels were significantly lower in hyperthyroid patient group when compared to control group ($p<0.001$, $p<0.001$ and $p<0.001$ respectively).

Our study results demonstrated that serum levels of ALT and total bilirubin were significantly higher in hyperthyroid case group when compared to control group ($p=0.006$ and $p=0.001$ respectively). These results were in agreement with **Navikala et al. (15)** regarding total bilirubin who found that there was a significant difference between newly diagnosed hyperthyroid patients and controls indicating total bilirubin values are comparatively high in newly diagnosed hyperthyroid patients ($p=0.03$). The diagnosis of elevated transaminases in hyperthyroidism is a challenge. This is due to the possibility of multiple etiologies including decreased cardiac output and/or liver congestion,

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concomitant primary liver disease or more specifically autoimmune hepatic disease such as primary biliary cirrhosis **(16)**

Our study results were also in agreement with **Xiao et al. (14)** who found that serum ZAG level was significantly higher in hyperthyroid patients compared to control group (66.51 ± 13.53 vs 47.81 ± 12.90 with p value<0.001). **Simo et al. (11)** explained that thyroid hormones increase the gene expression of zinca2-glycoprotein that contributes to lipolysis in hepatocytes.

Systolic Blood pressure (SBP) and DBP differed significantly before and after treatment (SBP was 145±5 vs 121±6) (DBP was 76±4 vs 72±4) in the clinical hyperthyroid group to be lower parameters after treatment. Similar finding was previously reported by **Iglesias et al. (17)** whereas SBP was 125.6 ± 2.9 vs 120.5 ± 3.0 and DBP was 71.0 ± 1.0 vs 71.0 ± 0.5. These results also agreed with **Xiao et al. (14)** who found that SBP and DBP differed significantly before and after methimazole treatment in clinical hyperthyroid patient group (p<0.001). **Berta et al. (12)** explained that Patients treated for hyperthyroidism and hypertension have reductions in cardiovascular related mortality and normalization of mean arterial blood pressure.

Our study results showed that ZAG level showed negative significance with fasting plasma glucose (P=0.027) in the clinical hyperthyroidism patients group. These results were in agreement with **Xiao et al. (14)** study who investigated the associations between ZAG and the other parameters in hyperthyroidism subjects.ZAG was negatively correlated with BMI, TC, LDLC and TSH.

Xiao et al. (14) reported that there was no significant correlation between serum ZAG and SBP or DBP, TG, HDLC, ALT, TBIL, DBIL which disagrees with point of correlation between ZAG and fasting blood glucose in our study.

Link between ZAG, blood glucose and insulin resistance was demonstrated by **Balaz et al. (18)** who reported that silencing ZAG resulted in reduced adiponectin (ADI), insulin receptor substrate-1(IRS-1) and glucosetransporters-4 (GLUT4) gene expression in primary human adipocytes indicating that ZAG plays an important role in modulating wholebody and adipose tissue insulin sensitivity.

Mracek et al. (**19)** found that ZAG mRNA positively correlates with ADI in adipose tissue in humans, and recombinant ZAG stimulates ADI release from human adipocytes.

Liao et al (20) found also that circulating ZAG can be regulated by SGLT2, and Dapagliflozin promotes the expression and secretion of ZAG in the liver via the activation of PPAR-γ. The changes in ZAG induced by DAPA may play a physiologic role in enhancing insulin sensitivity.

The results of our study showed that the decreased level of ZAG after treatment in hyperthyroidism patients group was significantly correlated with the decreased level of serum free T3 (p=0.049) and there was no significant correlation between serum ZAG and BMI or TSH, TC, TG LDL, AST, ALT in hyperthyroidism patients group after treatment. These results were in agreement with **Xiao et al. (14)** who reported that serum ZAG levels were decreased from 64.85 ±12.84 mg/l to 55.72 ± 8.83 mg/l after methimazole treatment (P<0.001) Interestingly, the decreased ZAG levels were significantly correlated with the decreased FT3, FT4 and increased TC levels, even after adjustment for the independent variables of age, gender and BMI.

To the best of our knowledge, there is only very few studies investigating serum ZAG concentration in hyperthyroid patients. We are considered from the first researchers who assessed ZAG level in hyperthyroidism patients before and after carbimazole treatment.

Conclusion:

Thyroid hormone up-regulates ZAG production in a dose-dependent manner. This up-regulation of ZAG induced by thyroid hormone in the liver leading to a significant increase in ZAG circulating levels. ZAG levels were positively related with serum free T3 levels after adjusting for age, gender and BMI in patients with newly diagnosed hyperthyroidism both before and after treatment with carbimazole. ZAG will return to normal values after obtaining normal thyroid function.

No conflict of interest.

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