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ASSOCIATION BETWEEN SERUM ZINC-ALPHA-2-GLYCOPROTEIN WITH THYROID HORMONE IN NEWLY DIAGNOSED HYPERTHYROIDISM

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ABSTRACT

Background: Thyroid dysfunction may contribute to lipids metabolic disorders. Zinc-alpha-2-glycoprotein (ZAG) function comes from its specific lipolytic action. This study aimed to find the association of serum ZAG levels with thyroxin and lipid profile in patients with hyperthyroidism before and after carbimazole treatment. **Patients and methods:** This study was conducted as a case-cohort study done on 23 patients suffering from typical symptoms of hyperthyroidism. In addition, 23 apparently healthy individuals were chosen as a control group. All patients were selected from Internal medicine department, Zagazig University Hospitals. **Results:** The present study showed fasting plasma glucose levels and SBP were significantly higher among case group. The difference in lipid profile in case group before and after treatment with carbimazole.There was significant higher levels in total cholesterol and TG Levels after treatment. The difference in lipid profile in case group before and after treatment with carbimazole. There was significant reduction in HDL Levels after treatment. While LDLwas significant higher after the treatment. Significant reduction in ZAG levels after treatment with carbimazole in case group. **Conclusion:** ZAG has a novel role in lipid metabolism as it has specific lipolytic action so it has a potential role in body weight regulation, protect against fatty liver by ameliorating hepatic steatosis, insulin resistance and inflammation.

Keywords: Zinc-Alpha-2-Glycoprotein;TSH; Hyperthyroidism; SBP; LDL

INTRODUCTION:

Hyperthyroidism is a clinical situation where there is excess thyroxin in the circulation due to increased synthesis of hormone from a hyperactive thyroid gland **(1).** 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 **(2).**

Adipose tissue secretes a variety of active biological substances called adipokines that act in an autocrine, paracrine and endocrine manner **(3).** Several lines of evidence have shown that adipokines, such as adiponectin, leptin, resistin and fibroblast growth factor 21, etc., play an important role in regulating energy expenditure and metabolism of lipids **(4).**

Researchers demonstrate that apart from abnormal circulating levels of thyroxin and thyroid-stimulating hormone (TSH), changes in profile of adipokines (like adiponectin, leptin and resistin, etc.) also have been found in patients with hyperthyroidism **(5).** Moreover, adipocytes express high levels of thyroxin and TSH

receptors which function similar to those in thyroid, suggesting thyroxin may participates in the regulation of adipocyte functions **(6).**

Thereby, thyroid dysfunction may influence secretion of adipokines, which contributes to lipids metabolic disorders. Zinc-alpha-2-glycoprotein (ZAG) is a characterized adipokine synthesized and secreted mainly by adipose tissues and liver **(7).** It is a 43-kDa soluble glycoprotein first isolated from human plasma and proposed as a tumor-derived cancer cachexia**.** It is found in various body fluids such as plasma, semen, sweat, milk and cerebrospinal fluid **(8).**

The functions of ZAG can protect against obesity-associated fatty liver by ameliorating hepatic steatosis, insulin resistance and inflammation, as well as promote browning in adipocytes, once again indicating its novel role in lipid metabolism **(9).** Given that, both 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 **(10)**. The aim of the present study was to find the better management of cases with hyperthyroidism and to decrease mortality and morbidity in patients with hyperthyroidism.

PATIENTS AND METHODS

A case-cohort study included 46 cases divided as 23 case groups with hyperthyroidism and 23 control group. This study was carried in Internal medicine department, Zagazig University Hospital during the year 2020. 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.

All patients were subjected to full history taking including detailed history was taken, with special considerations for: age, sex, comorbidities and medications. Special comment on symptoms of hyperthyroidism such as (heat intolerance ,palpitation ,weight loss ,insomnia ,irritability ,tremors , neck swelling).

Clinical examination:

Thorough physical examination was done especially:

1) Signs of hyperthyroidism such as (eye signs, tachycardia, fine tremors, hyperreflexia, flushing, wide pulse pressure)
2) Examination of thyroid gland for presence of goiter.
3) Signs of Grave's disease such as thyroid ophthalmopathy and dermopathy .

Blood sample collection:

10 ml of peripheral fasting venous blood were taken from each subject under complete aseptic conditions, the venous blood samples were collected and divided into:-5 ml left for 30 minutes for spontaneous clotting, then centrifuged at 3000 rpm for 5 minutes for measurement of liver function test. 2 ml taken from each subject left for 30 minutes for spontaneous clotting then centrifuged at 3000 rpm for 5 minutes to obtain serum samples and kept frozen until the time of assay of thyroid function (TSH, FT3 and FT4).

3 ml of peripheral venous blood were taken from each subject under complete aseptic conditions, after fasting 8-12 hour then left for 30 minutes for spontaneous clotting then centrifuged at 3000 rpm for 5 minutes to obtain serum samples and kept frozen until the time of assay of lipid profile (LDL-C, HDL-C, TC and TG) and ZAG.

Laboratory investigations:

The laboratory investigations included any investigations that verify inclusion and exclusion criteria.

1) Fasting plasma glucose.
2) Liver function tests [serum bilirubin (total and direct), serum alanine transaminase (ALT) and aspartate transferase (AST)].
3) TSH, free T3 and free T4.
4) Lipid profile.
5) Zinc α2 glycoprotein (ZAG)

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

RESULTS

The present study showed WC and BMI and were significantly lower among case group. While fasting plasma glucose levels and SBP were significantly higher among case group. Also, there was no significant difference between two groups as regarding Ageor DBP **(Table 1)**. Regarding thyroid profile, case group had significant higher levels of FT3, FT4, also had significant lower levels of TSH than control group **(Table 2)**.

Regarding lipid profile, case group had significant lower levels of total cholesterol, TG, LDL than control group. while there was no significant difference between two groups as regarding HDL levels **(Table 3)**.

Serum ALT, AST and total bilirubin were significantly higher in hyperthyroid case group **(Table 4)**. Zinc-alpha-2-glycoprotein (ZAG) was highly significant among hyperthyroid group **(Table 5)**.

Post treatment levels of TSH were signficant higher in case groups **(Figure 1)**. There was significant reducion in both FT3 and FT4 levels levels after treatment with carbimazole in case group **(Figure 2)**.

The difference in lipid profile in case group before and after treatment with carbimazole.There was significant higher levels in total cholesterol and TG Levels after treatment **(Figure 3)**.

The difference in lipid profile in case group before and after treatment with carbimazole. (FU:follow up means after treatment).There was significant reduction in HDL Levels after treatment. While LDL was significant higher after the treatment **(Figure 4)**.

Significant reduction in ZAG levels after treatment with carbimazole in case group **(Table 6)**.

Table (1): Comparison of clinico-demographic data in the studied groups

	Group		t	Sig.
	Control	Case		
	Mean ±SD	Mean ±SD		
Age(years)	4o ± 7	42 ± 8	o.1	0.9
SBP (mmHg)	120±5	145±6	15.4	**<0.001**
DBP (mmHg)	76±4	76±4	-0.1	0.941
BMI(kg/m²)	23.30±1.25	22.00±1.35	3.4	0.002
WC(cm**)**	83.3±2.8	75.7±3.2	8.6	**<0.001**
Fasting Plasma Glucose(mg/dl)	93.0±5.4	132±3.8	28.3	**<0.001**

Table (2): Comparison of thyroid profile in the studied groups

	Group		t	Sig.
	Control	Case		
	Mean ±SD	Mean ±SD		
Free T3(pmol/L)	4.7±0.4	13.6±2.7	-15.4	**<0.001**
Free T4(pmol/L)	16.4±1.1	39.8±4.1	-26.5	**<0.001**
TSH((uIU/mL))	2.397±0.596	0.020±0.015	19.1	**<0.001**

Table (3): Comparison of lipid profile in the studied groups

	Group		t	Sig.
	Control	Case		
	Mean ±SD	Mean ±SD		
Total Cholesterol(mg/dl)	173±19	149±21	4.1	**<0.001**
Triglycerides(mg/dl)	126±22	94±16	5.6	**<0.001**
LDL(mg/dl)	104±22	65±7	8.1	**<0.001**
HDL(mg/dl)	57±8	61±9	-1.8	0.086

Table (4): Comparison of liver enzymes & T. bil in the studied groups

	Group	t	Sig.

	Control	Case		
	Mean ±SD	Mean ±SD		
ALT (IU/L)	22.8±2.9	26.2±4.8	-2.9	0.006
AST (IU/L)	22±3	25±4	-2.3	0.026
T.Bil.(umol/L)	12.39±1.62	14.25±1.79	-3.7	0.001

Table (5): Comparison of ZAG level in the studied groups

	Group		t	Sig.
	Control	Case		
	Mean ±SD	Mean ±SD		
ZAG level (mg/L)	49.4±5.2	64.0±7.2	-7.9	**<0.001**

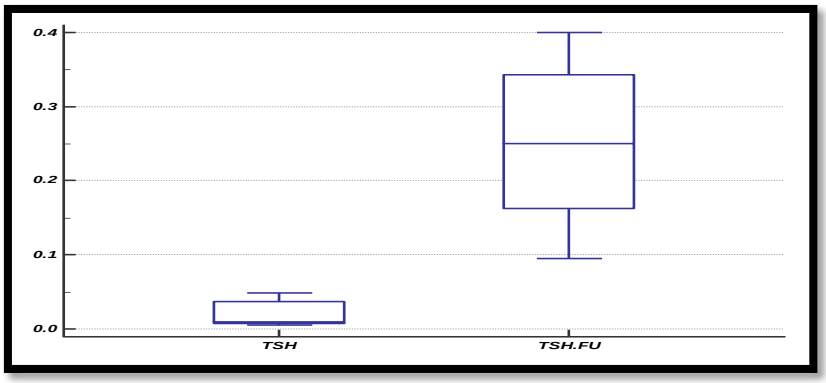

Figure (1): Box-plot diagram represents the range of TSH levels before and after treatment in the clinical hyperthyroid group; the upper & lower line in each box represents the 75th& 25th percentile respectively while the line through each box indicates the median. Whiskers represent the range between the minimum and maximum values excluding outliers (rounded markers).

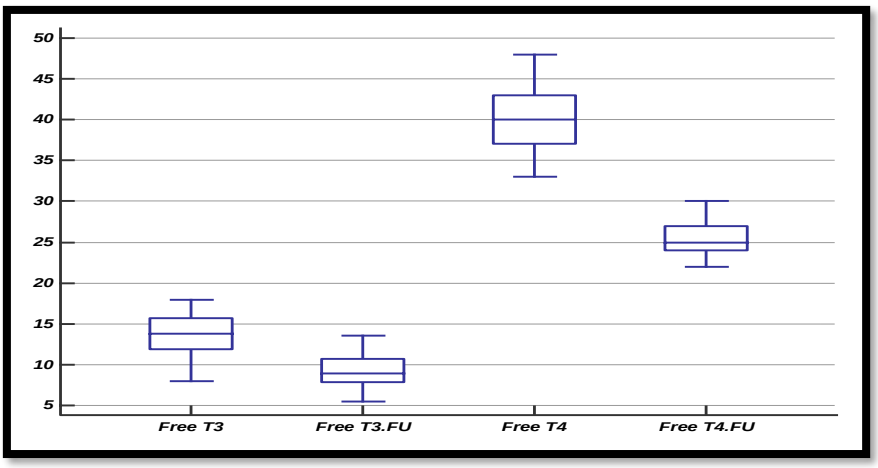

Figure (2): Box-plot diagram represents the range of Free T3 & T4 levels before and after treatment in the clinical hyperthyroid group; the upper & lower line in each box represents the 75th& 25th percentile respectively while the line through each box indicates the median. Whiskers represent the range between the minimum and maximum values excluding outliers (rounded markers).

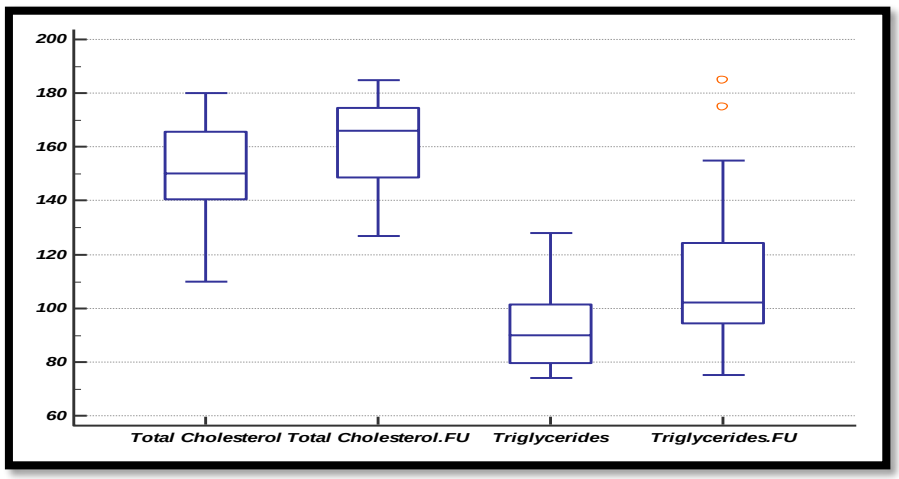

Figure (3): Box-plot diagram represents the range of TC & TG levels before and after treatment in the clinical hyperthyroid group; the upper & lower line in each box represents the 75th& 25th percentile respectively while the line through each box indicates the median. Whiskers represent the range between the minimum and maximum values excluding outliers (rounded markers).

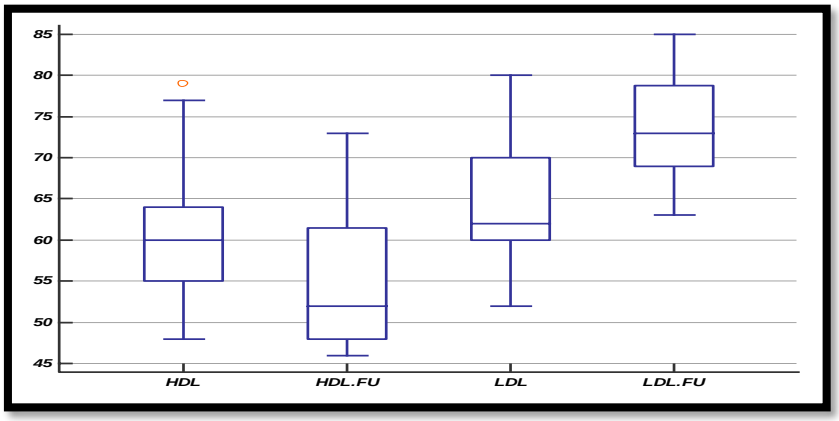

Fig (4): Box-plot diagram represents the range of LDL & HDL level before and after treatment in the clinical hyperthyroidgroup; the upper & lower line in each box represents the 75th& 25th percentile respectively while the line through each box indicates the median. Whiskers represent the range between the minimum and maximum values excluding outliers (rounded markers).

Table (6): Comparison of ZAG level before and after treatment in the clinical hyperthyroidgroup

	Clinical hyperthyroid group		Paired t test	P
	Baseline	After therapy		

ZAG level (mg/L)	64.0±7.2	52±4	9.5	**<0.001**

DISCUSSION

Thyroid dysfunction may influence secretion of adipokines, which contributes to lipids metabolic disorders. Zinc-alpha-2-glycoprotein (ZAG) is a characterized adipokine synthesized and secreted mainly by adipose tissues and liver. ZAG function comes from its specific lipolytic action and its potential role in body weight regulation **(7).**

Adipose tissue secretes a variety of active biological substances called adipokines that act in an autocrine, paracrine and endocrine manner. Researchers demonstrated that adipokines (like adiponectin, leptin and resistin, etc.) have been found in patients with hyperthyroidism **(5)**

In our study, we investigated the association of serum ZAG levels with thyroxin and lipid profile in patients with hyperthyroidism before and after carbimazole treatment.

In our study we used carbimazole (CMZ) instead of methimazole (MMI).Carbimazole was developed in 2004. Its action is identical to the action of MMI. CMZ is metabolized into MMI after absorption. It is marketed in Egypt, Europe, the United Kingdom, Australia and New Zealand but not in North America. While Methimazole was introduced in 1952. It also inhibits the enzyme thyroperoxidase but unlike PTU, does not inhibit the enzyme tetraiodothyronine 5'deiodinase. It is marketed in North America (not available in Egypt) **(11).**

The equivalent potency of MMI to PTU is approximately 1:20 (e.g., 5mg MMI =100mg of PTU) while ten milligrams of CM is rapidly metabolized to approximately 6mg of MMI **(12).**

Results of our study revealed that BMI and WC were significantly lower in the hyperthyroid case group ($p=0.002$ and $p<0.001$ respectively), while Fasting plasma glucose levels and SBP were significantly higher among case group when compared to control group ($p<0.001$).

Moreover, our study results were in agreement with **Chen et al. (13)** regarding BMI and WC who found that BMI, WC (23.76 ± 2.91, 74.41 ± 2.6 respectively) were lower in the hyperthyroid patient group when compared to control group (24.55 ± 3.36, 82.3 ± 1.9 respectively), while our results disagreed with **Chen et al. (13)** regarding fasting blood glucose that was found lower in the hyperthyroid patient group when compared to control group.

Also, **Xiao et al. (14)** reported that BMI and WC were significantly lower in case group when compared to control group ($p<0.001$, $p<0.001$ respectively), while our results disagreed with **Xiao et al. (14)** regarding fasting blood glucose that was found lower in the hyperthyroid patient group when compared to control group ($p<0.001$).

The likely explanations of these changes in glucose levels in hyperthyroidism are that hyperthyroidism has been associated with hyperglycemia, and different mechanisms are suggested including, reduced half-life of insulin due to increased insulin clearance rate, an enhanced release of biologically inactive insulin precursors, and increase glucose absorption from the gut, Moreover hyperglycemia in hyperthyroidism, is aggravated by elevated hepatic glucose production and increased glycogenolysis **(15).**

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 **(16).**

Results of the our study revealed that serum TC, TG and LDL levels were significantly lower in hyperthyroid case group when compared to control group ($p<0.001$, $p<0.001$ and $p<0.001$ respectively).These results were in agreement with results of the study done by **Chen et al. (13)** in which the serum TC, TG and LDL levels were lower in hyperthyroid patient group (4.55 ± 0.87, 1.50 ± 1.25, 2.32 ± 0.95 respectively) when compared to control group (4.65 ± 0.89, 1.53 ± 1.30, 2.50 ± 0.89 respectively).

Thyroid hormone can directly trigger a series of pathway mainly involved in lipid metabolism and energy homeostasis, such as PI3K/Akt, MAPK/ERK, SIRT1, peroxisome proliferator activated receptors (PPARs), also thyroid hormone participates in the regulation of adipocyte functions including secreting adipokines (like adiponectin, leptin and resistin) suggesting that serum ZAG which is a novel lipid-mobilizing adipokine could be changed in hyperthyroidism patients **(14).**

Additionally, in GD hyperthyroidism, TRAb could result in an inflammatory injury to hepatocytes since it is assumed that TRAb efficiently stimulate the TSHR of human hepatocytes. Thyroid hormone excess has also direct toxic effects on hepatobiliary system which induces cellular apoptosis and oxidative stress **(17)**.

Results of our study revealed that serum ZAG level was significantly higher in hyperthyroid case group (64.0±7.2 mg/L) when compared to control group (49.4±5.2mg/L) (p value<0.001). These results were in agreement with **Simo et al. (18)** who reported that higher serum levels of ZAG were detected when patients were in hyperthyroidism than when they were with normal thyroid function (47.08± 14.04 vs. 32.35 ±11.35; with p value 0.001).

Regarding thyroid profile before and after treatment in the clinical hyperthyroid group, the results revealed that free T3 and free T4 were significantly lower after treatment ($p<0.001$, $p<0.001$ respectively), while TSH was significantly higher after treatment ($p<0.001$). These results were in agreement with **Navikala et al., (19)** who found similar results regarding thyroid profile whereas free T3 and free T4 were significantly lower ($p<0.05$) while TSH was significantly higher ($p<0.05$) in patient group after treatment when compared to baseline group before treatment.

Results of our study revealed that total cholesterol, triglycerides and LDL were significantly higher while HDL was significantly lower after treatment when compared to the base line before treatment in the clinical hyperthyroidism patients group ($p<0.001$).These results were in consistency with **Oge et al. (20)** who found that after treatment of the hyperthyroid patients, total cholesterol, LDL cholesterol, triglycerides were increased significantly ($p<0.05$) while HDL decreased significantly ($p<0.05$) when compared to baseline patients group before starting the treatment.

The present study results revealed that ZAG level was significantly lower after carbimazole treatment in hyperthyroid patients groups when compared to ZAG level in baseline patients group before treatment ($P<0.001$). In good agreement with **Xiao et al. (14)** study, Serum ZAG levels was decreased from 64.85 ±12.84 mg/L to 55.72 ± 8.83 mg/L after methimazole treatment ($P < 0.001$).

CONCLUSION

Hyperthyroidism is a form of thyrotoxicosis due to inappropriately high synthesis and secretion of thyroid hormones by thyroid gland. Adipose tissue secretes

several adipokines that play an important role in regulating energy expenditure and metabolism of lipids**.** ZAG has a novel role in lipid metabolism as it has specific lipolytic action so it has a potential role in body weight regulation, protect against fatty liver by ameliorating hepatic steatosis, insulin resistance and inflammation.

No conflict of interest.

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