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Measuring the level of cytokines, micronutrients and some pituitary and thyroid hormones in females with anorexia nervosa at Ramadi Teaching Hospital

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Abstract

Anorexia nervosa distinguished as possessing a low body weight since limiting energy consume or compensating to an over rate intentionally so to reach or keep the thin ideal weight. The aim of our study is assessing the state of the hypothalamic-pituitary thyroid axis, the potential role of interleukins and micronutrients in females with anorexia nervosa comparison to healthy females. Thirty females with anorexia nervosa participated in the study and the control group was composed of thirty healthy females without eating disorders and any immunological disorders. The study was done in in Ramadi Teaching Hospital in Anbar governorate, Iraq from January to June 2024. Anthropometric Measurements (body mass index, Weight, Hight), concentration of thyroid hormones (triiodothyronine and thyroxine), pituitary hormone (Thyroid-stimulating hormone), micronutrient [trace elements (Selenium, Magnesium, Zinc, Copper) and vitamin E and vitamin D3], and interleukins (2, 4, 6, 10, Transforming Growth Factor-β). There was a significant (P-value ≤ 0.05) decrease in the concentrations of triiodothyronine, thyroxine, Vitamin D3, Vitamin E, trace elements, IL-2, IL-10 and Transforming Growth Factor-β in females with anorexia nervosa as compared to healthy females. While there were no significant (P-value≤0.05) differences of thyroid-stimulating hormone, IL-4 and IL-6 in females with AN as compared to healthy females. There was a significant increase in the concentrations of ghrelin hormone in females with anorexia nervosa as compared to healthy females. **Conclusion:** The low serum concentration of triiodothyronine in females with anorexia nervosa pointed that the peripheral conversion of triiodothyronine to thyroxine, is reduced during chronic starvation.

Introduction

Anorexia nervosa (AN) is an eating disorder distinguished by a pursuit of thinness, fright of gaining weight, restrictive eating behavior, and a distorted view of the body. In addition to other signs, including excessive exercise (or another compensatory actions) and other metabolic disorders resulting from malnutrition [1].

Anorexia nervosa is a disease can occur at any human age, but it can be seen in high rates in young adults and teenage girls as well. This condition greatly affects many physiological processes in the body, including metabolism and digestion, leading to digestive disorders such

as: postprandial distress or distension of the gut because of slow intestine motility which (at least in part) lead to decrease food intake of the patients [2].

Individuals with anorexia nervosa regulate their eating in compulsive and highly controlling ways, reflecting their profound disturbance in the relationship between food and the body. Their eating behavior is characterized by an excessive pursuit of thinness, which leads to avoiding foods rich in calories, careful calculation of calories and food components, strict nutritional routine such as controlling mealtimes, drinking plenty of water, focusing on eating fruits and vegetables, or focusing on a vegetarian diet. In addition to, cutting foods with high caloric carbohydrates as: cereals or bread is usual in patients with AN [3].

Hormonal abnormalities are common in anorexia nervosa (AN) and impacts on number of hypothalamic–pituitary paths. For examples: shorter in thyroid hormone levels, low testosterone levels in males, deficiency of menstrual cycle in females, also, anomalies in both anorexigenic and orexigenic hormones that effect on energy spending and food regulation, all these hormonal changes represent a response to hunger (undernutrition) and try to reassign limited substrate resources to the almost all crucial functions of life [4, 5].
This study aimed the understanding of how anorexia nervosa impact on each of the thyroid hormones and digestive system hormones, in addition to its effect on trace elements and vitamins.

Methodology

Study participants

This cross-sectional study was done in Ramadi Teaching Hospital/ Iraq. A total of 60 females have been involved over a period of 6 months (from January to June 2024) after approval from the scientific committee in the Hospital.

The study was included on Sixty females aged 19–24 years. The study group involved of 30 females aged 19–24 years with AN diagnosed according to the medical staff. 30 healthy-looking women were selected as a control group, matched in age to the patient group. The exclusion criteria for both group were females with chronic diseases, another psychological illnesses was make a diagnosis by a psychiatrist, also those who take medications, as hormonal medications or nutritional supplements within the past three months, and infection within the past month before the study, also, if they had lately had influenza.

Diagnosis

Doctors rely on many things to diagnose the disease, including: a physical examination that includes measuring weight in relation to height, measuring body mass index, heart rate, temperature, examining the skin and nails, in addition to blood pressure.
Psychological assessment: This includes questions about thoughts, feelings, and eating habits.
There were a number of common symptoms among women with anorexia nervosa, including: skipping or refusing to eat meals, eating certain “safe” types of food, usually low in fat or calories, fear of gaining weight, including constantly weighing oneself. Frequently checking one's body in the mirror, believing that there are defects in it, complaining of obesity or having fat in areas of the body, excessive exercise, low blood pressure, constipation and abdominal pain, dry or yellow skin.

Collection of blood

Five ml of venous blood was drawn from each female participant in the study using a medical syringe. The blood was transferred into a tube that did not contain anticoagulants and left for fifteen minutes at room temperature. Centrifuged was at 3000rpm for 5 minutes to collect sera, then resulting serum was separated and stored at -20 C till used for assessment of biochemical tests.

Estimation of Anthropometric Measurements

For calculating the anthropometric measurements were utilized the bathroom scale and height estimated tape, which included measuring both weight and height and were assessed to the nearest 0.1 kg c obesity data. BMI was measured according to the formula below (CDC Growth Charts, 200), and classifying the individuals as shown in Table 1.

$$BMI = \frac{weight\ (kg)}{square\ height\ (m)}$$

Table 1: BMI chart Adult (both Male and Female).

Weight Categories	BMI (Kg/m^2)
Under Weigh	<18.5
Healthy Weight	18.5 – 24.9
Overweight	25 – 29.9
Obese	30 – 34.9
Severally Obese	35 – 39.9
Morbidly Obese	≥40

Estimation of Thyroid Hormone Concentration

The concentration of thyroid-stimulating hormone (TSH), thyroxine hormone (T4), and triiodothyronine hormone (T3) in blood serum was estimated by Enzyme-Linked Fluorescent Assay (ELFA) using a compact automated immunoassay system, mini VIDAS® commercial kit (Biomerieux, France).

Estimation of Ghrelin Concentration

Ghrelin hormone concentration in blood serum was estimated by utilized enzyme-linked immunosorbent assay (ELIZA) kit commercially available from Phoenix Pharmaceuticals, INC an in vitro quantitative assay.

Estimation of Vit.D3 Concentration

Vit.D3 concentration in serum was measured by utilizing diagnostic kits prepared by Germany Immunodiagnostic systems (ids) company by the enzyme-linked immunosorbent assay (ELISA) technology.

Estimation of Vitamin E (Alpha-Tocopherol) concentration

Alpha-Tocopherol (Vit.E) concentration in serum was measured by utilizing diagnostic kits prepared by UK company by the enzyme-linked immunosorbent assay (ELISA) technology. This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antibody is pre-coated onto a 96-well plate.

Estimation of trace elements (Micronutrient) Concentration

Serum Cu, Zn and Mg were measured using special Chinese made kit by utilizing Spectrophotometer.

Estimation of Cytokines Concentration

The concentrations of cytokines in serum measured by utilizing Enzyme-Linked Immunosorbent Assay (ELISA) kit for In vitro diagnostic quantitative, manufactured by MyBioSource. A panel of cytokines was measured, namely IL-2, IL-4, IL-6, IL-10, and TGF-b.

Statistical Analysis

All data were analyzed using statistical Package for the Social Science (SPSS) software version 26. Quantitative variables were stated as a mean standard error (S.E). The accepted level of significance was $P \leq 0.05$

Results and Discussion

Table 2 summarized the characteristics of the females with AN and healthy females groups. Body mass index (BMI), Weight (kg), T4 (nmol/L), T3 (nmol/L), Vit.D3 (ng/ml), IL-10 (pg/ml), IL-2 (pg/ml), TGF- β (pg/ml) were significantly ($P \leq 0.05$) decreased in females with AN when compared to the healthy females, whereas there was Non-significant ($P<0.05$) differences in each of Age (year), Hight (m), TSH (IU/L), IL-4 (pg/ml), IL-4 (pg/ml), IL-6 (pg/ml) between females with AN when compared with the healthy females. The results in table 2 shows there is significant ($P \leq 0.05$) increase in serum ghrelin(pg/ml) concentration in in females with AN when compared to the healthy females.

Table2: Mean and SE of Biochemical parameters in all studied group.

Parameters	Group	N	Mean ± SE	95 %confidence interval for mean		Sig. Value
				Lower bound	Upper bound	
Age (year)	**AN**	30	20.341 **±** 0.324	19.175	22.209	N.S
	C	30	22.182 **±** 0.347	20.451	23.064	
Weight (kg)	**AN**	30	50.098 ± 1.003	45.012	53.995	0.0001*
	C	30	56.347 ± 1.853	54.128	57.998	
Hight (m)	**AN**	30	1.610 ± 0.420	1.539	1.598	N.S
	C	30	1.631 **±** 0.532	1.597	1.642	
BMI (Kg/m^2)	**AN**	30	19.339 **±** 1.023	19.235	21.424	0.0001*
	C	30	21.260 ± 1.019	21.476	22.087	
TSH (IU/L)	**AN**	30	1.429 ± 0.102	1.078	2.454	N.S
	C	30	1.608 ± 0.098	1.292	2.896	
T4 (nmol/L)	**AN**	30	96.982 ± 0.843	92.981	98.381	0.0001*
	C	30	106.987 ± 0.276	102.871	173.815	
T3 (nmol/L)	**AN**	30	1.119 ± 1.014	1.016	1.742	0.0003*
	C	30	2.428±0.591	1.267	2.570	
Vit.D3 (ng/ml)	**AN**	30	15.914 ±1.043	21.934	12.649	0.0002*
	C	30	29.871± 0.891	33.871	25.871	

Ghrelin(pg/ml)	**AN**	30	501.98 ± 0.34	439.09	586.87	0.0001*
	C	30	417.98 ± 0.01	349.61	442.09	0.0001*
Vit. E (µmol/L)	**AN**	30	15.014 ± 0.871	17.839	20.091	0.0009*
	C	30	22.719 ± 1.041	21.491	24.960	
IL-2 (pg/ml)	**AN**	30	200.517 ± 0.549	186.850	204.7651	0.0005*
	C	30	461.752 ± 0.719	456.871	482.987	
IL-4 (pg/ml)	**AN**	30	13.055 ± 1.188	10.730	13.127	N.S
	C	30	12.983 ± 0.497	11.012	13.549	
IL-6 (pg/ml)	**AN**	30	3.034 ± 0.512	0.951	3.704	N.S
	C	30	2.314 ± 0.105	1.116	2.983	
IL-10 (pg/ml)	**AN**	30	1.876 ± 0.287	0.793	2.104	0.0008*
	C	30	3.104 ± 0.431	1.109	3.916	
TGF-β (pg/ml)	**AN**	30	229.013 ± 0.127	201.925	274.183	0.0006
	C	30	341.194 ± 1.002	289.017	386.127	

***P ≤ 0.05 *SE-Standard Error * N.S-Not Significant *AN-Anorexia Nervosa group *C-Control group *BMI-Body Mass Index *TSH-thyroid-stimulating hormone *T4-thyroxine *T3-triiodothyronine *IL- interleukin**

The results of this study showed significantly decreases serum concentrations of T3 and T4 in females with anorexia nervosa (AN) than healthy females.

This study supports earlier pointed that the anorectics starved to low body weights have significantly lower concentrations of serum T3 and T4 than healthy females and males [6,7].

Starvation because of anorexia nervosa may be results in waked peripheral conversion of T4 to T3 with decreasing of serum T3 concentrations [8]. A linked defect in hypothalamic function leads to reduced TRH and thus TSH secretion as showed in the results of this study.
Thyroxine (T4) and triiodothyronine (T3) (thyroid hormones) are main regulators of metabolism activity that impact the tasks of organs all over in the body [9]. In some states like starvation as anorexia nervosa (AN), there is reduced conversion of T4 to T3, which lowers resting energy spending, and increased conversion of T4 to metabolically inactive shape of T3 [10].

The results of this study show there are ghrelin concentrations in plasms of females with AN are significantly higher than to concentration of ghrelin in healthy females. The high circulating ghrelin concentrations in AN appear to make it unlikely that ghrelin is straight responsible for the pathogenesis of AN [11].
Increased gastric ghrelin production in AN may mirror a physiological effort to compensate lack of food intake and stored energy.

According to a previous study, it was suggested that the ghrelin cues the central nervous system (CNS) when it is required to convert to additional energy storing metabolism and to become hungry [12].

Ghrelin is a stomach hormone, have ability to induce each of growth hormone secretion and positive energy balance resulting in adipocytes forming (abiogenesis). These impacts are mediated by hypothalamic neuropeptides [neuropeptide Y (NPY), Agouti-related protein (AGRP), proopiomelanocortin (POMC)] and are eventually a result of increased food consume and decreased of the fat oxidation [13].

The reason of the decreases for Vit. D3 in females with AN is possible that these young females, who limit their calories ingest for the reason that of fear of weight obtain, are further likely to ingest a no calories nutritional source as: a multivitamin than a healthy female [14]. Low concentration of vitamin D in serum blood of patients with anorexia nervosa may be because have restricted dairy consume [15].

It has also been hypothesized that these females with anorexia nervosa (AN), who have very small of body fat, possess declined metabolic clearance and decreased uptake of Vit. D3 via the adipose tissue. This hypothesis is reinforced by studies done by DiVasta and his colleagues that were conducted in obese individuals with overabundance adipose tissue [16]. The concentrations of Selenium (Se), Magnesium (Mg), Zinc (Zn), Copper (Cu) was significantly decrease in females with AN than in healthy females ($P<0.05$) as shown in Figure 1.

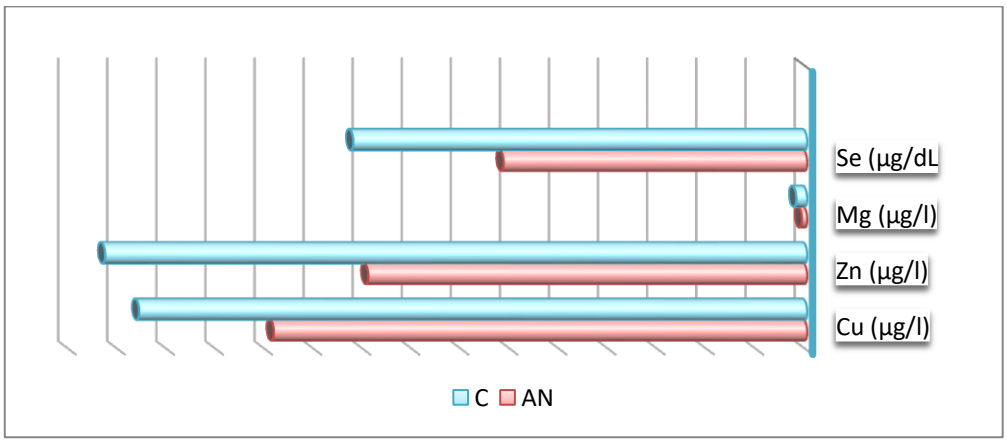

Fig 1: *Micronutrients (Trace elements) concentration in blood serum of female with anorexia nervosa and healthy female group. (*AN-Anorexia Nervosa group *C-Control group).*

The results above showed that the micronutrient (Se, Mg, Zn, Cu) concentrations were lower significantly in females to anorexia nervosa (AN) than in healthy females. The reason for this decreases is the reduced intake of micronutrients in patients with AN [17].

Selenium (Se) possess an principle role as cofactor of antioxidant systems and is as well share in myocardial function, defense against infection and regulation of the mood and anxiety [18].

In addition to, Se take actions synergistically with another antioxidant micronutrients like: ascorbic acid (Vitamin C), tocopherol (Vitamin E) and carotenoids [19].

Also there are decreasing concentrations of tocopherol (vitamin E) in females with anorexia nervosa (AN) as shown in results (Table 2) and thus the oxidative damage could contribute to anorexia nervosa (AN) disease [20].

Our study shown deficiency in Zn levels, this is in agreement with a previous studies in group of patients with AN, where authors suggested that the deficiency of Zn can be a causal factor leads to occurs or perpetuation anorexia nervosa [20,21].

IL-2, IL-10 and TGF- β serum concentrations were significantly lower in females with anorexia nervosa compared with the healthy females, while there was no significantly differences in the serum concentrations of IL-6 and IL-4**.**(Table 2) .

Rantala and his colleagues have pointed a psychoneuroimmunological model of disorders eating in which a dysregulation of the immune system, including some cytokines (an increase in TNF-a rates/a negative feedback of IL-2 and IL-4), could begin a cascade of actions and biological and chemical reactions that may result in either bulimia or anorexia nervosa [21]**.**

The results showed a limited role of these cytokines in anorexia, despite finding a significantly decrease in IL-2, IL-10 and TGF- β serum concentrations in females with anorexia nervosa (Table 2).

IL-2 this cytokine comes in the main from four main sources of synthesis: leukocytes (immune system), the central nervous system, the gastric mucous membrane and from the adipocytes (fat cells). It is the lowered concentrations of IL-2 may be largely associated to a lack of nutritional provides. Atrophy of gastric mucous membrane may also occur due to lengthy starvation and to the related undernutrition [22].

Conclusion

Body's adaptive response to starvation results in numerous endocrine abnormalities. The results shown the ghrelin correlates negatively with body mass index, as well the results of the study showed that there were an decrease in T3, T4, Vit.D3, Vit. E, IL-2, IL-10 and TGF-β concentration in the patients group. While there were no differences of TSH, IL-4 and IL-6 in patient group as compared with healthy one. While the results showed AN patients have trace elements decreases: Se are the most frequent, followed by Zn then Cu and Mg.

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قياس مستوى السيتوكينات والعناصر الغذائية الدقيقة وبعض هرمونات الغدة النخامية والغدة الدرقية لدى الاناث المصابات بفقدان الشهية العصبي في مستشفى الرمادي التعليمي

نور شاكر رزيج*

مديرية تربية الانبار الانبار، العراق

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معلومات المؤلف

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الخلاصة:

هو نوع من اضطرابات الطعام يتسم بانخفاض وزن الجسم بشكل غير طبيعي، والخوف الشديد من زيادة الوزن، ومحاولة الوصول الى المصالي النحيف او الحفاظ على الوزن. الهدف: الهدف من الدراسة هو تقييم هرمونات الغدة الدرقية وهرمون الغدة النخامية، ودور الإنترلوكينات والمغذيات الدقيقة في الإناث المصابات بفقدان الشهية العصبي مقارنة بالإناث الأصحاء. شاركت ثلاثون امرأة مصابة بفقدان الشهية العصبي في الدراسة وكانت المجموعة الضابطة تتكون من ثلاثين أنثى سليمة لا يعانين من اضطرابات الأكل وأي اضطرابات مناعية. أجريت الدراسة في مستشفى الرمادي التعليمي في محافظة الأنبار، العراق من يناير إلى يونيو 2024. القياسات الأنثروبومترية (مؤشر كتلة الجسم، الوزن، مرتفع)، تركيز هرمون الغدة الدرقية (T3 و T4)، هرمون الغدة النخامية (TSH)، العناصر الغذائية الدقيقة [العناصر النزرة (Se، Mg، Zn، cu) والفيتامينات (E و D3)]، والإنترلوكينات (IL-2، IL-4، IL-6، IL-10، TGF- β). كان هناك انخفاض كبير (قيمة P ≤ 0.05) في تركيزات T3 و T4 و Vit. D3 و Vit. E و IL-2 و IL-10 و TGF- β في الإناث المصابات بـ AN مقارنة بالإناث الأصحاء. بينما لم تكن هناك فروق معنوية (قيمة P ≤ 0.05) في مستويات TSH وIL-4 وIL-6 لدى الإناث المصابات بفقدان الشهية العصبي مقارنة بالإناث الأصحاء. الاستنتاج: يشير انخفاض تركيز T3 في المصل لدى الإناث المصابات بفقدان الشهية العصبي إلى انخفاض التحويل الطرفي لـ T4 إلى T3 أثناء المجاعة المزمنة..