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A biochemical study of angiotensin II, electrolytes and vitamin D in obese patients

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***Abstract*---**Background: Obesity is one of the most prevalent health issues impacting kids and teens in both industrialized and developing countries, yet it is a complex illness brought on by both inherited and environmental factors, Obesity develops as a result of a combination of low energy expenditure (such as exercise) and excessive calorie intake. Obesity-related problems such hypertension, metabolic disease, cardiovascular disease, and kidney disease are linked by Ang II and electrolytes. This study was designed to evaluate the alteration of Angiotensin II, electrolytes, Vit D in obese individuals. Methods: 88 persons with ages range between (20-55) years. Angiotensin II was determined by ELISA method. While serum Electrolytes estimated by GE300 Electrolyte analyzer, and Vitamin D determined by High pressure liquid chromatography (HPLC). Results: The findings of the present research reveals a significant increase in Ang II concentration ($p<0.001$) and decrease in Vit D ($p<0.003$), iCa ($p<0.000$) level in obese patients when compared with a control group. The results of linear regression analysis show positive correlations ($r = 0.37$, $p < 0.01$) of Ang II concentration with Sodium in obese group, and the same analysis show negative correlations ($r =-0.49$, $p < 0.001$) of BMI with Vit D in obese group.

***Keyword*---**biochemical study, angiotensin II, electrolytes, vitamin D

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Introduction

One of the really prevalent medical proplems affecting kids and teens in developing and industrialized nations [1],but it is a multifaceted conditions caused by both inherited and environmental factors. Obesity develops as a result of a combination of low energy expenditure (such as exercise) and a high calorie intake. This causes an excessive buildup of fat in the adipose tissue, which expands to handle the increased calorie intake and undergoes significant structural and cellular alterations[2,3].An persons wight (in Kg)devided by the square of their hight delivers the body mass index (BMI),a widly accepted indicator of obesity,it is defined as having BMI > 30[4,5].

The adipose tissue functions as endocrine organ ,creating avarity of chemicals that are necessary for controlling intake of food, calorie use and anumber of metabolic activities . Angiotensin II element of the RAAS that is also found in adipocytes, has a beneficial effect on adipocyte development and regulates adiposity due to its lipogenic properties and induces vascular smooth muscle contraction and hypertrophy, sympathetic nerve activation and release of adrenomedullary hormones, secretion of aldosterone, pituitary hormones release, salt and water conservation through its effects on kidney hemodynamics and tubular reabsorption [6– 8] .

Electrolytes are electrically charged substances that play a key role in acid-base balance, blood coagulation, and bodily fluid and muscle contractions in physiology. There are several common electrolytes found in the body, each with a distinct and significant function, although most are involved in maintaining the fluid balance between both the inside and outside environments in some way. This equilibrium is vital for concerns like as muscular function,hydration, ph concentration, nerve impulses,.Sodium, potassium, Magnesium, Calcium, and Chloride are the primary electrolytes in the body. Intracellular potassium and sodium activities have attracted attention in individuals who are obese since their deregulations increase the risk of hypertension [9]in this study we will see the changes of AngII concentration and the effect of Ang II on electrolytes (sodium, potassium, chloride, and calcium) as well as alteration AngII and electrolytes and relationship with Vit D in obese patient.

Materials and Techniques

Study Subjects

The study was conducted on 88 participants with age ranged between (20-55) years. Obese patients from who referred to Imam Ali hospital, Baghdad, Iraq. Including 44 obese individuals (BMI $\geq$30 Kg/m^2) and 44 normal individuals with (BMI $\leq$ 30 Kg/m^2). Any diagnosis of type1 or type2 of(D.M), Chronic hypertension, liver disease, kidney disease, CVD, cancer were among exclusion criteria. The study was approved by Ethics commission of the biochemistry department at the same collage Babylon new medical college (University of Babylon, Iraq) and the acceptance of the imam Ali hospital, scientific committee in al-Sadder city in the province of Baghdad.

Anthropometric and Clinical Features

All individuals anthropometric indexes such as, WC, BMI, WHR, were measured. To assess the individuals level of fatness,BMI which calculated as (weight in Kg /hight ic crest and the lowest rib were used to determine the WC.Additionally ,hip was measured at tha largest buttock circumference.

Biochemical Tests and Lab Measurements

(5 ml) of blood samples were aspirated without tourniquet from obese patients and control by using disposable syringe .Blood push slowly in gel tube and let to clot at room temperature for (10-15)min ,then centrifuged at 3000 rpm for 10 minutes .The serum was obtained put in eppendrof tubes (labeled) with number of sample then stored at -20 °C until the time of next experiments.

Estimation and performance of serum electrolytes (Sodium, Potassium, Chloride, ionized Calcium)

Tests are conducted using serum samples by auto analyzer Electrolytes (GE300). This method is dependent upon the ion selective electrode (ISE) principle: An ion-selective electrode (ISE) is an indicator electrode that responds (produces a potential) when it is placed in a solution containing a certainion. There is now large variety of ISE available which selectively respond to particular cat ions and anions, and certain gases; pH electrodes are by far the best known. The ion-sensing part consists of a membrane (which may be plastic, glass or an ionic crystal) which has sites capable of adsorbing the analyze ion. On other side of the membrane is a solution containing the ion of interest: one of these is the test solution, the other is a standard solution within the electrode itself. Inside the electrode body there is an electrical connection aelectrode – to monitor the response from the membrane. The relationship of the tested ion concentration and electrodes electric potential was described in equation

$$E=Eo+(RT/uF)*Ln(ax)$$

Where :
E: Electric potential of ion selective electrode during test
Eo: Standard electric potential of ion selective electrode
R:Gas constant (8.314/ j.mol(
T:absolute temperature (t+273 °C)
F:Faraday constant (96487 °C /mol)
ax: Tested ions concentration in solution.

Estimation and Performance Serum Angiotensin II

AngII measured by ELISA The quantitative ELISA (Sandwich technique) is used in this experiment The kit's Microtiter strip wells have been coated with an A monoclonal antibody specific for human Ang ll. The samples and standards are placed in the wells labeling and human Ang ll binds to the immobilized antibody. After eliminating unbound compounds with a wash phase, an HRP-conjugated anti-human Ang ll antibody is added, which binds to human Ang ll caught by the

During a wash step after incubation, unbound HRP-conjugated anti-human Ang ll antibody is removed. The wells are next filled with a chromogenic substrate solution, and color develops in proportion to the amount of Ang ll bound in the first phase. The reaction is completed when a colorful product is generated and a stop solution is added. At (450 nm), the color strength is determined spectrophotometrical.

Estimation and Performance Serum Vitamin D:

Vit D measured by HPLC

Sample preparation

400 microns were taken from the serum and treated with 400 microns of acetonitrile and placed in vortexes for 10 seconds for mixing, and then the sample was placed in Centrifuged for 10 minutes /10,000rpm at 25 °C for separation .The upper layer (supernatant) used for Vit D detection, and placed in the auto sampler unit of the HPLC. The extract was stable for at least 2 days at room temperature.

Mobile phase preparation

In the sonicator poll solvents of acetonitrile and methanol mixed in container by (9:1 ratio) as mobile phase with isocratic mode. The mobile phase was delivered with flow rate 1mL/minute

Preparation and Analysis of Slandered Solution

Stocks and standards solution of Cholecalciferol (vitamin D3): Stock solution was prepared by dissolving 1mg of cholecalciferol standard in 10 ml of methanol the intentional concentration (100 ppm). Serial standard solutions were prepared by using 10, 20, 30, and 40 (ppm). The stock and standard solutions were stored in brown flask with screw cap to avoid exposure to light and air at –4°C in refrigerator.

Statistical Analysis

(SPSS 24 version) and (Excel 2010) was utilized to analyze the results. For the purpose of comparing data between normal and obese patients values were given as (Means ±SE).The earson test was used for the correlation study ,ana a P-value of 0.05 or lower was regarded as statistically significant.

Results

The demographic and clinical characteristics of all participants are shown in Table (1).showing age groups per year, (BMI $Kg/M^{2)}$, waist to hip ratio. The (Means ±SE) and p-value for age . There is no significant ($p>0.05$) changes in age between control and patients. (Mean±SE) for the control is (33.75±1.564) and for patients (35.27±1.449). This age matching helps to eliminate variations in parameter results that may occur as a result of a large age difference and

According to the results of the data analysis(mean ± SE) for control and patient (0.82 ±0.007, 0.94 ±.001) respectively show significant difference ($p < 0.05$) in waist to hip ratio.

Table 1
Demographic & Biochemical characters of all participants in accurent study

Variable	Study groups	No.	Means ±SE	P- value
Age (years)	Control	44	33.75 ±1.564	0.477
	Ob. Patient	44	35.27 ±1.449	
BMI Kg/m^2	Control	44	23.02 ±0.25	0.000
	Ob. Patient	44	35.05 ±1.09	
Waist/ Hip ratio	Control	44	0.82 ±0.007	0.000
	Ob. Patient	44	0.94 ±.001	

BMI: Body Mass Index, SE:standared Error, Ob:Obese

Continuous variables descriptive (Mean± SE) and (p-value) showed in Table (2). The results of current study reveals a highly significant in Ang II concentration ($p < 0.001$)in obese patient compare with control group,and also significant decrease in Vit D ,iCa ($p < 0.003$,$p<0.000$)respectively in obese patients when compared with the control group.

Table 2
Means ±SE of Variables in control and Obese Group

Variable	Groups	NO.	Mean ± SE	P-value
AngII (ng/l)	Control	44	31.09 ± 1.26	0.001
	Ob. patient	44	40.45 ±2.47	
Sodium (mmol/dl)	Control	44	316.8± 0.59	0.680
	Ob. patient	44	318.2± 1.35	
Potassium (mmol/dl)	Control	44	15.81± 0.16	0.287
	Ob. patient	44	15.49 ±0.24	
Chloride (mmol/dl)	Control	44	351.1± 1.15	0.464
	Ob. patient	44	352.8 ±1.87	
iCa (mmol/dl)	Control	44	4.499± 0.53	0.000
	Ob. patient	44	3.380± 0.11	
Vit- D (ng/ml)	Control	44	25.20 ±0.45	0.003
	Ob. patient	44	11.53± 0.46	

No:number, iCa:ionized calcium, Vit-D:vitamin D, SE:standared error.

The results of linear regression analysis show positive correlations ($r = 0.371$, $p < 0.013$) of Ang II concentration with Sodium in obese group as show in figure (3) and negative correlations ($r = 0.523$, $p < 0.000$,$r =-0.49$, $p<0.001$)respectively of BMI with Sodium and Vit D in obese group as show in figure (6,4).Also negative correlation ($r =-0.99$, $P<0.001$)of AngII and Vit D in obese group.

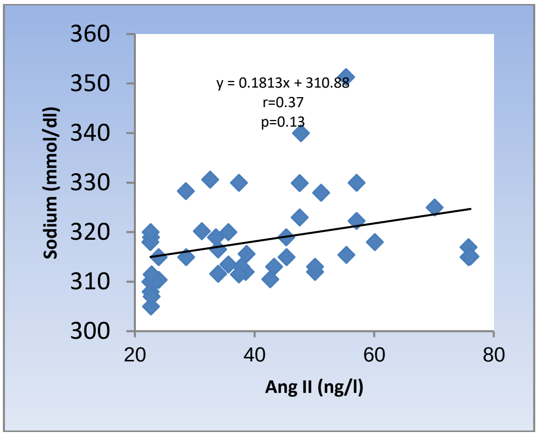

Figure 3. Correlation Between Angiotensin II and Sodium Group

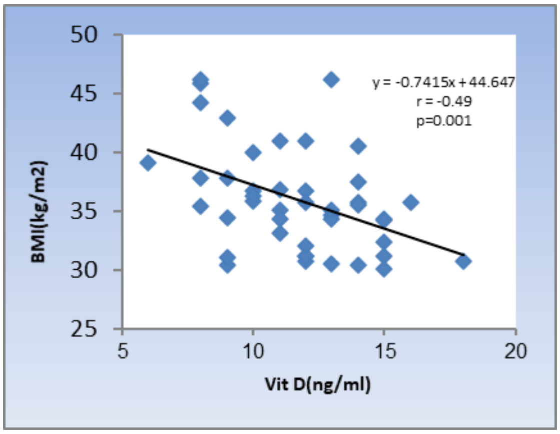

Figure 4. Correlation Between BMI and Vitamin D in Obese in Obese Group.

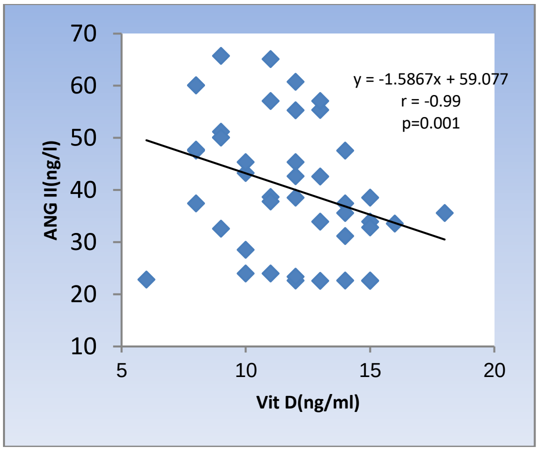

Figure 5. Correlation between Angiotensin II and Vitamin D

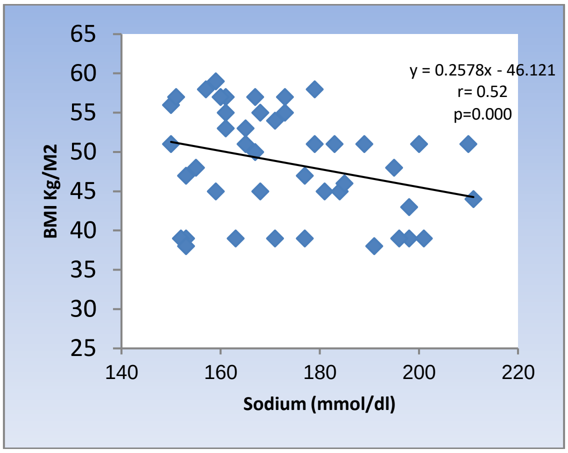

Figure 6. Correlation Between BMI and Sodium in Obese Group.

Discussion

In this study, the levels of Ang II in the obese and control groups were examined. In comparison to the control group, the obese group's Ang II levels were considerably higher. The results of current study support previous research that suggests Increased local production of Ang II in adipose tissue of human and animal [10] According to human research, rising obesity was associated with higher levels of plasma renin activity, angiotensinogen, Ang-II, and aldosterone. As a result, Acute stimulation with ANG II modulate salt/water homeostasis and vasoconstriction, modulating blood pressure, while prolong stimulation encourages the hyperplasia and hypertrophy of vascular smooth muscle cells (VSMCs) [11].In this study failed to show statistically any effect of AngII on electrolytes. On the other hand the results of the calculation for vit D level above in table(2)there was decreased in obese patient compared to control group.These results support previous research that suggests decrease Vit D in obese patients[12] .

Although the exact mechanism causing low serum calcidiol levels in obese people is unknown There are four processes that have been proposed in the literature to explain why people with obesity have low vitamin D levels: (1) Obese people get less sun exposure than lean people; (2)Vitamin D is stored in adipose tissue (3) negative feedback from an higher 1,25 (OH)D concentration in obese people reduces 25 (OH)D concentrations (4) lower 25(OH)D concentration is simply due to volumetric dilution[13]. Adipose tissue sequestration. (Wortsman et al.2000)[14]. Provided the first substantial, There is proof that vitamin D, a fat-soluble vitamin, can become trapped or buried in adipose tissue.

While iCa in studied groups showed decrease in obese patient as compare with control group. A stronger indicator of calcium status than total calcium is free calcium, which is a more significant metric. In this study, it was employed. The calcium-body weight effect's mechanism and amplitude are both unknown. Low calcium intake enhances dihydroxy vitamin D and PTH, and these calcitropic circulating chemicals, in turn, stimulate adipocyte calcium uptake, according to (Zemel et al.2000)[15]. Increased calcitriol production in response to low-calcium diets is also thought to contribute to Ca2+ influx in human adipocytes and obesity [16]. In the current study, significant negative correlation between vitD and BMI was found, this is in accordance with results of previous studies with(Brock et al.,) who reported that body mass index (BMI) >30 kg/is one of the major factors that affect vitamin D levels decreased in obesity [17].and also supporting these finding by(Khosravi, et al.:2018)founded that Weight, waist circumference, and BMI all fell dramatically after 6 weeks of taking 50,000 international units per week of Vitamin D supplementation, while serum Vitamin D increased[18].

The result of these study also showed positive correlation of BMI and Sodium in obese group. It's worth noting that our findings were in line with those of other studies conducted in different populations. In Australian children, a higher incidence of obesity was linked to salt imbalance. Interestingly, a subsequent investigation of Hispanic and Latino Americans discovered an association between salt and obesity metrics like BMI that was unrelated to caloric intake. [19]. The result of current study express negative correlation between Ang II and Vit D in obese patient these result agreement with(Forman et al.2010) Vitamin D deficit and insufficiency were associated with increased plasma angiotensin II levels and a trend toward higher plasma renin activity [20].

Conclusions

The study concludes that obesity is associated with decrease of Vit D cause activation of RAAS and increase Ang II that lead to retention of sodium in obese patient.

Ethical Approval

The Iraqi Ministry of Environment, Health, Higher Education, and Scientific Research have all ethically approved the research by the research ethics committee.

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