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CORRELATIONS BETWEEN SERUM OSTEOCALCIN, LEPTIN & INSULIN SENSITIVITY INDEX IN OBESE & NON-OBESE PCOS WOMEN

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ABSTRACT

Both lean and obese patients with PCOS have been found to be at risk for insulin resistance and type 2 diabetes mellitus. The exciting evidence, of particular interest is whether osteocalcin level in the circulation is associated with glucose metabolism in humans. Additionally, an adipocyte -secreted hormone affecting energy metabolism, was also a major determinant of bone remodeling, namely leptin indirectly inhibits bone accrual through a central pathway comprising hypothalamus and central nervous system. This newly identified feedback loop between bone and energy metabolism is mediated by osteocalcin (OC). The present study is aimed to evaluate the correlations between serum leptin and osteocalcin levels in relation to glucose homeostasis with hormonal changes (LH, FSH, Prolactin, Testosterone) in obese and non-obese women with poly cystic ovary syndrome (PCOS). **Methods:** This study included fifty women with poly cystic ovaries syndrome (PCOS) and thirty four apparently healthy control women with regular menstruation (28±2days). The diagnosis of PCOS was based on the revised Rotterdam Criteria. Both of PCOS patients and controls were divided into sub-groups according to their BMI into: twenty-five obese (BMI ≥30) with (Mean ±SEM BMI = 35.934±0.746) and another twenty five non-obese poly cystic ovaries syndrome women (Mean ±SEM BMI =25.074±0.456). Whereas, the controls were divided as: seventeen obese (Mean ±SEM BMI= 37.140±1.470) and seventeen non-obese (Mean ±SEM BMI= 25.022±0.683) healthy control women with regular menstruation and age range (20-40 years) and BMI matching that of the patients groups. Venous blood samples were collected at 9:00 am after an overnight fasting between the 3rd and 5th days of a spontaneous bleeding episode

of the PCOS group and of a menstrual cycle of the controls for analysis. **Results:** Although, serum osteocalcin levels were not significantly different from their corresponding controls, in both obese and non-obese PCOS patients, levels were significantly elevated in non-obese patients as compared to obese patients (p= 0.006). serum leptin levels were significantly elevated in obese PCOS patients as compared to their corresponding control group, non-obese control and non-obese PCOS patients respectively(18.338±0.538, 17.266±0.718 and 17.173±0.549 ng/ml, p <0.05). Furthermore, serum insulin levels were elevated significantly in obese patients as compared to non-obese patients and non-obese controls (p < 0.0001, 0.0001, respectively). Even so, there were no significant different in serum insulin levels between controls groups. The leptin / BMI ratio was significantly lowered in obese PCOS patients as compared to their control group, non-obese control and non-obese PCOS patients respectively(p <0.05). The insulin /osteocalcin ratio was not significantly different from their corresponding controls, in both non-obese and obese PCOS patients. The Estimation of Insulin Sensitivity Index (Quantitative Insulin Sensitivity Check Index – QUICKI) values showed a highly significantly difference in obese PCOS women as compare to obese controls (p < 0.0001), but there was no significant difference between non-obese PCOS women and their control (0.329±0.003, 0.335±0.003, respectively).Significant correlations among the studied parameter indicate that QUIKI values with Leptin/BMI ratio to be negatively correlated (r= -0.398, p=0.049). While QUIKI values was positively correlated with Leptin/insulin ratio (r= 0.764, p<0.000) in Non-Obese PCOS Women. Furthermore, data analysis indicates that in Obese PCOS Women QUICKI values were positively correlated to Leptin/Insulin ratio (r = 0.758, p <0.0000). Therefore, further studies of osteocalcin are required to elucidate it's role in metabolism can provide the fundamental basis of therapeutic strategies for metabolic disorders associated with PCOS among other disorders linked to insulin resistance.

KEYWARDS: PCOS, Osteocalcin, Leptin, OUICKI.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is considered as the most common endocrine abnormality of reproductive-age women and affects about 5–12% of women worldwide.[1-3] In addition, it was estimated that >42% of women with PCOS in the United States are overweight or obese and have a high risk of developing type 2 diabetes (T2D), atherosclerosis and cardiovascular

events.[4-9] PCOS subjects also appear to have a high risk of suffering lipid metabolism disorders[6,10], non-alcoholic fatty liver disease[4,11] and bone metabolic disorders.[4,6]

Although the root causes of PCOS remain to be identified, a popular hypothesis considers androgen excess as the primary defect in PCOS[12,13] and that hyperinsulinemia in PCOS patients could be associated with hyperandrogenism.[4,6,14-16] The hyperinsulinemia may cause hyperandrogenism by inhibiting hepatic synthesis of SHBG and by binding insulin like growth factor -1 (IGF -1) receptors in the ovary leading to increased androgen production by thecal cells. In fibroblasts of 50% PCOS patients (PCOS-ser) there is decreased insulin dependent receptor autophosphorylation of tyrosine residue and increased constitutive receptor serine phosphorylation. These defects may cause insulin resistance in women with PCOS-ser.[17] PCOS is a complex disorder and various features of this disorder may have an influence on bone metabolism.[18] Many previous studies have suggested that a relatively high estrogen concentration, higher insulin concentration, hyperandrogenemia and obesity are crucial bone growth stimulating factors in women with PCOS.[18] Estrogens play a key role in the development and maintenance of the appropriate bone mass in women, by acting on osteoblasts, as well as on osteoclasts. The influence of androgens on bone mass has not been fully elucidated. The main mechanism of androgen action on bones is believed to be linked to the aromatization of androgens to estrogens in the ovaries and extra glandular tissues. Moreover, all bone forming cells have receptors for both androgens and estrogens with a predominance of androgen receptors on osteoblast cells.[18] Estrogens and androgens production is significantly altered in PCOS, which probably has great importance for bone metabolism in young women.

In fact, the skeleton has been considered an endocrine organ because of its capacity to secrete osteocalcin, a bone specific protein, which has been implicated in energy and glucose homeostasis.[19] Osteocalcin (OC) is a 49 amino acid peptide synthesized exclusively by the osteoblasts and stored in the bone mineral matrix as hydroxyapatite crystals. It was recognized as a marker of bone formation at the time of its discovery and biochemical characterization.[19] Recently, osteocalcin levels have been positively associated with insulin sensitivity in both animal and human studies and therefore an interplay between bone tissue and carbohydrate metabolism has been revealed.[20] The novel description of Osteocalcin as a metabolic marker raised the question of its potential implication in the pathogenesis of PCOS. In one previous study, significantly higher carboxylated OC (cOC) concentration was

reported in patients with PCOS compared to controls thus suggesting a potential relationship between PCOS status and dynamics of the OC γ-carboxylation process.[21] Moreover, cOC and OC displayed by PCOS patients in that study correlated with several PCOS endocrine and metabolic components.[21]

The adipocyte derived hormone leptin is recognized as one of the most important regulators of bone remodelling, which emerged evolutionarily with the vertebrate animals. Ducy and cols discovered that leptin exerts a negative control on bone accrual mass, as leptin-deficient mice have a high bone mass, even in the presence of hypogonadism. This leptin effect is exerted through a hypothalamic relay using two neural mediators, the sympathetic tone and CART ('cocaine amphetamine regulated transcript'), both acting on the osteoblast.[19]

The present study is aimed to evaluate the correlations between serum leptin and osteocalcin levels in relation to glucose homeostasis with hormonal changes (LH, FSH, Prolactin and Testosterone) in obese and non-obese women with poly cystic ovary syndrome (PCOS).

METHODS

Study population

This study was carried out at Kamal Al-Samarrai Hospital (Center for Infertility treatment and In Vitro Fertilization "IVF"), for the period from November/2015 to April/2016. The study was conducted with approval from the human research ethics committee of the ministry of health of Iraq. Informed consent forms were obtained from each participant before beginning the research. The study included **fifty** women with poly cystic ovaries syndrome (PCOS) and **thirty four** apparently healthy control women with regular menstruation (28±2days). The diagnosis of PCOS was based on the revised Rotterdam Criteria.[22] Patients suffering from Cushing's syndrome, thyroid dysfunctions, androgen- secreting tumor, enzyme deficiency (21- hydroxylase in particular), decreased ovarian reserve (primary ovarian insufficiency), or type 1 or type 2 diabetes and smoking and alcohol habits were excluded.

All subjects did not take medications such as oral contraceptives, metformin, corticosteroids or other drugs or vitamins involved in bone and carbohydrate metabolism, none of the study subjects had a history of fracture the past 6 months and none was taking any drug known to affect vitamin K status (warfarin, ketoconazole, etc.) Because ingestion of lettuce and broccoli affects vitamin K status and subsequently serum intact osteocalcin or Gla osteocalcin

levels might be influenced, or could interfere with glucose and lipid metabolisms during the recent 3 months before the study.

Both of PCOS patients and controls were divided into sub-groups according to their BMI into: twenty-five obese (BMI ≥30) with (Mean ±SEM BMI =35.934±0.746) and another twenty five non-obese poly cystic ovaries syndrome women (Mean ±SEM BMI = 25.074±0.456). Whereas, the controls were divided as: seventeen obese (Mean ±SEM BMI= 37.140±1.470) and seventeen non-obese (Mean ±SEM BMI= 25.022±0.683) healthy control women with regular menstruation and age range (20-40 years) and BMI matching that of the patient groups. Anthropometric measurements (body mass, height, hip and waist circumference) were measured while participants wearing lightweight clothing without shoes.

Venous blood samples were collected at 9:00 am after an overnight fasting between the 3rd and 5th days of a spontaneous bleeding episode of the PCOS group and of a menstrual cycle of the controls. After centrifugation to obtain serum, all serum samples were stored at -80°C until analysis. The data of metabolic and hormonal characteristics of the control and PCOS patients are shown in table- I.

(Table- 1) Summary of the studied anthropometric, sex hormones and metabolic characteristics of the control and PCOS patients

Variable	Control	PCOS	P- Value
Number	34	50	-
Age (years)	33.559±0.937	28.220±0.786*	.0001
Weight (Kg)	79.382±3.567	76.922±2.161	.534
Height (M)	1.596±0.009	1.590±0.007	.596
Lean body weight (Kg)	52.992±0.599	52.584±0.483	.596
Body mass index (kg/m*2)	31.081±1.323	30.504±0.888	.708
Waist (CM)	94.735±2.361	98.02±2.110	.310
Hip (CM)	116.588±2.494	110.46±1.691*	.038
Waist Hip Ratio (WHR)	0.811±0.007	0.885±0.009*	.0001
Duration of PCOS(years)	-	5.244±0.441	-
Family History	-	0.920±0.039	-
Signs & Symptoms	0.058±0.041	0.880±0.046*	.000
Ultrasound for PCOS & Enlargement ovaries	-VE	+VE	-
Number of Gestations	2.724±0.232	1.565±0.270*	.004
Number of Abortions	0.1724±0.071	1.109±0.229*	.002
Number of Parity	2.552±0.214	0.457±0.119*	.0001
S.FSH (mIU/ml)	6.721±0.420	4.846±0.240*	.0001
S.LH(mIU/ml)	2.708±0.183	5.121±0.484*	.0001

LH/FSH Ratio	**0.412±0.017**	**1.080±0.089***	**.0001**
S. Prolactin(ng/ml)	**15.028±1.050**	**22.449±1.516***	**.0001**
S. Total Testosterone(ng/ml)	**0.214±0.025**	**0.481±0.040***	**.0001**
S.TSH(nmol/l)	**1.770±0.163**	**2.166±0.178**	**.121**

- **The data are expressed as the numbers or mean ± standard error of mean (SEM).**

*** P < 0.05 is significantly different**

Assays

Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), LH/FSH, prolactin (PRL), total testosterone (TES), thyroid stimulating hormone (TSH) were tested by automated quantitative test (ELFA) (VIDAS® PC Autoanalyzer, Biomerieux, Italia).[23-26] Fasting blood glucose was evaluated according to the method of Barham and Trindoer (1972).[27]

Serum osteocalcin {Demeditec Diagnostics GmbH, Germany}, Serum leptin {Demeditec Diagnostics GmbH, Germany}; and Serum insulin {Demeditec Diagnostics GmbH, Germany}; were determined with enzyme-linked immunosorbent assay (ELISA) according to manufacturers' instructions.[28-32]

Estimation of insulin sensitivity index was performed by using the quantitative insulin sensitivity check index (QUICKI) which is calculated from fasting glucose (FG) and fasting insulin (FI) levels using the following formula.[33]

QUICKI= 1/ {log (FI (µIU/ml) + log (FG (mg/dl)}

Statistical Analysis

Normality of the distribution of the variables was confirmed by the Shapiro-Wilk test. The results were expressed as mean ± standard error of mean (SEM) or percent changes. Student t-test and analysis of variance (ANOVA) were used to examine the degree of significance. P-values less than 0.05 were considered significant. Pearson's correlation analysis was employed to study the relationship between serum irisin levels and the studied hormonal and metabolic parameters. The statistical analysis was performed using **SPSS, version 22.**

RESULTS

Serum osteocalcin levels were not significantly different from their corresponding controls, in both obese and non-obese PCOS patients(Figure-1). However, serum osteocalcin levels were significantly elevated in non-obese patients as compared to obese patients (p= 0.006).

Meanwhile, non-obese controls were not significantly different from the obese controls, nor from the obese patients. As presented in Figure -2, serum leptin levels were significantly elevated in obese PCOS patients as compared to their corresponding control group, non-obese control and non-obese PCOS patients respectively(18.338±0.538, 17.266±0.718 and 17.173±0.549 ng/ml, p <0.05). But, serum leptin levels were not significantly different in non-obese women groups, both the PCOS and controls. Serum insulin levels were highly significantly elevated in obese PCOS women as compare to their corresponding controls (p < 0.0001), but there was no significant difference between non-obese PCOS women and their control (13.466±1.017, 10.719±0.440 **(µIU/ml),** respectively). Furthermore, serum insulin levels were elevated significantly in obese patients as compared to non-obese patients and non-obese controls (p < 0.0001, 0.0001, respectively). Even so, there were no significant different in serum insulin levels between controls groups. Table-2 show that, The leptin / BMI ratio was significantly lowered in obese PCOS patients as compared to their control group, non-obese control and non-obese PCOS patients respectively(p <0.05).Whereas, Leptin / BMI ratio was not significantly different among the non-obese women (p = 0.978). There was a highly significantly difference between obese control as compared with non-obese control and non-obese patients (p<0.01).

The insulin /osteocalcin ratio was not significantly different from their corresponding controls, in both non-obese and obese PCOS patients. Furthermore, there was no significant variation across studied groups.

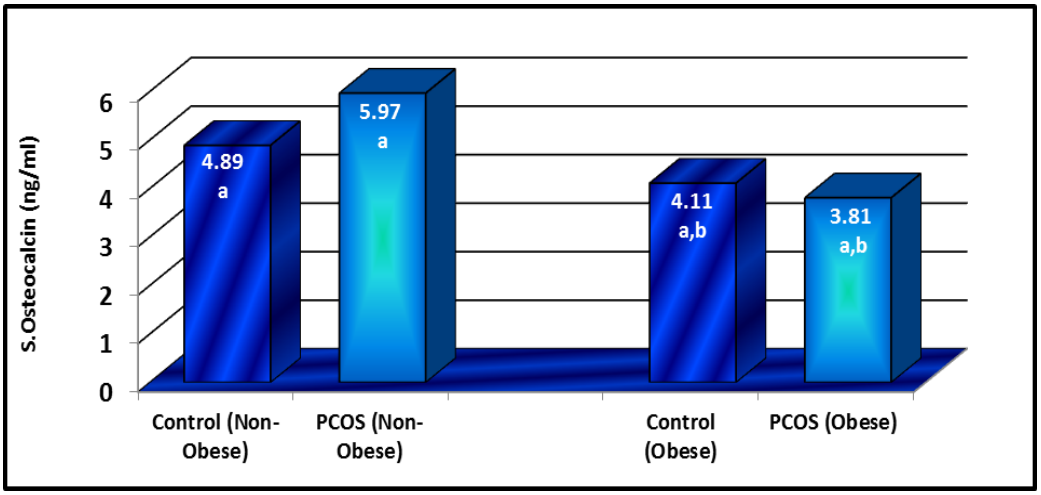
The groups mean values indicate that leptin / insulin ratio was not significant vary in PCOS groups from their corresponding control in non-obese (p = 0.094) but in obese PCOS patients there was significant difference as compared to its controls (p = 0.01). Meanwhile, leptin / insulin ratio was not significantly different between patients groups (non-obese & obese, p=0.133), nor between the studied controls (p=0.705).

The serum leptin / osteocalcin ratio was not significantly different in patients groups from their corresponding controls, in both non-obese and obese PCOS patients. Also, there were no significant variations among the studied groups.

(Table -2) Serum Hormone Measurements Among Various Groups

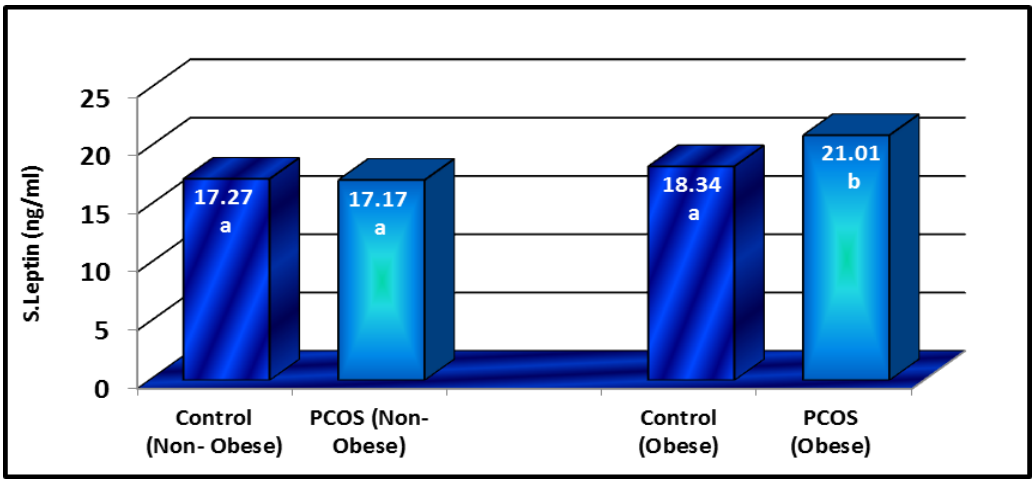
Groups (No.) / Variables	Control (Non-Obese) (17)	PCOS (Non-Obese) (25)	Control (Obese) (17)	PCOS (Obese) (25)
Osteocalcin (ng/ml)	4.891±0.577a	5.966±0.729a	4.110±0.471a,b	3.808±0.458a,b
Leptin(ng/ml)	17.266±0.718[a]	17.173±0.549[a]	18.338±0.538[a]	21.005±1.196[b]
Insulin(µIU/ml)	10.719±0.440[a]	13.466±1.017[a]	12.651±0.860[a]	22.002±2.430[b]
Leptin /BMI	0.689±0.024[a]	0.690±0.025[a]	0.506±0.024[b]	0.588±0.033[c]
Insulin/ osteocalcin	2.995±0.605a	6.981±3.613a	5.032±1.410a	8.835±1.684a
Leptin /Osteocalcin	5.088±1.082a	10.849±6.486a	7.362±2.167a	9.027±1.839a
Leptin / Insulin	1.676±0.121ab	1.392±0.084a	1.606±0.158a	1.163±0.114a,b

Data are presented as Mean±SEM, values with different letters are significantly different.



Data are presented as Mean; values with different letters are significantly different.

(Figure – 1): Serum osteocalcin Levels (ng/ml)



Data are presented as Mean; values with different letters are significantly different.

(Figure -2): Serum Leptin Levels (ng/ml)

As presented in table- 3, serum glucose levels were significantly higher in obese PCOS women as compared to their controls (p = 0.003), but there was no significant difference between non-obese PCOS women and their controls (4.832±0.100, 5.179±0.195 mmol/l, respectively). Furthermore, serum glucose levels were elevated significantly in obese patients as compared to non-obese patients and non-obese controls (p < 0.000, 0.001, respectively). Even so, there were no significant difference in serum glucose levels between control groups.

The Estimation of Insulin Sensitivity Index (Quantitative Insulin Sensitivity Check Index – QUICKI) values showed a highly significantly difference in obese PCOS women as compare to their controls (p < 0.0001), but there was no significant difference between non-obese PCOS women and their control (0.329±0.003, 0.335±0.003, respectively). Furthermore, QUICKI were elevated significantly in obese patients as compared to non-obese patients and non-obese controls (p < 0.0001, 0.0001, respectively). However, there were no significant difference in QUICKI among the control groups.

(Table -3) Summary of The Studied Glycaemic Indices Among Various Groups

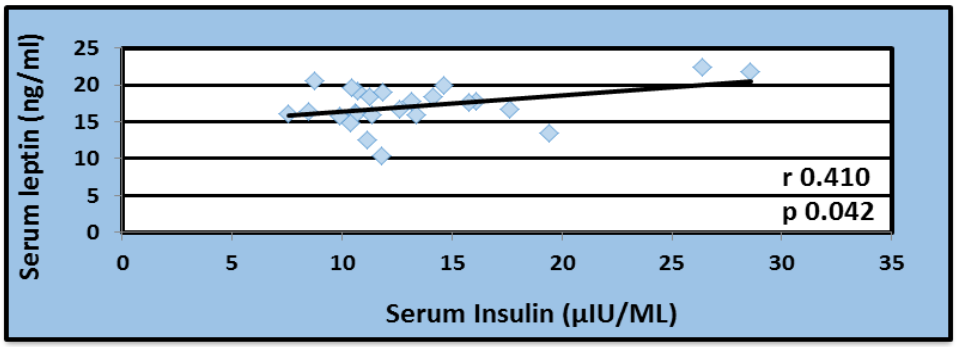
Groups (No.) / Variables	Control (Non-Obese) (17)	PCOS (Non-Obese) (25)	Control (Obese) (17)	PCOS (Obese) (25)
Serum Fasting Glucose (mmol/l)	5.179±0.195[a]	4.832±0.100[a]	5.232±0.161[a]	6.057±0.236[b]
QUICKI	0.335±0.003[a]	0.329±0.003[a]	0.328±0.005[a]	0.302±0.004[b]

Data are presented as Mean±SEM, values with different letters are significantly different.

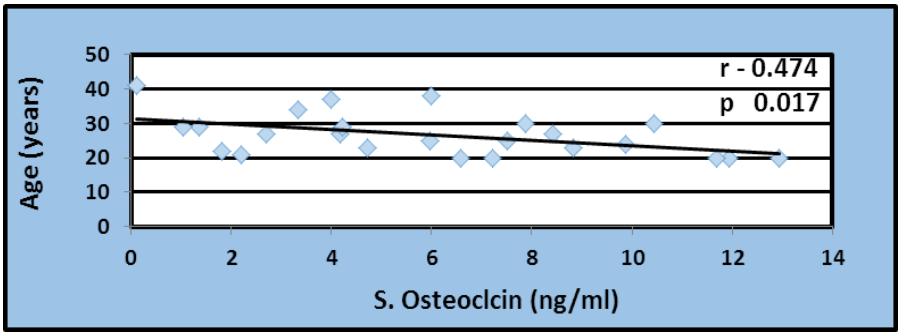
Correlation Studies

Correlation values that presented in this section were calculated using Pearson's correlation coefficient, considering P values < 0.05 as the level of significance are summarized as follows:

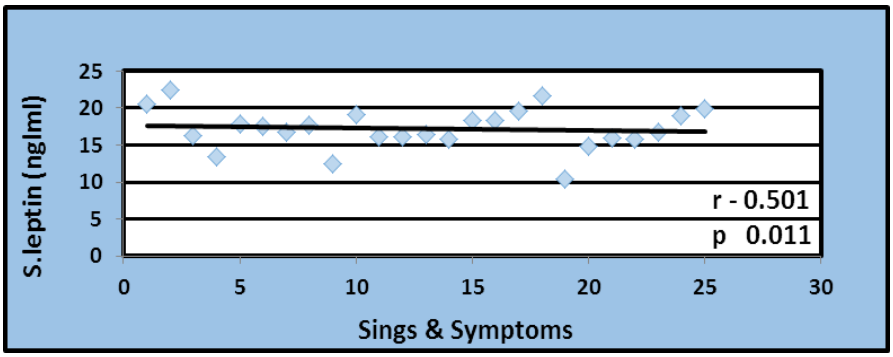
1. Pearson's Correlation In Non-Obese PCOS Women



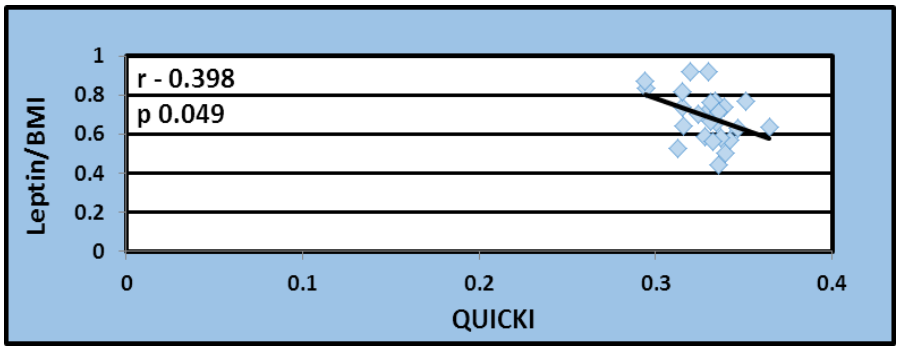
(Figure -3): Correlation between Serum Insulin and Serum Leptin (r = 0.410, p = 0.042) in Non-Obese PCOS Women



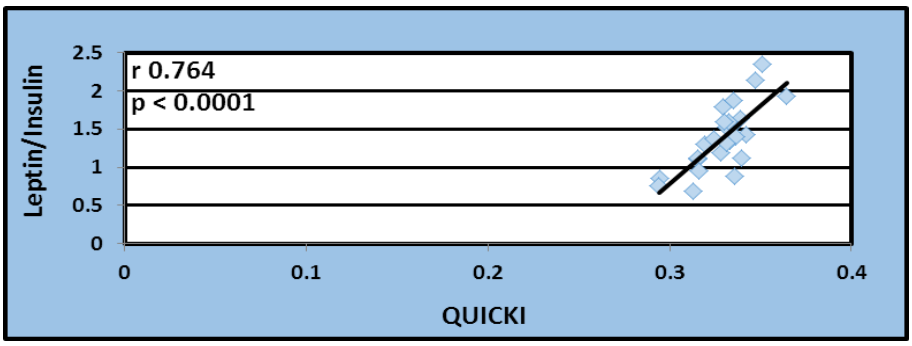
(Figure -4): Correlation between Serum Osteocalcin and Age (r = - 0.474, p = 0.017) in Non-Obese PCOS Women



(Figure -5): Correlation between Signs & Symptoms and Serum Leptin (r = - 0.501, p = 0.011)

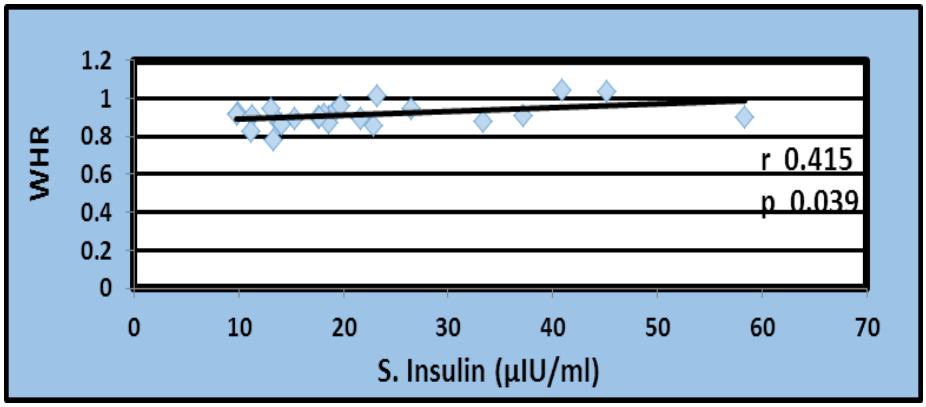


(Figure -6): Correlation QUICKI values and Leptin/BMI ratio (r= - 0.398, p=0.049) in Non-Obese PCOS Women.

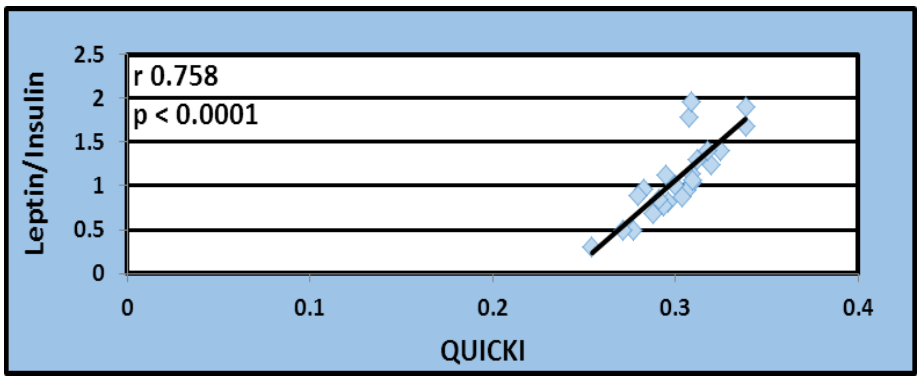


(Figure -7): Correlation QUICKI values and Leptin/insulin ratio (r= 0.764, p < 0.0001) in Non-Obese PCOS Women.

2. Pearson's Correlation In Obese PCOS Women



(Figure -8): Correlation between Serum Insulin and WHR (r = 0.415, p = 0.039) in Obese PCOS Women



(Figure -9): Correlation between QUICKI values and Leptin/Insulin ratio (r = 0.758, p <0.0001) in Obese PCOS Women.

DISCUSSION

Although, both lean and obese patients with PCOS have been found to be at risk for impaired glucose tolerance due to insulin resistance (IR) and later type 2 diabetes mellitus development. However, non-obese women exhibit lower insulinemia and IR.[34] Hyperandrogenism and polycystic ovaries have been described in non-obese patients with insulinomas.[35] Data in table-3 presents that fasting glucose levels were elevated in obese PCOS among all other studied groups. This was confirmed by the positive correlation between serum insulin levels with WHR in obese PCOS women referring to the associated insulin resistance status (figure-8). Insulin resistance has a central role in pathogenesis of several metabolic diseases, as it not only plays a major role in the development of type 2 diabetes mellitus (T2D) but is also a feature of a number of related disorders, including obesity, glucose intolerance, dyslipidemia and hypertension, clustering in the so-called metabolic syndrome[36], atherosclerosis and cardiovascular diseases (CVD).[37] Although,

insulin resistance is one of the most important metabolic dysfunction in PCOS, it is not considered as a parameter for diagnosis. The accompanied hyperinsulinaemia may play an important role in the aetiology of PCOS, as weight loss and insulin sensitizing drugs improve the clinical manifestations such as hyperandrogenaemia and restoring ovulation.[38]

There are exciting evidence of particular interest about whether osteocalcin level in the circulation is associated with glucose metabolism. As previously reported that serum osteocalcin was inversely associated with glucose and visceral fat mass and positively with serum adiponectin level, parameters of insulin secretion and its sensitivity in patients with type 2 diabetes. Kindblom et al. showed that osteocalcin level was inversely related to plasma glucose level and fat mass in elderly non-diabetic persons. Fernandez-Real et al. reported that serum osteocalcin level was associated with insulin sensitivity in non-diabetes subjects.[39] Similarly our finding that serum osteocalcin levels tends to be inversely related to BMI, than to be affected by PCOS state (figure-1). Such findings should be considered hypothesis generating and they need to be replicated in human studies designed to test the hypothesis that osteocalcin affects metabolism. Since, serum osteocalcin was reported to be inversely associated with markers of metabolic dysfunction including fasting hyperglycemia, insulin resistance and systemic inflammation as well as measures of adiposity, suggesting that osteocalcin may contribute to the dysmetabolic phenotype in humans.[40]

This relationship between fat and bone, initially thought to be unilateral, is now considered to be bilateral or reciprocal after the discovery of the role of bone in glucose and fat metabolism. This newly identified feedback loop between bone and energy metabolism is mediated by osteocalcin, an osteoblast-produced protein and has advanced academic progress in osteology and endocrinology. Furthermore, studies of osteocalcin have provided the fundamental basis of therapeutic strategies for metabolic disorders. In this aspect, we discuss the feedback loop between bones and fat and new discoveries related to novel metabolic roles of osteocalcin.[41]

There is feedback between glucose and bone metabolism. Adiponectin, a protein secreted by the adipose tissue with insulin-sensitizing and anti-atherosclerotic properties, has emerged as an element in the regulation of bone mass. Recent studies have closed this feedback by revealing a direct regulation of metabolic pathways by the skeleton through osteocalcin production.[42] As total osteocalcin was shown to be associated with lower fasting glucose and Hb_{A1C} in patients with diabetes and in older nondiabetics. Also osteocalcin was negatively

correlated with fasting glucose fasting insulin and HOMA-IR, in men and postmenopausal women. Hence, osteocalcin to be considered as an independent negative predictor of glucose level.[43]

Recent studies have revealed a new neuroendocrine circuit linking bone and energy homeostasis. Leptin, an adipocyte-derived hormone, has been previously shown to inhibit bone formation by acting on osteoblasts via central neural pathways, thereby closing the feedback loop between bone and peripheral organs involved in energy homeostasis. In elegant animal experiments, Lee et al. showed that mice lacking the gene that encodes osteocalcin (osteocalcin −/−), an osteoblast-specific secreting molecule, have an abnormal amount of visceral fat and exhibit glucose intolerance, insulin resistance and impaired insulin secretion compared with wild-type mice. In ex vivo studies, when pancreatic β-islets isolated from wild-type mice were co cultured with wild-type osteoblasts or in the presence of supernatants from cultured osteoblasts, insulin secretion increased, suggesting the presence of an osteoblast-derived circulating factor that regulates β-cell function. Furthermore, co-culture of wild-type osteoblasts with adipocytes increased adiponectin expression and action. In mice, administration of recombinant osteocalcin significantly decreased glycemia and increased insulin secretion. Taken together, these data support a regulatory role of the skeleton on glucose and energy homeostasis, which appears to be mediated by osteocalcin.[44]

Our data presented that, serum leptin levels tend to be higher in obese PCOS women rather than in non-obese PCOS women, but not in healthy controls (figure-2). Indicating the role of adipose tissue related cytokines, including leptin, in the pathogenesis of PCOS as presented by obesity as a major risk factor for PCOS development. Furthermore, serum insulin levels were significantly higher in obese patients (table-2). Other researchers have consistently shown that leptin regulates bone formation through a central pathway comprising the hypothalamus and central nervous system. Leptin is known to initiate intracellular signals within the hypothalamus through its binding to Ob-Rb, the leptin receptor isoform present in hypothalamic nuclei. At least two different central hypothalamic pathways, through which leptin influences bone formation in an antagonistic manner, have been identified. The first, anti-osteogenic, influence of leptin involves up-regulation of receptor activators of NF-kappa B ligand (RANKL), an osteoclast differentiation factor, through sympathetic signalling via β-adrenergic receptors, the only adrenergic receptor present on osteoblasts. Sympathetic signalling on β-adrenergic receptors induces phosphorylation of activating transcription

factor 4 (ATF4), a cell-specific cAMP response element binding (CREB)-related transcription factor that is essential for osteoblast differentiation and function. The second, more recently defined, osteogenic influence of leptin involves modulation of cocaine and amphetamine-regulated transcript (CART), a hypothalamic neuropeptide encoded by the *CARTPT* gene whose expression is increased by leptin. Down-regulation of RANKL expression by CART is unfavourable to bone resorption and inhibits osteoclast differentiation.[41]

Considering Leptin/BMI ratio showed reduced values among obese PCOS women compared to controls, whether obese or non, relating leptin levels to disorders associated with PCOS through body weight modification (table-2). As clearly presented by QUICKI values (table-3). Also the positive correlation between QUICKI and Leptin /insulin ratios in women with PCOS, both non-obese and obese subjects (figures-7 & -9, respectively). Meanwhile, Insulin/osteocalcin ratio showed no significant alteration among studied groups (table-2), which could be related to the number of population included in this study that to be considered before excluding the existence of variations related to PCOS or to BMI.

Although serum osteocalcin levels presents a negative correlation with age in non-obese PCOS (figure-4). An increased release of undercarboxylated osteocalcin into circulation enables it to act as a circulating hormone to stimulate insulin production and secretion by pancreatic β-cells and adiponectin by adipocytes. Insulin sensitivity increases, lipolysis and fat accumulation decreases while energy expenditure increases.[43]

Studies in animal models showed that undercarboxylated osteocalcin targets β cells in the pancreas to directly regulate insulin synthesis and regulates insulin sensitivity through adiponectin. And experiments in mice showed that undercarboxylated osteocalcin may be a potential therapy for diabetic patients. One of the regulators of osteocalcin is OST-PTP, which promotes the carboxylation of osteocalcin and reduces the concentration of undercarboxylated osteocalcin or the ratio of undercarboxylated osteocalcin / osteocalcin.[45]

Considering the interaction between bone and adipose tissue and there effects on insulin and hence glucose homeostasis, it was found that osteocalcin inactivation in mice results in increased visceral fat with carbohydrate intolerance, low insulin levels, changes in insulin response to glucose and a decreased mass of pancreatic beta cells.[46] Osteocalcin, especially undercarboxylated form of osteocalcin, function to regulate glucose metabolism by direct

stimulation of insulin secretion in pancreas and indirectly via increasing glucagon-like peptide-1 secretion in small intestine as well as adiponectin secretion in adipose tissue and enhances insulin sensitivity in muscle. Therefore, osteocalcin may be an important factor linking between bone and glucose homeostasis.[44] The Leptin/Osteocalcin ratio (table-2) showed no significant alterations among studied groups which might need larger populations to be studied in order to get a more clear picture considering their link in PCOS patients. Since there is a number of published studies have demonstrated that differentiation and functions of these bone-specific cells are regulated by leptin, that regulates energy intake and expenditure. Numerous other studies have examined the relationship between serum leptin levels and bone anabolism and have proposed a bimodal response in which moderate increases in leptin stimulate bone formation whereas higher levels actually inhibit it. This bimodal response is further complicated by the finding that adipocytes in both the periphery and in the bone marrow secrete leptin, which may induce apoptosis of bone marrow stromal cells and favours bone resorption at high local concentrations.[41]

As a conclusion, osteoclcin has emerged as an important contributor in glucose metabolism through considering bone as an endocrine organ to that affect the metabolic role of adipose tissue with their collective effects of bone and adipose tissue on insulin related metabolic pathways, considering specifically insulin resistance state associated with PCOS condition. Therefore, more investigations are needed to reveal the total functions of osteocalcin as a hormone in energy metabolism, that could open new promising future studies of osteocalcin to be the fundamental basis of therapeutic strategies for insulin resistance related metabolic disorders.

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