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CORRELATION BETWEEN SERUM LEVELS OF INSULIN-LIKE GROWTH FACTOR-1(IGF-1) WITH ESTROGEN AND FREE TESTOSTERONE LEVELS IN OBESE TYPE 2 DIABETIC WOMEN

[a]Shatha H. Ali, [a]Abdul-Rahim Ahmed Ali, & [b]Khalid Ibrahim Al-Luhaibi
[a]Department of clinical laboratory science, College of pharmacy/University of Baghdad, Baghdad, Iraq.
[b]The Specialized Al-Kindi Center for Endocrinology and Diabetes, Baghdad, Iraq.

ABSTRACT
There are several evidences that IGF-1 is a potent regulator of androgen production in mammalian theca cells, and estradiol synthesis by human granulosa and luteal cells. The association of obesity with some hormonal disorders in females could be attributed to altered metabolism in relation to changes in insulin like growth factor-1 (IGF-1) among others that might contribute to development of insulin resistance state. This study was aimed to study the correlations between serum levels of IGF-1 with female sex hormones (estrogen and free testosterone) in obese premenopausal women with type 2 diabetes mellitus (T2DM) compared to non-obese diabetics. Seventy one premenopausal T2DM women were studied, classified into two groups based on body mass index (BMI): Group D1 (≥30 kg/m^2, n=50) and Group D2 (<25 kg/m^2, n=21) in addition to thirty nine healthy premenopausal women as control and further classified into two groups depending on BMI: Group C1 (≥30 kg/m^2, n=20) and Group C2 (<25 kg/m^2, n=19). BMI and Waist: Hip ratios were calculated, in addition to estimation of glycemic indices [namely; fasting serum glucose (FSG), fasting serum insulin (FSI), HbA_{1C} %, quantitative insulin sensitivity check index (QUICKI), homeostatic model assessment of insulin resistance (HOMAIR)], serum IGF-1 and serum estradiol (E2) and free testosterone. Intra-group and inter-group correlation coefficients were calculated among these parameters. Data analysis showed that serum IGF-1 levels were lowered in obese diabetic as compared to non-obese diabetic patients. Similarly in non-diabetic obese women; the IGF-1 levels were lower than that of non-obese women. However, serum E2 levels were higher in obese diabetic than that of non-obese diabetic women, also higher than non-diabetic obese premenopausal women. While serum free testosterone levels expressed no significant differences among women participated in this study. Thus we could conclude that serum levels of IGF-1 and female E2 were markedly affected by obesity that might be attributed to insulin resistance as indicated by HOMA-IR values, but there was a mild, statistically non-significant correlation between IGF-1 and female hormones (estradiol and free testosterone) in obese type 2 diabetic premenopausal women.

KEY WORDS: Insulin-Like Growth Factor-1, Estrogen, Free Testosterone, Type 2 Diabetes Mellitus, Premenopausal Women.

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) or somatomedin C is a protein that is encoded by the IGF-1 gene [1], IGF-1that is a monomer of 70 amino acids (7,500 kDa), is involved in cellular growth, and it is a member of a family that also include insulin-like growth factor-2 (IGF-2) and pro-insulin[2]. IGF-1 has a similar molecular structure to insulin[3]. IGF-1 bind to its receptor, insulin-like growth factor -1 receptor (IGF-1-R), belongs to tyrosine kinase growth factor receptor family; it is structurally similar to, but distinct from, the insulin receptor (with which it shares a 70% homology). As expected, it cross-reacts with insulin and, vice versa, insulin receptor cross-react with IGF-1[4]. Circulating IGF-1 is produced primarily by the liver under GH control [5]. IGF-1 is an endocrine and autocrine /paracrine growth factor and is expressed in most cell types [6]. IGF-1 is a key regulator of cellular proliferation and differentiation[7], in conjunction with growth hormone (GH), insulin and sex steroids [8]. IGF-1, which considered as a principal marker of hepatic GH action, exerts a potent negative feedback to inhibit GH secretion[9].There is evidence that insulin-like growth factor-1 (IGF-1) is a potent regulator of estradiol synthesis by human granulosa and luteal cells [10]. Furthermore, IGF-1 is known to play a role in ovarian follicular development augmenting the action of FSH [11]. In vitro, IGF-1 acts synergistically with FSH, enhancing granulosa cell proliferation and stimulating aromatase enzyme activity[12]. Estradiol was found to cause a complementary augmentation of IGF-1 signaling by many ways, one of them, by increasing expression of IGF-1[13]. Both estrogen and androgen appear to be positively associated with abdominal obesity and the metabolic syndrome. The peripheral aromatization of androgens to estrogens in adipose tissue should be taken into consideration when interpreting the results of various clinical studies [14]. Aim of this study is to investigate the role of IGF-1 in obesity and/or type 2 diabetes mellitus in the metabolism of female sex hormones (estradiol and free testosterone) in premenopausal women. In addition to study the correlation of IGF-1 levels with the extent of insulin resistance as represented in this study by homeostasis model assessment of insulin resistance (HOMA-IR).

MATERIALS & METHODS

This study was carried out at the Specialized Al-Kindi Center for Endocrinology and Diabetes in Baghdad, Iraq for the period from December 2012 to May 2013. The study included 110 premenopausal women aging 20-45 years; 71 of them were type 2 diabetic and were grouped as: Group D1: composed of 50 obese women with BMI≥30 kg/m^2, and group D2: composed of 21 normal weight women (BMI<25 kg/m^2). In comparison to 39 healthy women as control, that were grouped into: Group C1: composed of 20 obese women (BMI≥30 kg/m^2) and group C2: composed of 19 normal weight women (BMI<25 kg/m^2) respectively. Subject's characteristics are summarized in table -1.

TABLE 1: Parameters included in the study among various groups

Groups / Parameters	Control non-diabetic women with BMI ≥30 kg/m^2 (C1) (N=20)	Type 2 diabetic women with BMI ≥30 kg/m^2 (D1)(N=50)	Control non-diabetic women with BMI<25 kg/m^2 (C2) (N=19)	Type 2 diabetic women with BMI<25 kg/m^2 (D2) (N=21)
Number	50	20	21	19
Age (yrs.)	35.42±6.16a	32.47±5.37a	33.8±9.92a	31.32±4.06a
BMI(kg/m^2)	35.73±4.96a	34.12±3.34a	22.64±2.25b	23.76±1.48b
HOMA-IR	5.92±4.02 b	10.51±7.48a	1.28±0.77c	8.80±7.49a
IGF-1 ng/ml	17.70±7.60 c	26.11±10.28 a	22.40±10.81 b	29.92±16.39 a
E2 pg/ml	28.69±9.26 b	47.25±43.88 b	81.67±89.87 a	37.93±36.47 b
F.T. pg/ml	1.45±1.01 a	1.81±1.27 a	1.31±1.27 a	1.57±1.66 a

Data are expressed as Mean ±SD. BMI: Body mass index. Yrs: Years, N: Number, DM: diabetes mellitus, HOMA-IR: Homeostasis model assessment of insulin resistance, IGF-1: Insulin-like growth factor, E2: Estradiol, F.T: Free testosterone.

Means with different small letters in the same row were significantly differed (P< 0.05).

Fasting blood specimens were collected and tested for: serum insulin-like growth factor-1 (IGF-1), serum glucose (FSG), serum insulin (FSI), glycated hemoglobin (HbA_{1C}), Homeostasis model assessment of insulin resistance (HOMA-IR), serum estradiol (E2),serum free testosterone. Biochemical hormonal investigations were done by utilizing ELISA technique. Statistical Package for Social Sciences version 21 (SPSS version 21) was used for data input and analysis. Continuous variables presented as mean ± standard deviation (SD). Pearson correlation coefficient was used to assess the correlation between variables. Findings with P value less than 0.05 were considered significant.

RESULT

Homeostasis model assessments of insulin resistance (HOMA-IR) values were significantly higher in obese diabetic premenopausal women as compared to values that estimated in obese non-diabetic premenopausal women. HOMA-IR mean also was higher in obese diabetic than that of non-obese diabetic premenopausal women, as summarized in table-1. But serum insulin-like growth factor (IGF-1) levels were slightly lower in obese diabetic than its levels in non-obese diabetic women (26.11±10.28) and (29.97±16.39) ng/ml, respectively. However, serum IGF-1 levels in obese diabetic women were significantly higher than that of non-diabetic obese women. The same was found in non-obese diabetic that showed higher IGF-1 levels than that of non-diabetic non-obese premenopausal women. Whereas, serum estradiol levels E2 were significantly lowered in diabetic women (both the obese & the non-obese) as compared to the normal body weight control women.

TABLE 2: Correlations between Serum IGF-1 and Different Parameters

Groups / Parameter	Type 2 diabetic women with BMI ≥30 kg/m^2 (D1)		Type 2 diabetic women with BMI<25 kg/m^2 (D2)	
	r	p	r	p
BMI kg/m^2	-0.15	0.29	0.13	0.56
W:H ratio	0.08	0.57	0.167	0.49
FSG	- 0.09	0.55	0.43	0.04
FSI	0.02	0.86	-0.16	0.46
HbA_{1C}	- 0.02	0.84	0.22	0.51
HOMA-IR	-0.002	0.992	-0.131	0.572
Estradiol pg/ml	-0.14	0.32	0.30	0.17
F.T. pg/ml	- 0.09	0.52	0.01	0.95

BMI: Body mass index, W: H: waist: hip ratio, HOMAIR: Homeostasis model assessment of insulin resistance, IGF-1: Insulin-like growth factor, E2: Estradiol, F.T: Free testosterone.

Statistically significant differences could not be found among the different group's means for serum free testosterone levels, despite higher values were observed in obese diabetic premenopausal women (1.81±1.27) pg/ml, in comparison to non-obese diabetic women (1.45±1.01) pg/ml. Also, non-diabetic obese women showed higher free testosterone level than levels estimated in non-diabetic non-obese premenopausal women (1.45±1.01) and (1.31±1.27) pg/ml respectively. Considering the correlation studies, (summarized in table -2) a statistically

significant positive correlation between serum IGF-1 and fasting serum glucose (FSG) (r=0.44, p=0.046) levels in diabetic women with normal body weight (BMI<25 kg/m^2). However, the data also revealed no significance in correlation between serum IGF-1 levels and other glycemic indices, nor with sex hormones in both obese & non-obese diabetic women

DISCUSSION

Both obesity and type 2 diabetes are sharing a feature of being associated with insulin resistance. Non-esterified fatty acids (NEFAs) originated from adipose tissues can induce insulin resistance and impair β-cell function. As a result of β-cell dysfunction and inadequate insulin secretion, postprandial and subsequently fasting glucose levels increase owing to incomplete suppression of hepatic glucose production and decreased efficiency of liver and muscle glucose uptake[15]. Thus diabetic women expressed significantly higher HOMA-IR than that of their corresponding controls. Furthermore, apparently healthy non-diabetic obese women with BMI≥30 kg/m^2, expressed obviously an elevated HOMA-IR level than normal weight non-diabetic women having BMI<25 kg/m^2, suggesting a higher level of insulin resistance. Whilst serum IGF-1 levels were found to be elevated in diabetics, whether these were obese or non-obese, over that of their control groups with significant differences ($p<0.05$). While no significant differences in serum IGF-1 levels were found between obese diabetic and non-obese diabetic groups, revealing a greater influence of the diabetic state on IGF-1 serum levels (table-1). Similar to other studies [16,17], only very weak correlations of BMI with IGF-1 were observed, (r=-0.15, p=0.29) in obese diabetic women and (r=0.13, p=0.56) in diabetic non-obese premenopausal women. The non-linear relationship of BMI with IGF-1 may be the expression of obesity-related changes in the synthesis of insulin, growth hormone (GH), GH receptor, IGFBP-1 and IGFBP-2 in lean individuals, the low endogenous insulin production is associated with a decreased GH receptor levels, resulting in resistance of IGF-1 synthesis in response to GH stimulation and a decrease in circulating IGF-1 levels[18,19]. Because insulin is partially controlling IGF-1 production in vivo. Given that there is a complex relationship between obesity, insulin, and IGF-1 (16). Our results revealed a statistically significant positive correlation between IGF-1 and FSG levels in diabetic women with normal body weight (BMI<25 kg/m^2). While a weaker correlation had been observed in obese diabetic women. A previous cross-sectional studies have found that free IGF-1 levels are, on average, elevated in patients with impaired glucose tolerance and type 2 diabetes[20]. As insulin resistance worsens, and insulin levels rise, these higher insulin levels result in lower serum IGFBP-1 levels, up regulation of hepatic IGF-I production, and higher levels of free (bioactive) IGF-I levels[21]. This may be the reason behind this positive correlation observed between serum levels of IGF-1 and fasting glucose that reflects extent of insulin resistance. But this correlation wasn't apparent in case of diabetic obese women, probably because of the presence of another abnormality that added which represented by obesity that can also affect serum IGF-1 levels because of its effects on GH levels[18,19], as mentioned previously. Serum estradiol (E2) levels for premenopausal women enrolled in present study demonstrated a significantly lower values in obese and in obese- diabetic women as compared to healthy normal body weight women. However, no statistically significant differences in serum E2 levels appear among obese and normal weight- diabetic women despite relatively higher E2 levels observed in premenopausal women having both obesity and T2DM than that of women having either obesity or T2DM. Circulating estrogen concentrations are increased due to increased aromatization of androgens in peripheral adipose tissue. So, women with upper body obesity had higher serum Testosterone and E2 concentrations than those with lower body obesity [22]. Tok *et al*., 2004, previously reported positive associations between serum estradiol levels and insulin resistance were detected in premenopausal women[23]. So the relatively elevated estradiol levels observed in obese diabetic as compared to non-obese diabetic premenopausal women may be attributed to the peripheral synthesis of estrogen that occur at a higher extent in these obese patients. Accordingly, diabetic premenopausal women participated in the present study still have lower serum E2 levels whether compared to healthy normal weight women reflecting derangements in female sex hormones metabolism particularly estrogens that accompany metabolic abnormalities including; obesity, insulin resistance and T2DM. Significant differences could not be found among the different groups concerning serum free testosterone levels, despite the higher values that observed in obese diabetic premenopausal women in comparison to diabetic non-obese women, as illustrated in (table-1). However, serum free testosterone levels also showed a pronounced elevation in its levels in obese diabetic as compared to that of obese non-diabetic women (1.81±1.27) and (1.45±1.01) pg/ml, respectively. Furthermore, non-obese diabetic women expressed higher serum free testosterone levels than in non-obese healthy premenopausal women (1.57±1.66) and (1.31±1.27) pg/ml, respectively. Also, non-diabetic obese women showed higher free testosterone level than levels estimated in non-diabetic non-obese premenopausal women (1.45±1.01) and (1.31±1.27) pg/ml respectively. In pre- and post-menopausal women, increased BMI, W:H ratio or abdominal obesity have been associated with either no change[24], or with an increase[25] in total testosterone concentrations. Most of the studies in pre-menopausal women have shown an increase in free testosterone with increasing body weight [26]. Our study also revealed no significant correlations between BMI and serum free testosterone concentrations were observed in diabetic pre-menopausal women, these data are consistent with other observations[27]. In a previous study during IGF-1 treatment, four out of the six patients (two girls and two adults) developed progressive clinical symptoms and signs of hyperandrogenism. The hyperandrogenism occurred concomitantly with an increase in serum IGF-1 and a decrease in serum insulin concentrations[28]. Furthermore, In vitro studies have shown that both insulin and IGF-1 can stimulate ovarian androgen synthesis[29].Our data analysis revealed weak inverse correlation between IGF-1 and E2 levels in obese diabetic .It had been proposed that

in obesity state, insulin and IGF-1 decrease SHBG production by liver, leading to an increase in the free testosterone fraction in both pre- and post-menopausal women[25]. However, this 'gonadotropic' effect of insulin and IGF-1 may be of less significance before menopause when circulating sex-steroid hormones are under the tight control of luteinizing hormone (LH) and FSH and regulated by powerful feed-back mechanisms[29]. Therefore, mild association that was observed in our study between IGF-1 and female sex steroid levels may lead us to conclude that IGF-1 has a modulatory but not essential role in female sex-steroid hormone metabolism, in agreement with other studies[30]. As conclusions, the results of this study provide evidence that an increase in BMI influences the circulating levels of IGF-1 and female sex-steroid hormones (estradiol and free testosterone) in premenopausal type 2 diabetic women and this seems to be related to the extent of insulin resistance. But, the association between IGF-1 and sex hormones levels was mild and statistically significant correlation between their levels couldn't be detected.

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