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Body Mass Index and Total Serum Leptin Level in Abortion

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Abstract

Background Adipose tissue secretes variety of adipokines, including leptin, which is involved in endocrine processes regulating reproduction and plays an important role in energy metabolism and fetal development during pregnancy.

Objectives To investigate the relationship between total serum leptin concentrations and anthropometric parameters including body mass index (BMI) in aborted women at the second trimester.

Methods A case control study was carried out from November 2011 to April 2012. The patients' group includes 30 aborted women at the second trimester. They were collected from Al-Elweyia, Al-Hakeem and Al-Khadhemiyia Teaching Hospital in Baghdad. Thirty healthy pregnant women (at their second trimester of gestation) were used as control. Patients and control were comparable in age. Blood HbA_1c and serum concentrations of total leptin, lipid profile, and glucose were measured in both groups.

Results Total serum leptin concentration were significantly lower in those with abortion at the second trimester compared with healthy pregnant control (3.5±0.8 pg/ml *vs.* 24.4±0.7 pg/ml, $P = 0.0001$) and leptin/BMI ratio *vs.* control (0.1±0.02 vs. 0.9±0.01 ml, $P=0.0001$). No correlation was found between leptin level and patient's age or gestational age in the case group. Highly significant correlation was found between patient's total serum leptin and their body mass index, HDL-C, total cholesterol/HDL-C ratio and atherogenic index ($P < 0.0001$).

Conclusion The significant correlation between patient's leptin and their BMI in addition to Leptin/BMI ratio even in non obese patient supports our objective that the unexplained abortion at second trimester is due to abnormality in their metabolic hormone action and reflect leptin resistance condition.

Keywords Second trimester abortion, Leptin, BMI, Gestational age.

Introduction

Leptin is the hormone product of the LEP gene and was originally thought to be produced only by adipocytes to aid in modulating satiety and energy homeostasis [(1)]. It is a polypeptide of 16 Kilo Dalton consisting of 167 amino acids, encoded by obese gene and is located on chromosome 7 in humans [(2)]. It is produced in large amounts by adipocytes and human placental trophoblasts. Its concentration is related to the mass of adipose tissue, and appears to be involved in modulating satiety and regulation of body weight homeostasis [(3)]. Moreover, leptin plays an important role in the reproductive system. It is involved in early embryogenesis, fat metabolism during pregnancy, and puberty [(4,5)].

Recent reports have demonstrated that leptin levels are elevated in serum during human and rodent gestations. Although increased adiposity during pregnancy might (as in obesity) be expected to underlie the hyperleptinemia of

pregnancy, leptin levels are elevated to an extent that cannot be attributed to the increased basal metabolic index (BMI), suggesting that during this state there is an additional source of leptin [6].

During pregnancy, plasma level of leptin starts to increase at the first trimester of gestation and then remarkably elevated during the second and third trimesters. These latter values are comparable to those found in obese humans. Within 24 h of delivery, the maternal plasma levels decline to normal values [7].

This study was designed to determine whether there is a relationship between total serum leptin level and BMI in women with unexplained abortion between 14-24 weeks and to estimate their leptin/BMI ratio and study its usage as an early predictive marker for abortion, which is due to abnormality in their metabolic hormone action.

Methods

A case control study was carried out from November 2011 to April 2012. The study included 30 women who had abortion at second trimester. They were collected from Al-Elweyia, Al-Hakeem, and Al-Khademiyia Teaching Hospital in Baghdad. Thirty healthy pregnant women (at their second trimester of gestation) were used as a control group. Patients' group and control were of a comparable age.

All patients who had hypertension, thyroid disease, diabetes mellitus, smoking, evidence of active infective, fever, chronic inflammatory diseases (including rheumatoid arthritis, joint pain, osteoarthritis, abdominal complain, inflammatory bowel disease); currently taking any medication, or having a positive test for cytomegalovirus (CMV) or toxoplasmosis were excluded from the study.

Blood samples were taken from patients during their admission to hospitals. Ten milliliters of venous blood were withdrawn from controls and all patients at the time of their abortion. One milliliter of it was transferred into Ethylene Diamine Tetra Acetic Acid (EDTA) tube for measuring glycosylated haemoglobin HbA_1c% by colorimetric method at 415 nm using (Bio-stand, France Kit) and the rest was transferred into a plain tube, allowed to clot, and then centrifuged for 10 min at 3000 rpm to collect serum.

Serum was used to determine, glucose, lipid profile including total cholesterol, triglyceride (TG), VLDL-C, and HDL-C [measured by the precipitation of chylomicrons] using colorimetric enzymatic method [8] (Biomaghreb, Sa, France kit). LDL-C was calculated if TG < 400 mg/dl by the formula of Friedewald *et al* [9]. Body mass index (BMI) was calculated by dividing study subjects weight (Kg) on their height (m^2).

Serum leptin concentrations were measured using an Enzyme linked immunosorbant assay (ELISA) method by using; Human Leptin (LEP) ELISA Kit (Catalog No. CSB-E04649h, CUSABIO BIOTECH CO., LTD, China). The minimum detectable concentration of human serum leptin is typically less than 1.56 pg/ml. Expected normal concentrations are between (3.5 - 12.5 pg/ml) at 450 nm.

Ethical approval and patient permission were obtained from the local ethics committee to conduct this study.

Statistical analysis

Data were statistically analyzed by SPSS version 17. All data were presented as a mean± SE. Statistical differences between value of patients and control groups were determined by student *t*-test. Correlation between the variables was performed by spearman correlation coefficient. *P* value < 0.05 was considered as significant.

Results

Sixty pregnant women at their second trimester, between 14-24 weeks of gestation, were divided into 2 groups: the first one included 30 second trimester abortion women and the second includes 30 normal healthy pregnant women.

It was conducted to determine the level of total leptin and other biochemical parameters in patient's sera and compared it with normal healthy pregnant at the same trimester.

According to analysis of clinical and anthropometric data, table 1 shows no

significant difference between study groups in regard to their age, gestational age (GA) and BMI.

A high percentage was found among aborted women aged between 20-24 years old (40%), while the lowest percentage was found among those aged < 20 years old.

Regarding BMI, the higher percentage of abortion was 63.3% found among women with normal BMI (18.5-24.9 Kg/m^2) compared with 50% healthy normal pregnant with overweight BMI (25-29.9 Kg/m^2, and no significance differences was found between BMI ranges among all study groups.

Table 1. Anthropometric characteristics and clinical criteria of study subjects

Parameter	Patient (N = 30) Mean±SE		Control (N = 30) Mean±SE		*P* value
	No	Range	No	Range	
Age (years)	26.2 ± 5.8	17.0 - 37.0	25.6 ± 6.7	16.0 - 42.0	NS
GA (weeks)	19.3 ± 0.7	14 - 24	19.9 ± 0.5	14 - 24	NS
BMI (Kg/m^2)	24.60 ± 3.14	18.8 - 30.85	25.94 ± 3.59	17.36 - 33.9	NS

SE = standard error, GA = gestational age, BMI = body mass index

Women with second trimester abortion showed significantly lower serum concentration of total leptin (mean 3.5 ± 0.8 pg/ml) *vs.* healthy pregnant control at same trimester (24.4±0.7 pg/ml, *P* = 0.0001), and leptin/BMI ratio (mean 0.1 ± 0.02) *vs.* control (mean 0.9 ± 0.01 ml, *P* = 0.001 (Table 2).

Table 2. Comparison of biochemical indices between patients and control group

Biochemical parameter		Patient (N = 30) Mean ± SE	Control (N = 30) Mean ± SE
Serum lipid profile(mg/dl)	S. Total Cholesterol	173.4 ± 6.5	181.3 ± 7.9
	S. Triglyceride	128.4 ± 6.2	127.8 ± 10.8
	S. HDL-C	52.1 ± 3.8	55.4 ± 2.3
	S. LDL-C	98.1 ± 8.3	103.3 ± 6.4
	S. VLDL-C	25.7 ± 1.2	28.3 ± 2.6
	Total Chol/HDL-C ratio	3.9 ± 0.3	3.4 ± 0.2
	AI = (LDL-C/HDL-C) ratio	2.4 ± 0.3	2.1 ± 0.3
Serum FBG (mg/dl)		82.1 ± 7.7	80.5 ± 2.6
HbA_1c %		5.3 ± 0.1	5.2 ± 0.1
Serum leptin (pg/ml)		3.5 ± 0.8	24.4 ± 0.7*
Leptin/BMI ratio		0.1 ± 0.02	0.9 ± 0.01*

SE= standard error, BMI= body mass index, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, VLDL-C=very low density lipoprotein cholesterol, AI= atherogenic index, S = serum, * *P*= < 0.001.

In table 3, highly significant differences (*P* < 0.001) were found between all patient's total leptin concentration and control sera when ranged according to subdivided ranges of age and BMI except no significant correlation was found in obese women with BMI ≥ 30 Kg/m^2 and this may be due to the small sample size of distributed subjects.

Correlations between total serum leptin and anthropometric and biochemical parameters in study subject are shown in table 4. Regarding leptin correlation among women with abortion

at second trimester group; a highly significant positive correlation was found between total serum leptin concentration and BMI (P = 0.0001, r = 0.682) as well as HDL-C (P = 0.008, r = 0.478) while a highly negative significant correlation was found between leptin and T.Chol/HDL-C ratio (P = 0.007, r =-0.479) as well as AI ratio (P = 0.007, r = -0.484).

Table 3. Distribution of serum leptin concentration according to subdivided ranges of age and BMI in the studied subjects

Parameter		Leptin (pg/ml)				*P* value
		Patient (N = 30)		Control (N = 30)		
		No	Mean±SE	No	Mean±SE	
Age (years)	< 20	3	5.0 ± 3.9	5	21.8 ± 1.6	0.003
	20-24	12	3.7 ± 1.1	10	22.8 ± 1.2	0.0001
	25-29	6	4.6 ± 2.0	6	25.1 ± 1.4	0.0001
	30-34	5	0.9 ± 0.2	5	27.3 ± 0.9	0.0001
	≥ 35	4	3.5 ± 2.2	4	26.5 ± 1.2	0.0001
P value		ns		0.050		
BMI (Kg/m^2)	< 18.5	-	-	-	-	-
	18.5-24.9	19	1.5 ± 0.2	13	20.8 ± 0.5	0.0001
	25-29.9	9	6.7 ± 1.5	15	26.9 ± 0.5	0.0001
	≥ 30	2	8.9 ± 5.7	2	28.1 ± 0.2	NS
P value		0.0001		0.0001		

SE= standard error, BMI= body mass index, ns = non significant

Table 4. Correlation between anthropometric and biochemical parameters with total serum leptin concentration in studied subjects

Parameters		Serum leptin (pg/ml)			
		Patient (N = 30)		Control (N = 30)	
		P	*r*	*P*	*r*
Age (years)		0.329	-0.184	0.005	0.500**
GA (weeks)		0.583	0.143	0.021	0.156*
BMI (Kg/m^2)		0.0001	0.682**	0.0001	0.897**
FBG (mg/dl)		0.682	0.078	0.133	0.281
HbA_1c %		0.213	0.234	0.392	0.162
Lipid profile	S. Total Cholesterol (mg/dl)	0.535	0.118	0.367	0.171
	S. Triglyceride (mg/dl)	0.721	0.068	0.089	0.316
	S. HDL-C (mg/dl)	0.008	0.478**	0.237	0.222
	S. LDL-C (mg/dl)	0.053	0.357	0.378	0.167
	S. VLDL-C (mg/dl)	0.744	0.062	0.270	0.208
	Total Cholesterol/HDL-C ratio	0.007	-0.479**	0.046	0.367*
	AI ratio	0.007	-0.484**	0.961	0.009

SE = standard error, GA = gestational age, BMI = body mass index, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol, AI = atherogenic index, S = serum, * = $P < 0.05$, ** = $P < 0.001$.

Discussion

A striking change was noticed in the total leptin level in women who had abortion in their second trimester. Leptin level seems to be significantly decreased compared to pregnant women at the same gestational age. During pregnancy, total leptin levels are substantially elevated [10,11] and serum leptin levels are significantly higher than levels in non-pregnant women, and increases from the first to the third trimester [12].

The increase in leptin level is due to the additional production of leptin by syncytiotrophoblast in the placenta and thus leptin synthesis is elevated with the increase of the placental mass with advancing gestation [13]. Yang (2005) reported that the elevation in serum total leptin level in the second trimester is not due to placenta and adipose tissue only, but also to mammary epithelial cells, fetal tissue, gastric mucosa, and hepatic stellate cells, and the production by these organs leads to additional increase in leptin concentration resulted in a significant change in its level related to GA [13].

Žaneta *et al.* also found an elevation in maternal serum total leptin levels during pregnancy with alterations particularly during the second and third trimesters of pregnancy. They mentioned that the occurring physiological hyperleptinemia is not associated with decreased food intake or reduced metabolic activity in pregnant women [14]. Al-Atawi *et al.* (2004) and Augustine *et al.* (2008) have found that maternal leptin concentrations increase during pregnancy, but the increase seems to occur during the first two trimesters and then leptin levels decrease slightly during the third trimester [15,16]. Whereas, Grattan *et al.* (2007) and Ladyman *et al.* (2010) suggested that a lack of increase in leptin level in the 3rd trimester is reflective of late pregnancy being a leptin-resistant stage [17, 18]. In addition, Grathar *et al* (2007) reported that during the third trimester, leptin levels do not rise although body weight increases, indicative of pregnancy induced leptin resistance, and this contributes to reduce insulin sensitivity seen during pregnancy [19].

Regarding the relation between leptin level in second trimester abortion group with their BMI *vs*. same parameters in control, lower significant correlation was found between patient's leptin and BMI with positive correlation. The higher percentage was found among patients with normal and overweight BMI.

In normal pregnancy, Kim *et al.* (2008) explained that the leptin elevation is due to an increase in maternal body weight, as the serum leptin level is dependent on body weight [20]. More explanation was done by Delia *et al.* who reported that leptin elevation in maternal serum is due to the gradual increase of BMI throughout gestation, which combines, with an increase in estradiol levels that stimulate leptin production from adipocytes [11]. On the other hand ,Sagawa *et al.* (2002) and Masayo *et al*. in 2003 mentioned that BMI did not necessarily reflect body fat mass in pregnant women, and the remarkable elevation serum leptin level during pregnancy was not explicable by an increase of fat mass alone. They suggested that the rise of leptin during pregnancy is caused by the placenta production and that maternal plasma leptin levels are not correlated with body mass index [21,22].

In this study, patient's and control BMI was nearly comparable but their leptin level was lower than control and was more obvious when patient's leptin distributed according to subdivided ranges of BMI as mention in table 3. Non-significant difference between patients and controls in leptin levels was found among obese women with BMI ≥ 30 Kg/m^2. This result may be due to the small sample size.

Regarding the ratio of total serum leptin to BMI, a significant difference was found between abortion L/BMI ratio *vs.* normal pregnant ratio. This ratio was first used in 2001 by Brannian *et al* [23] as a predictive marker of outcomes in women undergoing in vitro fertilization (IVF). They grouped the ratio into three categories, low (0.1-0.3), moderate (0.4-0.6), and high (≥ 0.7). They reported that, very few patients became pregnant when their leptin was ≥ 3×10^4 pg/ml, even if their BMI was relatively low. Finally, they

concluded that this relationship might assist clinicians in counseling patients and improving the success of assisted reproduction.

Abortion L/BMI ratio in this study was found ± 0.1 which is in agreement those reported Brannian *et al.* (2001). This due to their low leptin levels despite the BMI classifications (normal, overweight and obese). Normal pregnant women L/BMI ratio shows ± 0.9 which is above the high ratio recorded by Brannian *et al* (2001). This study is the first one that determines the serum total leptin levels in women with 2nd trimester abortion.

Although age of both studied groups were comparable, a highly significant correlation was found between serum leptin levels *vs*. aborted maternal age *vs*. control age even when subdivided into ranges. Although leptin level was found higher among patients age range 20-24 years old, no correlation was found between GA and leptin level. On the contrary, other research has found a significant correlation between GA and maternal serum leptin levels [13,14]. They concluded that maternal serum leptin concentration throughout the pregnancy course correlates not only to body weight and BMI but also to GA. Furthermore, they didn't found a significant relationship between maternal serum leptin and GA in the first trimester but serum leptin was correlated to GA in the second trimester and is inversely related to GA in the third trimester. Also, maternal BMI is related to GA in the second trimester and the whole pregnancy, but not in the first and third trimesters [13].

In conclusion, obviously at second trimester abortion, maternal serum leptin level was found within the normal range and not elevated as expected during second trimester of healthy pregnancy. No correlation between patient's GA and leptin level compared with positive correlation in control, which might be due to lack of leptin production by the placenta. Finally, the significant correlation between patient's leptin and their BMI, even if they were not obese, support our objective that the unexplained abortion at second trimester is due to abnormality in their metabolic hormone action. Also, the low level of L/BMI ratio in patients group compared with high ratio found in normal pregnant group confirm the usage of this ratio as an early predictive marker for 2nd trimester abortion especially when it becomes ≤ 0.1.

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