Comparison of Serum Leptin Level in Ectopic and Normal Pregnancy

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ABSTRACT

Background: Leptin hormone has an important role in reproduction; its secretion increased during pregnancy from the first trimester and decrease after parturition. Alterations in leptin level are an indicator of cessation of pregnancy either normally at term or due to pathology.

Objective: To assess maternal serum leptin level in women with ectopic pregnancies and compare it with that of normal pregnant women.

Methods: A case-control study carried out in Al Emamin AlKadmain medical city, Department of Obstetrics and Gynaecology from the first of February 2014 to the first of January 2015. One hundred fifty pregnant females were included in this study, seventy five women with ectopic pregnancies considered as study group and seventy five women with singleton uncomplicated pregnancy considered as control group with maximum gestational age in both was eight weeks. Maternal age, gestational age, parity, body mass index and leptin levels, were recorded for both groups.

Results: The mean leptin level (ng/ml) in the study group was (15.25±2.84) while the mean leptin level (ng/ml) in the control group was (12.51±2.11), there was statistically significant difference between the two groups as P value < 0.0001. There was no statistically significant difference in maternal age, parity, body mass index in both study and control groups, but significant difference was found in gestational age between study and control groups as P value < 0.0001. There was a positive correlation between leptin level and body mass index in both the study and the control groups as r value 0.504, 0.637, respectively, with statistically significant difference as P value < 0.001 in both groups. There was no statistically significant difference between leptin level and maternal age, parity in both groups. While statistically significant difference was found between leptin level and gestational age only in control group as P value 0.018.

Conclusion: Maternal serum leptin level was higher in women with ectopic pregnancy compared to that of normal pregnancy.

Keywords: Serum Leptin, Ectopic pregnancy.

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Leptin is a hormone made by white adipose tissues, structurally it is non-glycosylated polypeptide of 167 amino acids discovered in 1994 by Zhang et al. It regulates the amount of fat stored in the body and with many other hormones, participate in the complex process of energy homeostasis[1,2].

Leptin circulates in blood in free form and bound to proteins at a level of 5 to 15 ng/mL in lean subjects[3]. Serum leptin levels in pregnant women are significantly higher than in non-pregnant women. The human placenta expresses high amounts of leptin mRNA and protein in early, mid, and late gestation[4].

Leptin concentrations rise along with estrogen and are correlated in early

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pregnancy with those of human chorionic gonadotrophin hormone[5].

Leptin produced by the placental trophoblasts participates in a number of processes in pregnancy including implantation, proliferation of cytotrophoblasts, and nutrient transfer across the placenta[6].

Leptin displays a synergistic effect with FGF-2 or VEGF in stimulation of blood vessel growth[7] and modulates the expression of adhesion molecules and metalloproteinases involved in the invasive process[8] thus it acts directly as a critical "placental hormone" in the control of human placental growth, development, and functions. Accordingly, leptin is now commonly used as a biochemical marker of trophoblast differentiation[9].

Ectopic pregnancy is a pregnancy that implants outside the uterine cavity[10].

The estimated prevalence is 1-2% worldwide[11]. A dramatic increase in incidence over time has been reported in several countries[12]; this rising incidence is strongly associated with an increased incidence of pelvic inflammatory disease[13].

Ectopic pregnancy has a relative risk of death 10 times as great as that of childbirth. The fact that this condition can result in the death of women of childbearing age who are free of disease and for the most part otherwise healthy increases the personal tragedy and medical concern the early recognition of an ectopic pregnancy[14].

When a woman presents with a suspected ectopic pregnancy, in up to half the cases the diagnosis is not made based on the initial consultation[15], therefore a serum biomarker of tubal implantation, which could accurately identify ectopic pregnancy at first presumption, would be a major clinical advance[16].

Methods

A prospective case-control study conducted at Al-Emamain Al-Kadhemain medical city, from the 1st of February 2014 to the 1st of January 2015.the study included 150 pregnant women who were divided into two groups:

- Study group of seventy five women with ectopic pregnancies, maximum gestational age eight weeks.
- Control group of seventy five women with uncomplicated singleton pregnancies, maximum gestational age eight weeks.

The women in the study group were selected from the Gynaecology ward at Al-Emamain Al-Kadhemain Medical City .The women in the control group were selected from the outpatient clinic, while attending for booking visit.

Inclusion criteria included maternal age 18-40 years, gestational age 5-8 weeks, maternal body mass index 19-30 kg/m^2, stable general condition, pregnancy with singleton viable fetus (for the control group).

Exclusion Criteria: multiple pregnancies, unstable general condition patients with chronic illness at the time of the study (like diabetes and hypertension), cigarette smoking, patient on steroid therapy.

Verbal consent was obtained from all pregnant women included in the study. The demographic characteristics of each patient were assessed including maternal age, parity, gestational age for the two groups. The gestational age up to 8 weeks was calculated from the first day of the last menstrual period and confirmed by transvaginal ultrasound scan. Full history was taken and complete examination (general and obstetrics examination) was done for all participants, Body mass index was calculated to all patients by dividing the weight of the participant in kilograms over the square of her height in meters.

Investigations: Full blood count, fasting blood sugar, midstream urine sample, β-HCG titer, transvaginal ultrasound and serum leptin level, the sample collection and preparation of serum leptin done by

collected five ml of blood by venipuncture using disposable plastics syringes from antecubital fossa, the blood was kept in plastic tubes then centrifuged at 5000 rpm for ten minutes to obtain the serum which was then stored at – 20 C^0. All the specimens were clearly labeled with names of participants then measurement done by Abcam's Leptin Human ELISA kit which is designed for the quantitative measurement of leptin concentration in serum, samples applied at sandwich ELISA HRP labeled.

Statistical Analysis of data was carried out using a variable of SPSS-17 (Statistical Package for the Social Sciences version 17) + Microsoft Excel 2013.

Data were presented as mean ± standard deviation. Comparisons of means were done using unpaired standard t-test, P value < 0.05 was considered significant.

Person correlation was done to find correlation coefficient (r) between two parameters within the same group. P value < 0.05 was considered significant.

The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value, <0.3 represent no correlation, 0.3 - <0.5 represent week correlation, 0.5 - <0.7 moderate correlation, >0.7 strong correlation. Box plot and ROC (receiver operating characteristic) was used for serum leptin. Sensitivity and specificity were calculated.

Results

The demographic characteristics of the participants in the two groups are shown in table 1. There was no significant difference in the maternal age between the study group and the control group as P value was 0.5291. There was no significant difference in the mean BMI (kg/m^2) between the two groups as P value was 0.1456. There was no significant difference in the mean parity between the two groups as P value was 0.4531. Regarding the gestational age there was significant difference between the two groups as the P value < 0.0001.

Table 2 shows the serum leptin levels in the study and the control groups, there was statistically significant difference between the two groups as the P value <0.0001.

The results in the table 3 show that the mean leptin level (ng/ml) ±SD in the study group was significantly higher than its level in the control group in the gestational age (5-5.9) weeks, as P value <0.0001.

The mean leptin level (ng/ml) in the study group was significantly higher than its level in the control group at (6-6.9) weeks, as P value < 0.0001.

The mean leptin level (ng/ml) in the study group was significantly higher than its level in the control group at (7-7.9) weeks, as the P value 0.0297.

The results in table 4 show a positive correlation between the leptin level (ng/ml) and the maternal age (years) in the study group with no statistically significant difference as r value 0.145 and the P value 0.213; while a negative correlation between the leptin level (ng/ml) and the maternal age (years) in the control group with no statistically significant difference as r value -0.026 and P value 0.826.

Positive correlation between the leptin level (ng/ml) and BMI (kg/m^2) in the study group, statistically significant difference was found as r value 0.504 and P value < 0.001, a positive correlation between leptin level (ng/ml) and BMI (kg/m^2) in the control group with statistically significant difference as r value 0.637 and P value < 0.001.

A negative correlation was found between the leptin level (ng/ml) and the gestational age (weeks) in study group but, it was not statistically significant as r value -0.118 and P value 0.312. A positive correlation was found between the leptin level (ng/ml) and the gestational age (weeks) in the control group which was statistically significant as r value 0.273 and P value 0.018.

Table 5 shows serum leptin level median in the study group which was 15.45 (ng/ml) with leptin level 13.06 (ng/ml) at 25% and 17.49 (ng/ml) at 75% while serum leptin level median in the control group was 12.44 (ng/ml) with leptin level 10.93 (ng/ml) at 25% and 13.92 (ng/ml) at 75%, (Figure 1).

Figure 2 shows ROC curve of serum leptin level as diagnostic test for ectopic pregnancy including its sensitivity and specificity.

the sensitivity and specificity of serum leptin level between study group and control group at cut off value 13.81 (ng/ml), there was 73.3% specificity and 68.0% sensitivity, significance difference between the two groups found as P value < 0.001.

Table 1: Demographic characteristics of the women in both study and control groups.

Parameter	Study (n=75) Mean ±SD (Range)	Control (n=75) Mean ±SD (Range)	*P* value
Age (y)	28.01±6.83 (18-40)	27.33±6.37 (18-39)	0.5291
BMI (kg/m^2)	26.31±2.72 (18-30)	25.64±2.9 (19-29)	0.1456
Parity	2.15±1.63 (0-4)	2.35±1.62 (0-6)	0.4531
Gestational age (w)	5.97±0.61 (5.3-6.5)	6.46±0.67 (5.1-7.9)	**<0.0001**

Table 2: Serum leptin levels in the study and the control groups.

Parameter	Study (n=75) Mean ± SD (Range)	Control (n=75) Mean ± SD (Range)	P value
S. Leptin (ng/ml)	15.25±2.84 (10.22-19.71)	12.51±2.11 (8.39-16.93)	**<0.0001**

Table 3: Comparison of leptin levels according to the gestational age between the study and the control groups.

Parameter	study Mean ±SD (n = 75)	Control Mean ±SD (n = 75)	*P* value
5-5.9 (week)	15.49±3.18 No.=42	11.13±1.94 No.=14	**<0.0001**
6-6.9 (week)	15.0±2.68 No.=22	12.76±2.08 No.=40	**<0.0001**
7-7.9 (week)	15.67±2.24 No.=11	12.95±1.95 No.=21	**0.0297**

Table 4: Correlation of serum leptin with other parameters in the study and the control groups.

Parameters	Study		Control	
	R	P	R	P
Age (y)	0.145	0.213	-0.026	0.826
BMI (kg/m^2)	0.504	< 0.001	0.637	< 0.001
Parity	0.035	0.765	0.113	0.335
Gestational age (w)	-0.118	0.312	0.273	0.018

Table 5: Median of serum leptin (ng/ml) in the study and the control groups.

	Study (n=75)	Control (n=75)
Min(ng/ml)	10.23	8.39
25%	13.06	10.93
Median(ng/ml)	15.45	12.44
75%	17.49	13.92
Max(ng/ml)	24.22	16.93

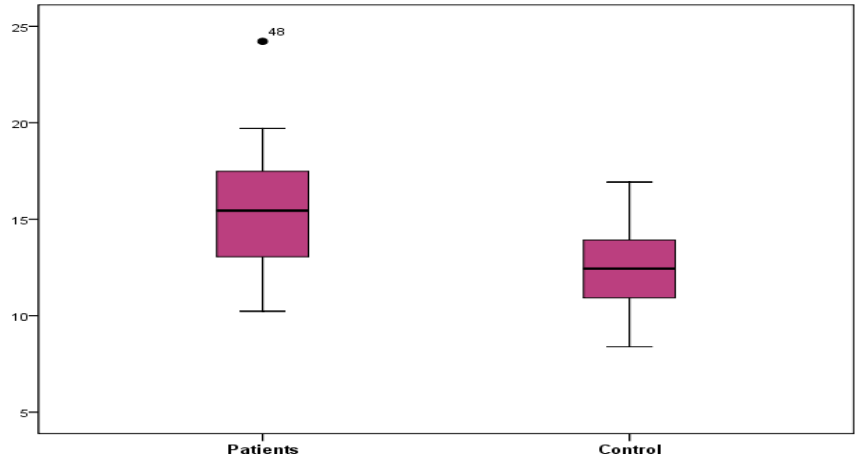

Figure 1: Box plot of serum leptin in the study and the control groups.

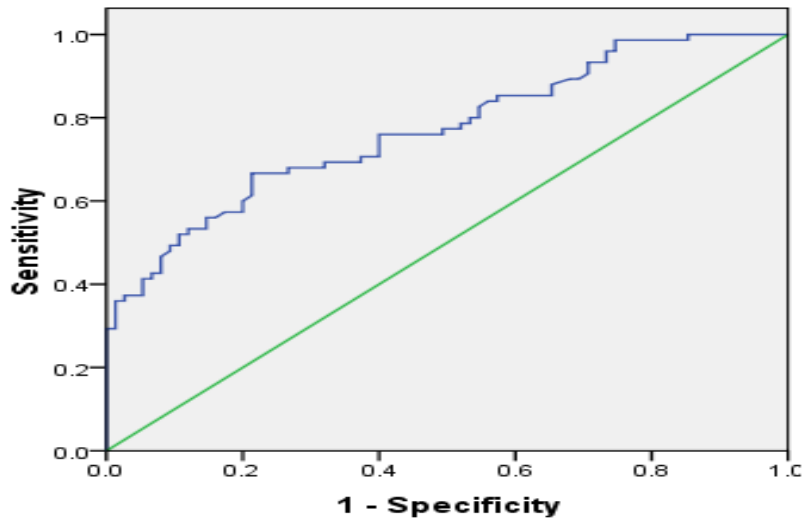

Figure 2: ROC curve for serum leptin between the study and the control groups.

Discussion

Ectopic pregnancy is one of the most common public health problems. It may be the life-threatening disease in which incidence has been increased[17]. Early diagnosis of first trimester hemorrhage presents an important challenge. Transvaginal ultrasounds and serial β-hCG determinations are currently the most common methods used for diagnosis[18]. Investigation of new serum markers could result in earlier diagnosis, leading to timely implementation of medical treatment and prevention of tubal rupture and its complications[16].

A study done by Senaris, et al in 1997[19] revealed strong staining for immune reactive leptin in 1st trimester chorionic villi and 3rd trimester syncytiotrophoblast, respectively, and this indicate that the placenta is an important source of leptin in pregnancy.

In the current study there was no significant difference between women ages, BMI and parity in both the study and control groups, a significant difference in the mean gestational age between the two groups has been demonstrated, because of a later presentation of women of the control group to our consultation clinic and this would made the comparison of leptin level between the two groups unreliable; therefore we compared the leptin level between the two groups at the same gestational age week where a significantly higher level was found in patient with ectopic pregnancy than those with normal pregnancy. Also, our observations of a statistically significant positive correlation between leptin level and gestational age in the control group was in agreement with Henson et al 2000[20] who demonstrated increased maternal serum leptin concentration throughout most of pregnancy. While not necessarily follow the same progressively increased pattern in impaired placentation as evidenced by the statistically non-significant negative correlation with the gestational age in the study group.

Hiroko Mise et al 1998 [21], founded an augmented placental production of leptin in patients with preeclampsia, these observations suggest that in pre-eclampsia placental production of leptin is increased in response to hypoxia, there by supporting the idea that augmented plasma leptin level in severe preeclampsia reflect placental hypoperfusion and/ or hypoxia.

In a study done by Hempstock et al 2003[22] showed that both preeclampsia and missed miscarriage were associated with impaired placentation and placental oxidative stress, a common mechanism may responsible for increased production of leptin. The results of these studies could explain the finding of current study in which increased leptin level found in ectopic pregnancy may be due to impairment of placental oxygenation that stimulate production of leptin .

Another study done by G Makrydiamas et al 2005[23], which investigate the possible role of leptin in early pregnancy failure, they compared pregnancy with missed miscarriage to those with live fetuses. The median leptin concentration in coelomic fluid of missed miscarriage was higher. The high coelomic fluid leptin concentration suggests that the embryonic death may be preceded by impaired oxygenation of the placenta that stimulates production of leptin. While according to Giovanni et al 2006[24], there was no difference in leptin level in the first trimester of threatened abortion cases, even those that led to complete abortion or normal pregnancy.

In a study done by Baban et al 2010[25], which measured serum leptin and insulin level in recurrent pregnancy loss, serum leptin level found to be higher in women with pregnancy loss in their three trimester, high significance correlation was found between total serum leptin and insulin in women with recurrent pregnancy loss during first, second, third trimester, the number of women who lost their pregnancy during the first trimester was higher than those losing their pregnancy during other trimester.

A study of Kamyabi et al 2011[26] included 47 females with normal pregnancies and 47 females with ectopic pregnancies at maximum gestational age eight weeks showed significantly higher serum leptin level in females with ectopic pregnancy compered to normal pregnancy, which support our study. With regards to the correlation

between BMI and serum leptin levels, the results of the current study showed a positive correlation with statistically significant difference between study and control groups, these findings agree with in Aka et al 2006[27] who reported a significant correlation between leptin level and BMI.

While disagree with Sagawa et al 2002[28] who concluded that maternal plasma leptin level was not correlated with BMI and Arselan et al 2003[29] who reported that there was significance inverse correlation between BMI and leptin level in the first trimester.

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