Leptin concentration during different trimesters of pregnancy and its relation to other pregnancy hormones

Fatma S. Al-Atawi, BSc, Mohammed H. Addar, ABOG, Arjumand S. Warsy, PhD, Zainab A. Babay, ABOG.

ABSTRACT

Objective: To determine the levels of leptin and other pregnancy hormones (progesterone, estradiol, folliculi stimulating hormone, luteinizing hormone and beta human chorionic gonadotropin) in pregnant females during different stages of pregnancy and to correlate these levels to maternal weight, body mass index (BMI), babies weight and babies BMI.

Methods: Leptin level and other pregnancy hormones were measured in 36 pregnant females and 30 non-pregnant females followed at King Khaled University Hospital, Riyadh, Kingdom of Saudi Arabia in the year 2001 in a prospective study. Blood samples were collected at the first, 2nd and 3rd trimester and after delivery. Correlation analysis between leptin level and pregnancy hormones, in addition to maternal weight, BMI, babies weight and BMI.

Results: The mean leptin levels during pregnancy and postnatally were significantly higher in pregnant females compared to the non-pregnant controls. Serum concentration of leptin increased significantly (p=0.01) in the pregnant females from 21.24 ± 9 ng/ml during the first trimester to 26.3 ± 8.69 ng/ml during the 2nd trimester, but insignificantly decreased to 23.29 ± 8.62 ng/ml during the 3rd trimester (p=0.073). After delivery leptin concentration significantly decreased to 17.36 ± 7.95 ng/ml (p=0.0025). The changes in levels of leptin during pregnancy were independent to other pregnancy hormones which showed a different pattern of variation.

Conclusion: The changes in levels of leptin during pregnancy were independent to other pregnancy hormones which showed a different pattern of variation.

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Leptin is produced by the white fat cells by a specific gene called the obese (ob) gene.[1] It has important effects in controlling body weight, metabolism and reproductive functions.[2,3] The role of leptin in the induction of puberty is believed to be through its action on hypothalamic cells, where it stimulates release of luteinizing hormone-releasing hormone (LHRH) hence triggering the release of gonadotropins luteinizing hormone (LH), follicle stimulating hormone (FSH), which subsequently lead to induction of puberty.[4] During different stages of the menstrual cycle changes in the level of leptin have been reported and are highest at the luteal phase and lowest at the follicular phase. In this respect, a close correlation was seen between leptin and progesterone levels, which also peak during the luteal phase of the menstrual cycle.[5,6] This study was carried out to determine the levels of leptin and

From the College of Science (Al Atawi, Warsy), Department of Biochemistry (Addar, Babay), College of Medicine, Department of Obstetrics and Gynecology, King Saud University, Riyadh, *Kingdom of Saudi Arabia*.

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Address correspondence and reprint request to: Dr. Zainab A. Babay, Consultant and Associate Professor, Department of Obstetrics and Gynecology, King Khaled University Hospital, PO Box 7805, Riyadh 11472, *Kingdom of Saudi Arabia*. Tel. +966 (1) 4670818. Fax. +966 (1) 4671945. E-mail: zbabay@hotmail.com

other pregnancy hormones (progesterone, estradiol (E2), and beta human chorionic gonadotropin (βHCG) in pregnant females during different stages of pregnancy and to correlate these levels to maternal weight, body mass index (BMI), babies weight and babies BMI.

Methods. The study included 36 Saudi pregnant females regularly attending antenatal clinic and 30 non pregnant healthy females of the same age attending King Khaled University Hospital (KKUH), Riyadh, Kingdom of Saudi Arabia (KSA) in the year 2001. All women gave informed consent before recruitment. The history and the essential information's were recorded on specially designed forms. Twenty-six of the 36 pregnant females delivered at KKUH, where as the rest delivered in other hospitals. Gestational age of the pregnant females was calculated from the first day of last menstruation period and was confirmed by mid trimester ultrasound scan. The mean height of the pregnant females was 1.57m ± 0.1 and for the controls was 1.62 m ± 0.35. The mean maternal weight was 69.3kg ± 14.4 in the first trimester, 72.55kg ± 14.6 in the second trimester, 75.5kg ± 13.7 in the third trimester, 72.2kg ± 15.1 after delivery and 53.9kg ± 16.5 for the controls. Body mass index was calculated by dividing the weight in kg by the height in meter squared. Fetal growth was evaluated by clinically and by ultrasonography. Pregnancy complications, outcome of pregnancy, and sex of babies, length and weight were also recorded. The mean placental weight was 578.5gm± 131.8, mean neonatal height 48.45cm ± 3.1, mean neonatal weight 2.75kg ± 0.52 and mean neonatal BMI 13.6 ± 1.72. Blood samples was obtained from pregnant females at 4 different times; at booking time (first trimester), second and third trimester of pregnancy, and postnatally (on second day after spontaneous vertex delivery, and on third day after cesarean section delivery). Only a single sample was obtained from non-pregnant females. The collection of blood samples was between 8 am and 5 pm without overnight fasting. Blood samples (5 ml) were drawn by venepuncture in plain tubes (red top tubes). The blood was allowed to clot, and serum was separated by centrifuge at 4000 rpm for 7 min at room temperature. The serum samples were stored frozen at -20°C until required for analysis. Serum leptin concentrations were estimated in duplicate by a human leptin RIA (RIA, Linco Research, Saint Charles, Missouri, United States of America). The within batch C.V for leptin ranged from 1.6-13.0%, while the between batch C.V was 3.9%. Serum Progesterone, E2, FSH, LH and βHCG concentrations were estimated in duplicated by Auto-analyzer (Elecsys 2010, Roche, Immunoassay System). For progesterone the within batch C.V ranged from 0.354-2.67%, while the between batch C.V was 10.9%. For E2 the within batch C.V ranged from 0.05-1.857%, while the between batch C.V was 10.4%. For βhCG the within batch C.V ranged from 0.29-4.08% while, the between batch C.V was 12.4%.

Statistical analysis. The data analysis was carried out using SPSS Program. Data are presented as the mean ± SD. Student's "t" test was used to determine the statistical significance of the difference between the means in any 2 trimesters and after delivery. Bivariate correlation studies were carried between all the studied parameters in the different groups. Pearson's correlation coefficient (r) and *p* values were obtained. $p \leq 0.05$ was considered statistically significant. Multiple regression analysis was conducted to determine the influence of the different parameters on the level of leptin.

Results. The demographic characteristics of the pregnant females and non-pregnant controls are presented in **Table 1**. The ages of the pregnant and non-pregnant females were similar. In the pregnant females, the weight and BMI showed a gradual increase during the 3 stages of pregnancy, while a reduction was observed after delivery. In the pregnant females, mean level (±SD) of leptin during first trimester was 21.24 ± 9 ng/ml, while in the control group it was 12.4 ± 7.155 ng/ml. The leptin mean during first, 2nd and 3rd trimester and postnatal stage were significantly higher in pregnant females compared to the non-pregnant controls ($p<0.05$) **(Table 1).** Serum concentration of leptin increased significantly ($p=0.01$) in the pregnant females from 21.24 ± 9 ng/ml during first trimester to 26.3 ± 8.69 ng/ml during the 2nd trimester, but insignificantly decreased to 23.29 ± 8.62 ng/ml during 3rd trimester ($p=0.073$). After delivery leptin concentration significantly decreased to 17.36 ± 7.95 ng/ml ($p=0.0025$) **(Table 1)**. In the pregnant females the serum concentration of progesterone increased significantly from 107.99 ± 77.45 nmol/l during first trimester to 317.71 ± 219.9 nmol/l during 2nd trimester. Further significant increase ($p=0.0001$) to 1877.4 ± 768.41 nmol/l during 3rd trimester was observed ($p=0.0001$). After delivery progesterone concentration significantly decreased to 31.86 ± 30.75 nmol/l ($p=0.0001$) **(Table 1)**. The estradiol levels during first, 2nd and 3rd trimesters and postnatal stage are presented in **Table 1**. Each value was significantly higher compared to the controls ($p<0.05$). Serum concentration of estradiol increased significantly among pregnant females from 11084 ± 14676 pmol/l during first trimester to 8702 ± 106975 pmol/l during 2nd trimester, and to 185267 ± 198172 pmol/l in 3rd trimester ($p=0.0001$). After delivery, estradiol concentration

Table 1 - Demographic characteristics of pregnant females during different stages of pregnancy and after delivery compared to control.

Variable	Pregnant females (mean ± SD) First trimester (n=36)	2nd trimester (n=36)	3rd trimester (n=36)	PA (n=26)	Controls (n=30)
Maternal age (years)	29.0 ± 5.0	-	-	-	28.0 ± 3.0
Maternal BMI (kg/m2)	30.1 ± 12.34	31.5 ± 13.01	32.77 ± 12.93	28.7 ± 5.26	1.62 ± 0.35
Gestational age (weeks)	10.4 ± 2.57	20.82 ± 3.3	33.67 ± 2.57	38.9 ± 1.9	
Hormones assay					
Leptin (ng/ml)	21.24 ± 9*	26.3 ± 8.69*	23.29 ± 8.62*	17.36 ± 7.95*	12.4 ± 7.155
Progesterone (nmol/L)	107.99 ± 77.45	317.7 ± 219.9*	1877.4 ± 768.4*	31.86 ± 30.8*	10.76 ± 17.77
Estradiol (pmol/l)	11084 ± 14676*	87026 ± 106975*	185267 ± 198172*	502.89 ± 653	243 ± 228.6
βHCG (IU/L)	71906 ± 47829*	17455 ± 14033*	21298 ± 20174*	1654 ± 2638*	-

* statistically significant difference (p<0.05) compared to control, BMI - body mass index
βHCG - beta human chorionic gonadotropin, PA - postnatal

Table 2 - Pearson's correlation (r) and significance of correlation (P) between serum leptin concentrations and other parameters during different trimesters and after delivery.

Parameters	T1 r	T1 p	T2 r	T2 p	T3 r	T3 p	PA r	PA p
Age	0.21	0.16	0.19	0.225	0.426*	0.005	0.92	0.33
Maternal height	-0.13	0.21	0.001	0.5	0.078	0.33	0.21	0.16
Maternal weight	0.42*	0.003	0.32*	0.027	0.265	0.059	0.245	0.20
BMI	0.36*	0.01	0.3*	0.03	0.18	0.14	0.117	0.29
Progesterone	0.22	0.13	-0.15	0.18	-0.11	0.23	-0.08	0.34
E2	0.35	0.4	-0.23	0.085	0.03	0.43	0.23	0.18
βHCG	-0.16	0.13	0.15	0.28	-0.13	0.22	-0.48*	0.05

T1 - first trimester, T2 - second trimester, T3 - third trimester, PA - postnatal
BMI - body mass index, E2 - estradiol, βHCG - beta human chorionic gonadotropin

significantly decreased to 502.9 ± 652.99 pmol/l (p=0.0001) **(Table 1)** Serum concentration of βHCG decreased significantly among pregnant females from 71906 ± 47829 IU/L during first trimester to 17455 ± 14032 IU/L during 2nd trimester (p=0.001), while it insignificantly increased to 21298 ± IU/L during 3rd trimester (p=0.175). After delivery, βHCG sharply decreased to 1654 ± 2638 IU/L (P=0.003) **(Table 1)**. In the pregnant females there were no values recorded for LH and FSH hormones. The level of leptin, progesterone, E2 and βHCG were correlated with each other and with the demographic parameters using Pearson's correlation and the value of Pearson's Correlation Coefficient (r) and p values were obtained during the different stages of pregnancy **(Table 2)**. A significant correlation was observed between maternal BMI and leptin concentration during first trimester (r =0.362, p=0.01) and 2nd trimester (r=0.304, p=0.034). On the other hand, the correlation between leptin and BMI during 3rd trimester and at postnatal stage was statistically not significant. Maternal age and leptin concentration correlated positively only during the 3rd trimester (r=0.426, p=0.0025), and the correlation was statistically significant. During the first trimester a positive correlation was found between leptin and progesterone and E2 but the results were not significant while, during 2nd trimester a negative non-significant correlation was found between leptin and progesterone and E2. During 3rd trimester an insignificant negative correlation was found between leptin and progesterone, and a very weak correlation was found between leptin and E2. After delivery a negative but not significant correlation was found between leptin and

progesterone, and a positive non significant correlation was found between leptin and E2. With βHCG a non-significant correlation was observed during first trimester to 3rd trimester, however, after delivery there was a negative correlation, which was statistically significant. Interestingly, after delivery, a maternal leptin showed no correlation with both babies BMI and placenta weight ($p>0.05$). There was no significant correlation between the serum leptin and neonatal height ($p=0.34$), neonatal weight ($p=0.32$), neonatal BMI ($p=0.21$) and placental weight ($p=0.3$).

Discussion. Leptin is a protein which is predominantly produced by adipocytes. It plays an important role in regulation of body weight and reproductive functions.[1-3] Leptin has been shown to play a role in the regulation of pubertal onset, which is a phenomenon highly coupled to nutritional and metabolic status of mammals.[4-6] Results of our study showed that serum leptin concentrations are significantly elevated in pregnant females at all stages of pregnancy, compared to non-pregnant females. In the pregnant females, serum leptin levels are the lowest during the first trimester, but increase significantly from first trimester to 2nd trimester, while a slight insignificant decrease occurs during the 3rd trimester. After delivery the plasma leptin levels decreased significantly, reaching almost the non-pregnant levels within few days after delivery. These results confirm the previous reports by Hardiel et al[5] and Sivan et al,[6] who also showed elevated leptin levels during pregnancy and a significant decrease after delivery. Our results also showed that the changes in plasma leptin concentrations during pregnancy were independent of the concentration of other pregnancy hormones (such as progesterone, E2, βHCG), which showed a different pattern of variation during the 3 stages of pregnancy and after delivery. The serum βHCG levels reached a peak value during first trimester and from first trimester to 2nd trimester there was a significant decrease in its concentration, while during 3rd trimester there was a slight increase in its concentration, which was not significant. After delivery a sharp decrease occurred in serum βHCG concentrations. On the other hand, progesterone and E2 levels increased continuously throughout pregnancy until they reached a peak value during the 3rd trimester. After delivery there was a significant decrease in both serum progesterone and E2 levels. Several factors may contribute to the elevation in leptin concentrations during pregnancy in females. 1. A significant rise in serum leptin is probably due to the increase in body weight, which accompanies the pregnant state, and the accumulation of body fat in the first 2 trimesters of pregnancy (such as a positive correlation between leptin level, body weight and BMI during 1st and 2nd trimesters of pregnancy).[7-9] On the other hand, from the late second trimester to early third trimester, skin fold thickness begins to decrease at all sites as fat is mobilized to support fetal growth.[10] This observation can explain the slight though insignificant decrease observed, during our study, in leptin concentrations during the 3rd trimester. 2. This may be a consequence of an influence of βhCG on the leptin level. A stimulatory effect of βHCG on the production of leptin has been shown in vitro, and studies have confirmed that when adipose tissues are incubated with βHCG there is an induction of leptin secretion.[6] During the first trimester the increase in leptin and βHCG levels occur concomitantly. This may explain the increase of leptin concentrations in early pregnancy before the occurrence of any notable increase in body weight. 3. The fetoplacental unit may contribute to the circulating maternal leptin level.[11-13] 4. The increase of leptin levels during pregnancy may be due to a decease rate of leptin clearance from maternal circulation.[14,15] 5. Gestational hormones, most of all progesterone and E2, are also reported to stimulate leptin production by adipose tissue and studies in vivo carried out by Messinis et al,[16] reported that E2 and progesterone administration to normal cycling women causes significant increase of plasma leptin concentrations. Furthermore, in vitro, 5μg E2 administered to ovariectomized rats for 2 days significantly enhanced both leptin mRNA levels in adipose tissues and circulating leptin levels, over that of untreated controls.[17] In contrast, Wu-peng et al,[18] reported that no changes in transcription or leptin concentration occurred in ovariectomized rats receiving 2-5 E2 for 1-2 week. It has been shown that short term E2 replacement increases leptin levels in postmenopausal women.[19] However, long term E2 treatment does not have the same effect and a study reported that, serum leptin levels were depressed in women receiving E2 replacement therapy over several months.[20] Similarly, Grueso et al,[21] reported that there was no changes in plasma and cerebrospinal fluid leptin levels in female rats receiving progesterone over 30 days. Thus long term elevation of E2 may be the cause of inhibition of leptin secretion. This suggestions made from the results of the above mentioned studies may be used to explain the increase in leptin levels during first and 2nd trimester, when E2 and progesterone have not reached their peak levels, while a decrease in leptin level during the 3rd trimester when E2 and progesterone reach their peak values. 6. Studies have shown that insulin and cortisol increase with plasma leptin during pregnancy, and are associated with increased maternal lean and fat mass as well as with considerable changes in glucose metabolism.[22]

Sivan et al[6] noted a surge in leptin between 12 and 24 weeks of gestation, which correlated positively with cortisol level. Furthermore, in pre eclampsia there was a positive correlation between leptin and insulin concentrations.[23] 7. Increase in food intake, which is known to stimulate leptin levels may play a role during pregnancy.[24] As is well known there is frequently an increase in food intake with the advancement of pregnancy and this may lead to elevation in leptin levels with the advancement of pregnancy. Our study confirmed that a positive correlation occurs between plasma leptin level, maternal weight and BMI during the 3 stages of pregnancy. Leptin levels during the first trimester correlated significantly with maternal weight and BMI. A positive correlation was found between leptin and progesterone and E2 but the results were not statistically significant. Depending on our results, it is suggested that βHCG which positively correlates with progesterone levels during the first trimester, has a stimulating effect on progesterone secretion from placenta. Progesterone favors a positive energy balance by enhancing food intake and inhibiting the enhancement in CSF leptin level and thereby increases fat mass. Increase of both food intake and body fat mass may lead to increase of leptin levels during pregnancy.[25] During 2nd trimester, a significant correlation was observed between maternal weight and BMI, while in contrast, a negative non-significant correlation was found between leptin and progesterone, E2 and βHCG. The peak of maternal fat accumulation occurs during 20-30 week's gestation, which is associated with a peak value of leptin level. A negative correlation between leptin and progesterone and E2 may reflect inhibition effect of leptin on progesterone and E2 as seen in vitro, when the placental tissues were incubated with leptin there was a significant decrease in progesterone and E2 secretion into the media.[26] But the mechanism and the importance of this effect still remains largely unknown and needs more investigations for elucidation of the underlying mechanism and possible importance. During 3rd trimester, leptin levels correlated positively with maternal weight, and BMI. On the other hand, a negative correlation was found between leptin and progesterone and βHCG levels, while, no correlation was found between leptin and E2 levels. Previous studies have reported that from the late second trimester to early third trimester, skin fold thickness begins to decrease at all sites as fat is mobilized to support fetal growth[10] and this observation can explain the slight decrease in leptin concentrations during 3rd trimester as leptin concentration in plasma is more strongly correlated to total fat mass than to the BMI.

After delivery, a positive but not significant correlation was found between leptin and maternal weight, maternal BMI, while, a non-significant negative correlation was found between leptin and progesterone and placental weight. In contrast, significant negative correlation was found between leptin and βHCG. The possible reason for the negative correlation between leptin and placenta weight, progesterone and βHCG still remains largely unclear. The reversible correlation between maternal leptin concentrations and placental weight was reported by other studies.[6] However, Yura et al,[27] reported that leptin secretion during first trimester from placental villous tissue is approximately 50 folds greater than that by tissue collected at term. Thus suggesting that leptin should be higher initially during pregnancy compared to the later stages. However, we, and others did not obtain such findings.

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