Cord Blood Leptin Levels in Gestational Diabetes

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OBJECTIVE: To evaluate the cord leptin levels of newborns in relation to birthweight and Gestational Diabetes (GD) either regulated by insulin or diet.

STUDY DESIGN: A total of 62 patients were included in the study. Patients with impaired oral glucose tolerance test were included with diagnosis of GD (n=41). Control group consisted of women with normal plasma glucose level and 50-gr oral glucose challenge test (n=21). Twenty six of women with GD had blood glucose control by diet, while 15 required insulin. The blood samples were taken from the umblical cord of the newborns of GD and the control group for leptin levels.

RESULTS: Birthweight of babies born to healthy mothers, mothers with GD regulated by diet and insulin were 3197.14±348.2 gr, 3496.11±734.9 gr and 3642.86±843.8 gr, respectively (p=0.05). Percentages of macrosomic babies were 4.8%, 11.5% and 33.3% in healthy women, women with GD regulated by diet and insulin, respectively (p<0.05).). Cord blood leptin levels were higher in women with GD requiring insulin (6.1 ng/ml, range: 0.48-28.5) compared to women with GD regulated by diet (2.7 ng/ml, range: 0.5-54.7) and healthy mothers (2.0 ng/ml, range: 0.2-6.4, p<0.05). Cord blood leptin level was positively correlated with BMI (r:0.44, p<0.01), weight of mother (r:0.43, p<0.01), birthweight (r:0.37, p<0.05), and presence of GD and necessity of insulin (r:0.41, p<0.01).Cord blood leptin level was the only factor defining maternal GD requiring insulin in ROC analysis (AUC:0.77, p<0.01). Cord blood leptin level equal to or higher than 4.1 ng/ml had a sensitivity of 80% and specificity of 79% in defining GD with insulin requirement (positive likelihood ratio:3.68)

CONCLUSION: In this study there is a minimal clinical effect of cord blood leptin on macrosomia in women with GD, although it is increased in GD and associated with birthweight. Therefore overgrowth may be a result of direct anabolic effect of insulin, rather than indirect effect via leptin.

Key Words: Gestational diabetes mellitus, Leptin.

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Introduction

Leptin, encoded by ob gene in adipocytes, is a polypeptide hormone regulating energy expenditure.[1] Leptin levels are continuously increased with a state of relatively leptin resistance during pregnancy.[2] Deviations of leptin levels from the normal values expected in pregnancy can be associated with pathophysiological changes of preeclampsia, abortion. An important site of non-adipocyte production of leptin is found to be placenta and a significant amount of leptin is found in cord blood which is secreted by fetus.[3,4]

Maternal metabolic factors may be independent factors influencing fetal growth, aside from maternal weight gain during pregnancy and maternal constitution. Glucose intolerance including gestational diabetes (GD) in pregnancy is associated with fetal macrosomia and this effect of metabolic disturbances in glucose regulation seems to be independent of the maternal size.[5] Objective of the present study was to compare cord blood leptin levels in relation to birthweight and GD either regulated by insulin or diet.

Material and Method

A total of 62 patients admitted to Zekai Tahir Burak Women's Health Research and Education hospital between 2003 and 2004 were included. Local ethical committee of the hospital approved the study and informed consents were ob-

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tained from all patients. The diagnosis of GD was based on 100 gr oral fasting glucose tolerance test of National Diabetes Data group (100 gr oral glucose tolerance test).[6] All the samples for 100 gr oral glucose tolerance tests were collected at 24-28 gestational ages from women with plasma glucose higher than 130mg/dl or higher than 140mg/dl one hour after 50 g load of glucose. Patients with impaired 50 gr oral glucose challenge test and normal 100 oral glucose tolerance test were excluded from study. Patients with impaired oral glucose tolerance test were included with diagnosis of GD (n=41). Control group consisted of women with normal plasma glucose level and 50-gr oral glucose challenge test (n=21). Twenty six of women with GD had blood glucose controlled by diet, while 15 required insulin.

Demographic and obstetrical characteristics of patients, and body mass indexes (body weight (kg)/(body height (m))^2 were recorded. Blood samples were taken from the umbilical cord of the newborns of healthy women and women with GD in five minutes after the labor and before the placenta were separated. The blood samples were centrifuged at 5000 rpm for 5 minutes and serum samples were stored at -70 °C until further analysis. Serum leptin levels were analyzed by a solid phase sandwich Enzyme-Linked ImmunoSorbent Assay kit (Leptin ELISA kit Hu, BioSource International, Inc., California, USA), catalog number KAP 2281) which detects leptin at a minimum of <3.5 pg/ml.

Kolmogorov-Smirnov test was performed to assess normality of data. One-way ANOVA were used to compare continuous data and expressed as mean ± standard deviation. Kruskal-Wallis test were used to compare continuous variables and expressed as median and range. The chi square test was used for non-continuous data. Spearman and Pearson correlation tests were applied to reveal if there was any correlation. Regression models were used to assess the contribution of various predictors on cord blood leptin level and birthweight. ROC analyses were performed to designate cut-off value with sensitivity and specificity. Likelihood ratio test was further used to analyze clinical impact of factors on macrosomia. The level of significance was chosen to be $p < 0.05$. Data were analyzed using the Statistical Package for Social Sciences, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographical and obstetrical parameters are shown in table I. Mean age of healthy women (25.3±4.6 years-old) was significantly lower than women with GD either regulated by diet (31.0±4.4 years-old) or insulin (30.2±3.7 years-old) ($p<0.05$). Gravida, para, gestational age at birth, percentage of smoking mothers and height measurements were similar. Weight of women with GD requiring insulin was higher than healthy mothers ($p=0.05$). However, BMI of women included in the study were not significantly different. Forty-three percent and 28.6% of healthy mothers were obese (BMI>30) and overweight (25<BMI<30), respectively. Fifty percent and 30.8% of women with GD regulated by diet were obese and overweight, respectively ($p>0.05$). Sixty-seven percent and 26.7% of women with GD regulated by insulin were obese and overweight, respectively ($p>0.05$). Weight gain during pregnancy was not different in study groups and healthy controls. Although statistically not significant, history of GD in previous pregnancies was increased in women with GD compared to healthy women (Table 1).

Table 1: Demographical and obstetrical features of patients included in the study

	GD regulated by diet	GD requiring insulin	Healthy	p value
Age	31.0±4.4	30.2±3.7	25.3±4.6	<0.05†
Gravida	3 (1-7)	2 (1-6)	2 (1-7)	>0.05‡
Para	1 (0-3)	1 (0-3)	1 (0-2)	>0.05‡
Height (cm)	159 (145-175)	158 (150-175)	157 (149-178)	>0.05‡
Weight (kg)	75(48-110)	80 (57-124)	68 (55-103)	=0.05‡
BMI	30.7±6.2	31.7±5.6	28.4±4.2	>0.05†
Smoking	11.5% (n=3)	13.3% (n=2)	23.8% (n=5)	>0.05א
GA	39 (36-41)	38 (36-39)	39 (36-41)	>0.05‡
Weight gain(kg)	12 (8-24)	14 (9-20)	12 (5-18)	>0.05‡
History of GD	15.4% (n=4)	13.3% (n=2)	n=0	>0.05א
Birthweight (gram)	3496.11±734.9	3642.86± 843.8	3197.14± 348.2*	=0.05†
Macrosomic fetus	11.5% (n=3)	33.3% (n=5)	4.8% (n=1)	<0.05א
Sex	69.2%(n=18) male	53.3%(n=8) male	52.4% (n=11) male	>0.05א
Cord Blood Leptin (ng/ml)	2.7 (0.5-54.7)	6.1(0.48-28.5)	2.0 (0.2-6.4)	<0.05‡

† One- way ANOVA analysis (mean±standard error), ‡ Kruskal-Wallis analysis [median (range)], א Ki-square analysis. Alpha level:0.05.

Male-female ratio were similar in healthy women and women with GD. Birthweight of babies born to healthy mothers, mothers with GD regulated by diet and insulin were 3197.14±348.2 gr, 3496.11±734.9 gr and 3642.86±843.8 gr, respectively (p=0.05). Percentages of macrosomic babies (birthweight higher than 4000gr) were 4.8%, 11.5% and 33.3% in healthy women, women with GD regulated by diet and insulin, respectively (p<0.05). Cord blood leptin levels were higher in women with GD requiring insulin (6.1 ng/ml, range: 0.48-28.5) compared to women with GD regulated by diet (2.7 ng/ml, range: 0.5-54.7) and healthy mothers (2.0 ng/ml, range: 0.2-6.4, p<0.05).

Cord blood leptin level was positively correlated with BMI (r:0.44, p<0.01), weight of mother (r:0.43, p<0.01), birthweight (r:0.37, p<0.05), and presence of GD and necessity of insulin (r:0.41, p<0.01). The association between leptin and these factors were not linear. Regression analysis yielded that heavier mothers and babies have more levels of cord blood leptin (p<0.01). Besides, as the BMI and severity of GD increases, cord blood leptin levels increases (p<0.01). (Figure I.)

Cord blood leptin level was the only factor defining maternal GD requiring insulin in ROC analysis (AUC:0.77, p<0.01). Cord blood leptin level equal to or higher than 4.1 ng/ml had a sensitivity of 80% and specificity of 79% in defining GD with insulin requirement (positive likelihood ratio:3.68). None of the parameters were useful to distinguish GD regulated by diet from healthy mothers and mother with GD regulated by insulin.

Birthweight and fetal macrosomia were found to be correlated with cord blood leptin level, presence of GD and insulin requirement, gestational age at birth, weight of mother, BMI, history of GD in previous pregnancies. Birthweight of babies born to women with GD regulated by insulin was higher than the others (p<0.05). Higher the cord blood leptin levels, gestational age, maternal weight, BMI and presence of history of GD was associated with higher birthweight.

Best cut-off value for cord blood leptin level to diagnose fetal macrosomia was found to be 3.3 ng/ml at a sensitivity of 66% and specificity of 62% (positive likelihood ratio:2.0). Weight of mother higher than 78 kg had a sensitivity of 77% and specificity of 70% (positive likelihood ratio:2.5). Likelihood ratios of other factors correlated with macrosomia is shown at table II.

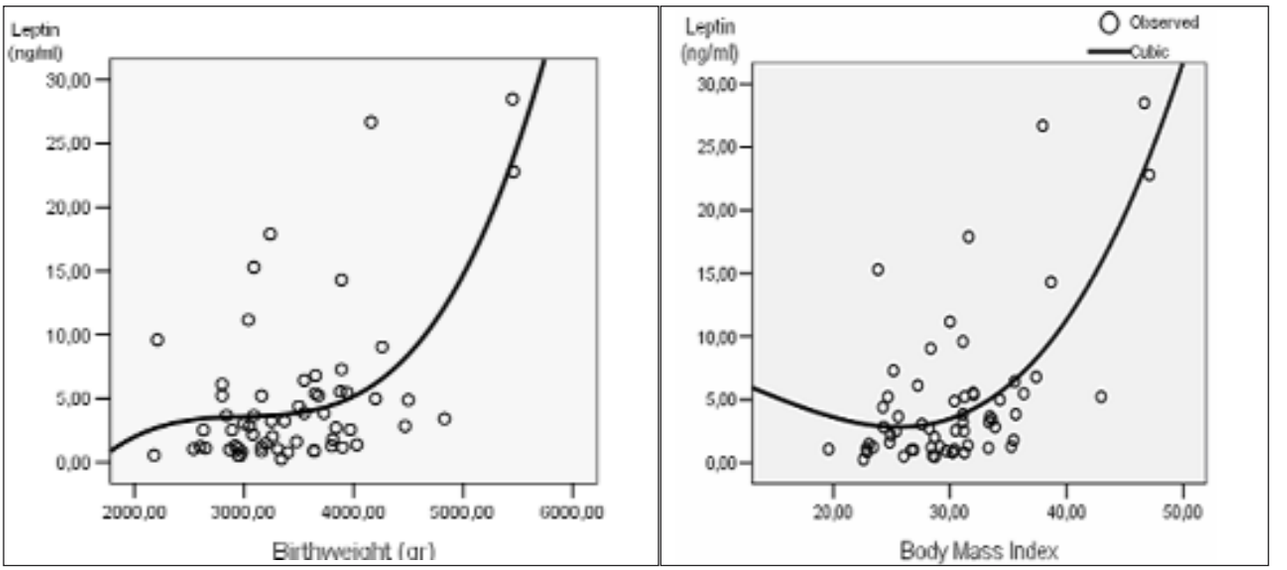

Figure I. Factors (birthweight and BMI of mother) significantly affecting cord blood leptin in regression analysis.

Table 2: Likelihood ratios of predictors on fetal macrosomia

Predictors of Fetal Macrosomia	Positive Likelihood Ratio	Negative Likelihood Ratio
Cord Blood Leptin: ≥3.3 ng/ml	2.0	0.3
Presence of insulin requiring GD	2.8	0.5
BMI >30	1.9	0.2
History of GD in previous pregnancies	5.7	0.7
Weight of mother >78 kg	2.5	0.3

Impact on likelihood 1:no change, >10 or <0.1:high, 5-10 or 0.1-0.2: moderate, 2-5 or 0.2-.5: small, <2 or >0.5 tiny.

Discussion

In the present study, cord leptin level in babies born to mothers with GD and factors effecting birthweight are studied. Leptin in umbilical cord blood originates exclusively from fetal and/or placental sources.[7-9] Fetal leptin concentrations are lower than maternal levels and no correlation appears to exist between maternal peripheral concentrations and cord levels.[10] Okereke et. al reported that there was no significant difference in cord leptin concentration between male and female neonates, in accordance with our results.[11] It is reported that leptin is highly related to the nutritional status already during the fetal and neonatal periods.[12] Wiznitzer et. al reported that there is a statistically significant correlation between plasma leptin levels and neonatal birthweight.[13] Devaskar et.al reported that fetal ovine white adipose tissue leptin mRNA amount are regulated by fetal development and circulating insulin concentrations.[14] In consistent with previous studies,[7,12-17] we found positive correlations between birthweight and cord blood levels of leptin. But the present study showed that the impact of cord blood leptin on macrosomia could be clinically neglected.

Farhead et.al reported that leptin infusion did not affect linear skeletal growth or body, placental and organ weight in ovine fetus.[18] Fang et.al reported that the serum levels of neuropeptide-Y (NPY), insulin and insulin like growth factor 1(IGF-1) had positive correlations with leptin levels as well as body mass index at early periods of life, which suggested that NPY, insulin and IGF-1 cooperating with leptin might take part in the regulation of development of premature infants [19]

Many fetuses in pregnancies complicated by GD display accelerated intrauterine growth, so their birth weight exceeds the normal range. Normal maternal glucose levels have usually been considered as the main target of any protocol for the management of pregnancies complicated by GD. However, fetal overgrowth may occur in pregnancies complicated by GD despite rigorous glycemic control in modern clinical management of these patients.[7] In the present study, cord leptin levels increased if the mother has GD and insulin requirement.

Gender-specific differences with higher leptin concentrations in females than in males have also been recognized in children and adults.[20,21] This may be a result of different proportion of body fat between females and males. Laml et. al reported that leptin concentrations in female newborns are higher compared to male newborns.[22] However, total body fat is similar in both sexes in neonates.[23] Shekhawat et. al reported that there are no gender differences in cord blood leptin,[16] in accordance with our results.

In conclusion, our results show a minimal clinical effect of cord blood leptin on macrosomia in women with GD, although it is increased in GD and associated with birthweight. Increased neonatal weight of babies born to mothers with GD may involve another pathogenesis other than leptin. Therefore, it may be hypothesized that overgrowth may be a result of direct anabolic effect of insulin, rather than indirect effect via leptin. Further studies are needed to validate our results and document the role of leptin on birthweight.

Gestasyonel Diyabette Kordon Leptin Seviyeleri

AMAÇ: İnsülin ya da diyetle regüle edilen gestasyonel diabette (GD) yenidoğan kordon leptin seviyelerinin doğum ağırlığına göre değerlendirilmesi.

GEREÇ VE YÖNTEM: 62 hasta çalışmaya dahil edildi. Oral glukoz tolerans testi bozuk olan 41 GD hastası çalışmaya dahil edildi. Glukoz seviyesi ve 50 gram oral glukoz tolerans testi normal olan 21 vaka control grubunu oluşturdu. GD hastalarında kan glukoz seviyesi 26 vakada dietle, 15 vakada insülinle kontrol altına alındı. Leptin seviyesi için kan örnekleri yenidoğanların umblikal kordonlarından alındı.

BULGULAR: Sağlıklı, diyetle kontrol edilen ve insülinle kontrol altına alınan vakaların yenidoğanlarının doğum kiloları sırasyla 3197,14±348,2 gr, 3496,11±734,9 gr ve 3642,86±843,8 gr, idi (p=0.05). Sağlıklı, diyetle kontrol edilen ve insülinle kontrol altına alınan vakaların yenidoğanlarında makrozomi oranları sırasıyla 4,8%, 11,5% ve 33,3% olarak bulundu (p<0,05). İnsüline ihtiyaç duyan GD hastalarında (6,1 ng/ml, 0,48-28,5) diyetle regüle GD hastalarına (2,7 ng/ml, 0,5-54,7) ve sağlıklı annelere (2,0 ng/ml, 0,2-6,4) göre kordon leptin seviyeleri daha yüksekti. (p<0,05). Kordon leptin seviyesi ile vücut kitle indeksi (r:0,44, p<0,01), anne kilosu, (r:0,43, p<0,01), doğum ağırlığı (r:0,43, p<0,01), GD varlığı ve insulin ihtiyacı (r:0,41, p<0.01) arasında pozitif korelasyon saptandı. ROC analizinde kordon leptin seviyesi insulin bağımlı GD için tek tanımlayıcı faktördü (AUC:0,77, p<0,01). Kordon leptin seviyesinin 4.1 ng/ml'ye eşit ya da büyük olması 80% sensitivite ve 79% spesifite ile insulin bağımlı GD tanımlıyordu.

SONUÇ: Bu çalışmada kordon leptin seviyeleri GD'te yükseldiği ve doğum ağırlığı ile ilişkili olduğu halde GD hastalarında makrozomi üzerinde minimal klinik etkisi bulunmuştur. Bu nedenle makrozomi leptinin indirek etkisinden çok insülinin direk anabolic etkisinden kaynaklanabilir.

Anahtar Kelimeler: Gestasyonel diabetes mellitus, Leptin

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