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Cord blood sex hormones concentration: relation to birth weight and pregnancy complications

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Objectives Umbilical cord blood can be taken at birth and largely gives indication of fetal and maternal conditions. The aim of the study was to investigate the relation between sex hormones in cord blood and birth weight of newborns and pregnancy complications.
Methods Fifty cord blood samples were collected from newborns at labor room of Baghdad Teaching Hospital between May and October 2018. Blood was withdrawn from their mothers for lead analysis. Five milliliters (ml) of cord blood was taken, 3 ml was used for testosterone and estradiol analysis (using enzyme-linked immunosorbent assay) and 2 ml for lead measurement by lead care analyzer. Newborns weight and head circumference were measured. Delivered women were divided into four groups: Women with normal pregnancy, women with pre-eclampsia, diabetic women and polycystic ovary syndrome (PCOS) women.
Results There was no significant difference in age between women in all groups ($P > 0.05$). Birth weights, estradiol, and testosterone were significantly different between groups. Estradiol was higher in cord blood of newborns of PCOS women ($P < 0.05$) than others. Testosterone was higher in cord blood of babies of PCOS and pre-eclampsia women compared with those of diabetes ($P < 0.05$). There were no significant differences between male and female neonates regarding cord estradiol (3596.27 ± 1934.69, 3714.57 ± 1581.47 pg/ml respectively), and testosterone (393.18 ± 87.14, 361.43 ± 102.14 ng/ml respectively) ($P > 0.05$). Maternal lead levels correlated positively with cord lead ($r = 0.905$, $P < 0.05$), which correlated negatively with head circumference ($r = -0.766$, $P < 0.05$). Birth weight correlated negatively with estradiol ($r = -0.295$), but positively with testosterone ($r = 0.006$) ($P > 0.05$).
Conclusion Cord blood estradiol and testosterone levels do not differ between males and females. Estradiol was high in cord blood of PCOS mothers. Testosterone was high in cord blood of PCOS and pre-eclampsia mothers. The increase in cord lead causes decrease in babies head circumference.
Keywords cord blood, pregnancy, birth weight, estradiol, testosterone

Introduction

Pregnancy is a special stage of life in which circulating hormone levels are obtained from maternal, placental, and fetal origins. The placenta is a steroidogenic organ which has a role in the synthesis of great quantities of free androgens and estrogens from the adrenals of the fetus and gonadal sources.[1,2] Steroids are lipophilic substances and can pass placental barrier in both directions.[3] Fetal blood goes out of the placenta (full of steroids) through the umbilical vein and goes back from the fetus to the placenta through the umbilical artery.

Measuring the hormones in maternal blood does not actually reflect the concentrations in fetal circulation.[4] In addition, measuring hormone concentration through amniocentesis does not give exact indication and it is invasive procedure. For these reasons cord blood is currently the practical way to measure fetal hormones.[5]

Cord blood (both serum and plasma) is a specially challenging medium to measure because it has unique steroid concentrations due to the mix of placental and fetal steroid production and metabolism.[6]

Umbilical cord blood is usually obtained after delivery, so the hormone levels in the cord plasma or serum are said to represent the levels in the fetal circulation near the end of pregnancy.[7] Umbilical cord blood has about equal quantities of venous and arterial contents, in spite of the fact that the relative amounts are not yet estimated exactly.

It was noted that the estimation of umbilical cord hormones is influenced by several obstetric and maternal factors.[1]

Sex hormone levels in fetal blood have been the subject of interest over many years because of their relation to maternal metabolic disorders, cancer risk later in life, reproductive, and behavioral/neurodevelopmental disorders.[7]

The aim of this study was to investigate the relation between sex hormones in cord blood and birth weight of the baby and pregnancy complications.

Patients and Methods

In this prospective study, 50 cord blood samples had been collected from the labor room of Baghdad teaching hospital in the period between May 2018 and October 2018. An informed consent was taken from all pregnant women. The work was approved by the Ethical Committee of College of Medicine/ University of Baghdad. It was in agreement with the Helsinki Declaration of 1975 that was revised in 2000. All pregnancies were singleton.

Immediately after birth, the umbilical cord was clamped and cut. Another clamp was put 20–25 cm from the first one. The part between the clamps was cut and a mixed arterial and venous blood sample (5 ml) was collected into a plastic tube. About 2 ml of each sample was analyzed for lead using lead care analyzer device and 3 ml of each sample was centrifuged and kept in −20°C till the time of analysis of estradiol and tess tosterone by enzyme-linked immunosorbent assay (ELISA). At the same time whole blood (2 ml) was drawn from the pregnant women for lead measurement. There is no normal level of lead in blood since it does not constitute the human body. Little exposure to lead in adults are not thought to cause poisoning [blood lead <10 micrograms (μg) per deciliter (dl)], but it can harm on the long term.[8]

Testosterone and estradiol concentrations were measured by ELISA (Cloud–Clone Corp., CEA461Ge, using IMMULITE 2000 XPi, Siemens) as showed in Fig.1.

Full medical and obstetrical history was taken from the pregnant women, including age, pregnancy complications like diabetes (already having or gestational diabetes confirmed by fasting blood sugar), pre-eclampsia (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥90 mmHg, measured after 20 weeks of pregnancy, Proteinuria ≥ 0.3 g or more in 24 h urine),[9] or having polycystic ovary syndrome (PCOS) (diagnosed according to Rotterdam criteria).[10]

Gestational age was determined by directly counting days since the start of the last menstrual period or by early pregnancy ultrasound. Normal pregnancy period ranges from completed 37 to 42 weeks.[11] Gender of the baby was also recorded.

The weight of the placenta and the baby were measured using digital balance in the place. Normal range of birth weight is between 2.5 and 5 kg,[12] and placenta ideally weighs about 500 g. It was weighed after removing the membranes and cutting the cord.[13] Head circumference was recorded using measuring tape (normal head circumference of newborn is about 35 cm).[14]

Maternal and cord blood lead measurements were achieved by lead care analyzer, that contains the lead care kit, and the Lead Care Analyzer Device version 3.3 (ESA, Inc., USA). A total of 50 µl of whole blood was put in the EDTA tubes to be mixed with treatment reagent in the tubes provided by the kit. The blood was mixed with the reagent, so the color of the mixture became brown. Then the tubes were left to stand up for a minute giving the chance for the mixture to drain down to the bottom. By a pipette, 35 µl of the mixture was drawn and put on the device sensor after calibration and the process of analysis began.[15]

Statistical Analysis

Data were analyzed by SPSS version 20. Analysis of variance (ANOVA) test was applied for comparing parameters between groups, results were expressed as mean ± standard deviation. Correlations were analyzed by Pearson's correlation test. A *P*-value of <0.05 was considered significant in this study.

Results

Descriptive data concerning all pregnant females was shown in Table 1.The mean age was 29.66 ± 4.94 ranging between 22 and 40 years. Mean body mass index (BMI) was 24.8 ± 2.08. The study included 22 males and 28 females. There were 18 normal pregnancies, 13 with pre-eclampsia, 8 with diabetes and 11 with PCOS.

A comparison between normal pregnancy and those pregnancies of women with pre-eclampsia, diabetes and PCOS was done (Table 2). There was no significant difference between the groups regarding age. BMI was significantly different between the groups. Birth weights were significantly higher in babies of diabetic patients compared with other groups (Table 2). Estradiol was significantly higher in cord

Fig. 1 **The device used to assess estradiol and testosterone concentration.**

Table 1. **Descriptive data for all patients.**

Parameter	Mean ± SD
Age (years)	29.66 ± 4.94
BMI (kg/m²)	24.8 ± 2.08
Birth weight (kg)	3.25 ± 0.56
Gender	
Males	22
Females	28
Estradiol (pg/ml)	3794.52 ± 1775.62
Testosterone (ng/ml)	359.60 ± 102.34
Diseases	
Normal pregnancy	18
Pre-eclampsia	13
Diabetes	8
PCOS	11
Blood lead (mg/dl)	
Maternal	3.03 ± 1.06
Cord	0.38 ± 0.30
Gestational age (weeks)	38.35 ± 1.08
Head circumference (cm)	37.28 ± 0.56
Placental weight (g)	529.46 ± 29.57

PCOS: Polycystic ovary syndrome, BMI: body mass index, SD: standard deviation.

Table 2. **Comparison of age, BMI, birth weight, estradiol and testosterone levels in cord blood and gestational age between groups**

	Normal pregnancy (*n* = 18)	Pre-eclampsia (*n* = 13)	Diabetes (*n* = 8)	PCOS (*n* = 11)	*p*
Age (years)	30.83 ± 3.07	28.15 ± 3.69	29.75 ± 5.78	27.55 ± 3.17	>0.05
BMI (kg/m²)	24.76 ± 2.10	25.34 ± 1.99	23.50 ± 1.60	25.50 ± 2.23	<0.05
Birth weight (kg)	3.80 ± 0.46	3.29 ± 0.39	4.77 ± 7.20	3.26 ± 0.23	<0.05
Estradiol (pg/ml)	4014.56 ± 1902.29	3904.92 ± 1806.48	1800 ± 840.52	4154.55 ± 1103	<0.05
Testosterone (ng/ml)	351.28 ± 64.87	364.92 ± 89.46	257 ± 36.42	477.27 ± 46.92	<0.05
Gestational age (weeks)	38.66 ± 0.99	38.09 ± 1.17	37 ± 0.1	39.13 ± 0.1	<0.05

PCOS: polycystic ovary syndrome, BMI: body mass index.

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Gestational age	PCOS	11	39.1273	0.10090	0.03042
	NO	18	38.6556	0.99186	0.23378

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Gestational age	diabetes	8	37.0000	0.00000	0.00000
	NO	18	38.6556	0.99186	0.23378

Group statistics					
	Diseases	N	Mean	Std. Deviation	Std. Error Mean
Gestational age	Pre-eclampsia	13	38.0923	1.16795	0.32393
	NO	18	38.6556	0.99186	0.23378

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Testosterone	PCOS	11	477.2727	46.92354	14.14798
	NO	18	351.2778	64.86530	15.28890

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Testosterone	Diabetes	8	257.0000	36.42213	12.87717
	NO	18	351.2778	64.86530	15.28890

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Testosterone	Pre-eclampsia	13	364.9231	89.46364	24.81275
	NO	18	351.2778	64.86530	15.28890

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Estradiol	PCOS	11	4154.5455	1103.05361	332.58318
	NO	18	4014.5556	1902.29262	448.37467

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Estradiol	Diabetes	8	1800.0000	84.51543	29.88072
	NO	18	4014.5556	1902.29262	448.37467

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Estradiol	Pre-eclampsia	13	3904.9231	1806.47513	501.02606
	NO	18	4014.5556	1902.29262	448.37467

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
BWT	PCOS	11	3.2636	0.22923	0.06911
	NO	18	4.7694	7.20651	1.69859

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
BWT	Diabetes	8	3.8000	0.45981	0.16257
	NO	18	4.7694	7.20651	1.69859

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
BWT	Pre-eclampsia	13	3.2923	0.39256	0.10888
	NO	18	4.7694	7.20651	1.69859

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
BMI	Diabetes	8	23.5000	1.60357	0.56695
	NO	18	24.7588	2.10284	0.49564

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
BMI	Pre-eclampsia	13	25.3374	1.99897	0.55441
	NO	18	24.7588	2.10284	0.49564

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
BMI	PCOS	11	25.5009	2.23358	0.67345
	NO	18	24.7588	2.10284	0.49564

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Age	Pre-eclampsia	13	28.1538	3.69338	1.02436
	NO	18	30.8333	3.07265	0.72423

Not

Group statistics					
	Diseases	N	Mean	Std. deviation	Std. error mean
Age	diabetes	8	29.7500	5.77556	2.04197
	NO	18	30.8333	3.07265	0.72423

E2

ANOVA					
Diseases					
	Sum of squares	df	Mean square	F	Sig.
Between groups	65.453	15	4.364	89.017	0.000
Within groups	1.667	34	0.049		
Total	67.120	49			

Test

ANOVA					
Diseases					
	Sum of squares	df	Mean square	F	Sig.
Between groups	54.231	16	3.389	8.678	0.000
Within groups	12.889	33	0.391		
Total	67.120	49			

blood of fetuses of PCOS mothers than those of pre-eclampsia, diabetes and normal pregnancy (4154.55 ± 1103, 3904.92 ± 1806.48, 1800 ± 840.52, 4014.56 ± 1902.29 respectively). On the other hand, testosterone was significantly higher in babies of PCOS patients and pre-eclampsia compared with those of diabetes and normal pregnancy (477.27 ± 46.92, 364.92 ± 89.46, 257 ± 36.42, 351.28 ± 64.87 respectively).

Males and females cord blood were compared for estradiol and testosterone levels and nonsignificant differences was found between them although testosterone was higher in male newborns (Table 3).

Cord blood lead correlated positively and significantly with maternal lead, but negatively and significantly with babies' head circumference (Table 4). Birth weight correlated negatively with estradiol and positively with testosterone in the cord blood, however both correlations were not significant (Table 5).

Gestational age had a nonsignificant positive correlation with estradiol, but a significant negative correlation with testosterone (Table 6).

Discussion

Cord blood sex hormones measurements and their relations to maternal complications and fetal birth weight were studied in this work. Birth weight was higher in babies of diabetic mothers, this agrees with other studies that reported high birth weight in babies born to diabetic mothers.[16,17] It was reported that infants of diabetic women are at high risk of being overweight and they may become obese at a young age.[18]

Polycystic ovary syndrome women had their fetuses with a cord blood significantly higher in estradiol and testosterone. This result goes with that found by Daan et al.[19] who suggested that high androgen environment in pregnancy of PCOS women may increase androgen in their fetuses. The high insulin levels in pregnant women who have PCOS predispose to the increase in fetal androgen and this is due to the inhibition of placental aromatase action that makes the conversion of the androgen in maternal and fetal circulation to estrogens low.[20] Other studies had also reported increase androgen in cord blood of babies born to PCOS mothers.[21,22] On the other hand some studies found a decrease in androgen concentrations[23,24] or observed no differences in comparison to controls.[25] The explanation for the decreased androgens in cord blood may indicate a modification of fetal steroid metabolism,[23] and the differences in the amount of testosterone may be affected by variations in the tissue activity of placenta in PCOS. In addition, there are differences in the diagnostic criteria of PCOS, differences in ethnicity and in the applied statistical tests.

Cord blood testosterone was higher in fetuses of mothers with pre-eclampsia than in those born for normal mothers, this was in accordance with the results of Chinnathambi et al. who reported a high testosterone in plasma of women with pre-eclampsia and in cord blood of their fetuses and they attributed that to the effect of testosterone on vascular endothelia since they changed vascular adaptation throughout pregnancy by decreasing nitric oxide effect as a vasodilator, in addition to the fact that testosterone increases vascular resistance in these patients. Also, testosterone suppresses the relaxation of blood vessels induced by acetylcholine in mesenteric arteries.[26] Other studies also demonstrated positive correlation between cord blood testosterone and high blood pressure in the mother.[27,28]

Estradiol was high in cord blood of fetuses of PCOS mothers, this goes with Caanen et al.'s[29] study, they reported a disturbance in the function of placental enzymes in PCOS, especially aromatase which catalyze the conversion of 16-hydroxytestosterone to estradiol.

On the other hand, Kallen[30] showed that estradiol is low in cord blood of PCOS women. Estrogen levels in cord blood are affected by fetal adrenal and placental steroidogenesis, and decreased estradiol levels in cord blood of babies of PCOS women was due to abnormal placental steroidogenesis.[31]

There were no differences in cord blood estradiol and testosterone between male and female fetuses, this agrees with other studies which reported that estrogen in umbilical cord is not significantly different between males and females,[24,32–34] while other studies found higher estradiol levels in females cord blood,[35] and higher testosterone

Table 3. **Comparison of estradiol and testosterone concentrations in cord blood between males and females**

	Males ($n = 22$)	Females ($n = 28$)	P
Estradiol (pg/ml)	3596.27 ± 1934.69	3714.57 ± 1581.47	>0.05
Testosterone (ng/ml)	393.18 ± 87.14	361.43 ± 102.14	>0.05

Table 4. **Correlations between cord blood lead and maternal lead, cord blood lead and newborns' head circumference**

	Cord blood lead (mg/dl)	
	r	p
Maternal lead (mg/dl)	0.905	<0.05
Head circumference (cm)	−0.766	<0.05

Table 5. **Correlations between cord blood estradiol and testosterone with birth weight**

	Birth weight (Kg)	
	r	P
Cord blood estradiol (pg/ml)	−0.295	>0.05
Cord blood testosterone (ng/ml)	0.006	>0.05

Table 6. **Correlation between cord blood estradiol, testosterone and gestational age**

	Gestational age (weeks)	
	r	P
Cord blood estradiol (pg/ml)	0.830	>0.05
Cord blood testosterone (ng/ml)	−0.703	<0.05

concentrations in males.[35,36] These findings were in assumption that they are reflection of their levels in utero in the early pregnancy stages, which refers to the exposure to high androgen during fetal development that occurs in male more than female fetuses.[37]

Exposure of pregnant females to lead is one of the important factors affecting pregnancy outcome. In this study there was significant increase in cord lead with the increase in maternal lead levels, this agrees with a study that reported a significant positive correlation between maternal and cord lead.[38] Lead level correlated negatively and significantly with neonates' head circumference which was also found by Akbari-Nassaji et al.[39] On the other hand some studies found no significant relationship between cord blood lead and head circumference.[40,41]

Cord blood estradiol correlated negatively but not significantly with birth weight, this was found in another study which suggested that this correlation is modified by some adjustment of insulin like growth factor level in these fetuses.[42] Whereas other studies documented that the greater estradiol levels were obtained from cord blood of high birth weight babies.[31,43]

Testosterone in cord blood correlated positively with birth weight which was found by another study that assumed testosterone effect from early in utero period.[44] Keelan et al.,[7] reached the same result in their work that studied the metabolic changes in PCOS women who had increased testosterone levels in cord blood of their babies and a corresponding increase in their birth weights. Another study found no relation between birth weight and androgen levels.[22]

However, factors affecting fetal growth and birth weight that is related to sex hormones are complicated and have many aspects, which makes it difficult to determine.

The negative correlation between cord blood testosterone and gestational age had met results of other studies who emphasizes on testosterone and other androgen types.[21,45] This correlation is related partly to the amount of sex hormone binding globulins (SHBG) that have positive correlation with gestational age, and their increase will cause the decrease in testosterone which explains our results.[45] The correlation of estradiol with gestational age had not been demonstrated clearly in other studies [43] thus more work to investigate this correlation is needed.

Using ELISA for analyzing sex steroids could be considered as a limitation since it was suggested that mass spectrometry method is more accurate and can detect lower androgen concentrations than ELISA.[7] Also, determination of biologically active fractions of sex steroids by adjusting albumin and SHBG concentrations during gestation was not done in this study, nonetheless, it is important in order to have more valid conclusions.[44]

Conclusion

Cord blood estradiol and testosterone levels do not differ significantly between male and female fetuses. Estradiol was significantly high in cord blood of neonates of PCOS mothers. Testosterone was significantly high in cord blood of newborns of PCOS and pre-eclampsia women. The increase in cord lead causes decrease in neonates' head circumference.

Conflict of Interest

None.

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