Evaluation of serum granulocyte colony stimulating factor levels in infants of preeclamptic mothers

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In this study we aimed to evaluate the relationship between serum granulocyte colony stimulating factor (G-CSF) levels and absolute neutrophil counts (ANC) in infants of preeclamptic mothers.

The study group consisted of 31 infants of preeclamptic mothers while the control group consisted of 24 gestational age-adjusted infants of normotensive mothers. G-CSF levels were determined by enzyme-linked immunosorbent assay (ELISA).

The mean G-CSF level was 981.8±1682.5 (25.7-5924) pg/ml in the study group and 770.8±1779 (18-8526) pg/ml in control group (p>0.05).

There was no correlation between G-CSF levels and absolute or total neutrophil counts on the 1st, 2nd and 7th days in infants of preeclamptic mothers. There were positive correlations between G-CSF levels and ANC on the 1st and 7th days of life in infants of normotensive mothers. Neutropenia developed in 42.3% of the study group and in 21.7% of the control group on the 1st day of life (p>0.05). On the 2nd day, neutropenia was observed in 61.5% of the study group and 26.1% of the control group (p=0.013).

Serum G-CSF levels were not low in neutropenic babies of preeclamptic mothers. In contrast, higher G-CSF levels in neutropenic infants suggest impaired G-CSF response in infants of preeclamptic mothers.

Key words: preeclampsia, newborn, neutropenia, granulocyte colony stimulating factor, very low birth weight.

Preeclampsia is a syndrome of unknown etiology that is characterized by the sequential development of facial and hand edema, hypertension, and significant proteinuria after the 20th week of gestation[1]. It occurs in 2-8% of all pregnancies[2-4]. Preeclampsia is a major cause of both maternal and perinatal morbidity and mortality, and probably more than 50,000 maternal deaths per year can be attributed to preeclampsia[4]. Neutropenia is present in 40-50% of infants at birth who were born to preeclamptic mothers[5,6]. Neutropenic neonates are more likely to have mothers with severe preeclampsia[7], and severe preeclampsia is closely related to intrauterine growth retardation and premature delivery[8,9]. Preeclampsia-associated neonatal neutropenia (NN) is well recognized, but its etiology and clinical importance are poorly understood[5-7]. Although 80% of preeclampsia-associated neutropenia resolves by 60 hours of postnatal age, it may persist for as long as 30 days[6,10]. Preeclampsia-associated neutropenia is a risk factor for an increased incidence of infection in preterm neonates even after the recovery of neutropenia[7,10,11]. Preeclampsia-associated neutrophil function disorders also contribute to the high incidence of infection in neutropenic infants[6,7,11].

Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are hematopoietic growth

factors for proliferation, differentiation, survival and functional activation of phagocytes[12-14]. Recombinant human G-CSF treatment has been shown to increase circulating neutrophils and bone marrow neutrophil storage pools, improve neutrophil function and result in fewer bacterial infections in neutropenic infants of preeclamptic mothers[15,16]. There are limited studies investigating the relationship between G-CSF blood levels and neutrophil counts in infants of preeclamptic mothers, but the results are controversial[17-21]. In this study, we aimed to evaluate the relationship between serum G-CSF levels and absolute neutrophil counts (ANC) in infants of preeclamptic mothers

Material and Methods

Study Population

This study was conducted in the Neonatology Unit, Hacettepe University İhsan Doğramacı Children's Hospital, Ankara, Turkey from January 2002 to December 2002. A total of 55 infants were included in the study. The study group consisted of 31 infants of preeclamptic mothers and the control group of 24 gestational age-adjusted infants of normotensive mothers.

Preeclampsia was determined by increased blood pressure (gestational blood pressure elevation) accompanied by proteinuria. The blood pressure measurement was standardized as defined by the American Heart Association[22]. Proteinuria was defined as urinary excretion of ≥0.3 g protein in a 24-hour specimen. Severe preeclampsia was described as blood pressure >160 mm Hg systolic and >110 mm Hg diastolic in at least two measurements within six hours and the urinary excretion of ≥0.5 g protein. Neonatal neutropenia was defined as ANC below the -2 standard deviation described by Manroe et al.[5].

Infants with evidence of maternal infection, premature rupture of membrane, Rh-rh isoimmunization, hydrops fetalis, major congenital anomalies and inherited metabolic disorders or infants who died in the first seven days of life were excluded from the study.

Small-for-gestational age (SGA) was defined as birth weight below the 10th percentile according to Lubchenco intrauterine growth scale. Approval for the study was obtained from the Clinical Research Ethical Committee of Hacettepe University. Written informed consent was obtained from parents of the infants.

Blood Samples

For serum G-CSF analysis, peripheral venous blood samples were collected from an upper extremity within the first 12 hours of life. Blood samples were centrifuged at 2000 cycle/minute for 10 minutes within 30 minutes after sample collection and serum samples were kept at -80°C until the time of analysis. Complete blood count and peripheric blood smears were obtained within the first 12 hours, on the 2nd and 7th days of life.

Measurements

Serum G-CSF level was quantified by an enzyme-linked immunosorbent assay (Quantakine Human G-CSF Immunoassay; R&D Systems, Minneapolis, Minn, USA) with lower limit of sensitivity of 20 pg/ml. The complete blood count was measured with a Coulter STKS counter (Coulter Electronics, Hialeah, FL). Peripheral blood smears were evaluated by a collaborating pediatric hematologist.

Statistical Analysis

Data were evaluated for significance with Student's t test, ANOVA, Spearman correlation test and chi-square analysis and expressed as the mean ±SD. The G-CSF values were not normally distributed; therefore, they were log transformed before the statistical analysis. p values lower than 0.05 were accepted as statistically significant.

Results

Five (16%) infants from the study group and one (4%) infant from the control group were excluded since they died in the first seven days of life. The characteristics of the study and the control groups are presented in Table I. The number of very low birth weight (VLBW) infants (birth weight <1500 g) was higher in the study group (p=0.016). In the study group, the number of primiparous women was significantly higher than in the control group (p=0.001).

There was no statistically significant difference in log[1] G-CSF levels between the study and control groups (Table II).

Table I. Demographic Characteristics of the Study and Control Groups

		Study group (n=26) n (%)	Control group (n=23) n (%)	p
Gender	Female	12 (46.2)	9 (39.1)	>0.05
	Male	14 (53.8)	14 (60.9)	>0.05
Mode of delivery	Vaginal	2 (7.7)	4 (17.4)	>0.05
	Cesarean	24 (92.3)	19 (82.6)	>0.05
Birth weight (g)*		1487±601 (770-2650)	1662±408 (780-2250)	>0.05
Gestational age (week)*		31.5±2.6 (26.3-36)	32.0±2.3 (25.2-36)	>0.05
SGA		7 (26.3)	2 (8.7)	>0.05
VLBW†		18 (69.2)	8 (30.8)	0.016
Apgar score (<7 at 5 min)		9 (34.6)	6 (26.1)	>0.05

*: Mean±Standard Deviation (range); †: Very low birth weight (<1500 g).

Table II. Serum G-CSF and Log_G-CSF Levels in Study and Control Groups

	Study group (n=26)		Control group (n=23)		
		Median		Median	p
G-CSF (pg/ml)*	981.8±1682.5 (25.7-5924)	223.3	770.8±1779 (18-8526)	226.6	0.672
Log_G-CSF*	1.15±0.37 (0.6-1.75)	2.35	1.07±0.3 (0.6-1.78)	2.34	0.45

*: Mean±Standard Deviation (range). G-CSF: Granulocyte colony stimulating factor.

The percentage of neutropenic infants was not higher in the study group than the control group on the 1st day of life. On the 2nd day of life, the percentage of neutropenic infants was statistically higher in the study group (61.5%) than the control group (26.1%) (p=0.013). Neutropenia resolved in nearly all cases by the 7th day of life. ANCs obtained on the 7th day of life showed only one neutropenic infant in the control group.

There were no correlations between serum G-CSF levels and ANCs on the 1st, 2nd and 7th days of life in the study group. However, positive correlations were found between serum G-CSF levels and ANCs on the 1st (p=0.006) and 7th days (p=0.037) in the control group.

Mean gestational age of neutropenic and non-neutropenic infants was similar in both control and study groups. Mean birth weight of neutropenic infants was significantly lower than of non-neutropenic infants (p=0.048), and 85.7% of the SGA infants were neutropenic. The rates of neutropenia in infants of severe and mild hypertensive mothers were similar (p>0.05).

Although there was no statistically significant difference between the serum mean G-CSF levels of neutropenic and non-neutropenic infants in the study and control groups, mean serum G-CSF levels of neutropenic cases in the study group were higher than in the control group (Tables III, IV).

Discussion

An increased incidence of neonatal neutropenia in infants of hypertensive mothers was first documented by Manroe et al.[5]. Neutropenia is seen in about 40-50% of infants of hypertensive mothers. Mouzinho et al.[7] pointed out that this rate could increase to 80% in infants whose gestational ages are below 33 weeks. In this study, neonatal neutropenia rate was found to be 42.3% (p=0.125) on the 1st day of life and 61.5% (p=0.013) on the 2nd day of life in

Table III. Serum G-CSF Levels in Infants with Neutropenia in the Study Group

	G-CSF (pg/ml)*			
	n		Median	P
1st day Neutropenia	15	467.9±713.4 (25.7-2354)	192.8	0.108
	11	1682.5±2329.8 (96-5924)	339.4	
2nd day Neutropenia	10	203±147.5 (25.7-478)	188	0.068
	16	1468.5±2011.9 (35.9-5924)	371	

*: Mean±Standard Deviation (range). G-CSF: Granulocyte colony stimulating factor.

Table IV. Serum G-CSF Levels in Infants with Neutropenia in the Control Group

	G-CSF (pg/ml)*			
	n		Median	P
1st day Neutropenia	18	924.1±1991.7 (33-8526)	247.8	0.126
	5	219.1±248.6 (18-499.1)	77.2	
2nd day Neutropenia	17	612.6±2061.5 (15-8526)	194.7	0.703
	6	369±254.9 (77.2-738)	375.1	

*: Mean±Standard Deviation (range). G-CSF: Granulocyte colony stimulating factor.

the study group. The NN rate determined by Manroe et al.[5] was in accordance with other studies[1,5-7,11,21,23-25].

During the neonatal period, various factors can affect ANC. Davies et al.[26] showed the blood neutrophil count starting to increase after the 28th week of gestation and with a higher rate after the 32nd week of gestation. Similarly, Forestier et al.[27] also reported that the fetal leukocyte count did not change between the 18th and 30th weeks of gestation. It seems that that gestational age does not affect the fetal leukocyte counts in infants born before the 32nd week of gestation. In this study, we observed that gestational age was not an effective factor on ANC and NN. The number of VLBW infants (birth weight <1500 g) was higher in the study group (p=0.016); however, the number of SGA infants was not higher in the study group (p>0.05). Various studies have indicated that gestational age and birth weight had an effect on the development of NN[6,7,28]. In our study, mean birth weight of neutropenic cases was significantly less than of non-neutropenic cases, and most of the SGA infants in the study group were neutropenic. Similarly, some studies have reported a higher incidence of NN in SGA infants whose pregnancy was complicated with severe hypertension and the existence of HELLP syndrome (hemolysis, elevated liver enzymes and low platelets)[6,11].

Schelonka et al.[29] reported that the birth weight and gestational age did not affect ANC, and ANC increased significantly only after a prolonged labor. Some studies[6,7] stated that the VLBW infants born by cesarean section (C/S) were more likely to be neutropenic, and increased C/S rate in hypertensive mothers may contribute to the decreased neutrophil count. These results suggest that event of labor is an important factor for the neutrophil count in fetal circulation We were unable to detect a statistical significance between neutrophil counts of infants who were delivered vaginally or by C/S because of the high C/S rate in the preeclamptic mothers in this study.

It has been shown that there is no relationship between endogenous maternal serum G-CSF levels and cord G-CSF levels[30]. There are some conflicting studies about the effects of gestational age, birth weight, infection and maternal hypertension on serum G-CSF levels[18-21,23,31-35]. In this study, we did not find any relationship between gestational age and birth weight and serum G-CSF levels. Some studies[20,31] have indicated a positive

correlation between gestational age and serum G-CSF levels; however, Bailie et al.[35] found a negative correlation. Similar to our study, Tsao et al.[21], Shimada et al.[32], and Bedford Russel et al.[19] demonstrated that gestational age has no effect on serum G-CSF level.

It has been shown that NN associated with maternal hypertension is due to transiently reduced neutrophil production[6]. However, it is unclear whether the etiology of this diminution is due to decreased production of neutrophilic growth factors, reduced responsiveness of neutrophil pro-genitors to these factors, or the presence of inhibitors. Tsao et al.[21] found that cord blood G-CSF levels were significantly lower in neutropenic infants of preeclamptic mothers. G-CSF levels were positively correlated with both ANC and total white blood cell count in this study. Koenig and Christensen[23] indicated that serum G-CSF levels were similar in normotensive and hypertensive gestations. They concluded that the disorder in neutrophil production should be related to a decreased biologic activity, but not to decreased serum concentrations of G-CSF, possibly caused by an inhibitor of neutrophil production by the placenta in hypertensive gestations.

Similarly, in this study, we did not find any difference in serum G-CSF levels between infants of preeclamptic and normotensive mothers. Although we could not determine corresponding serum G-CSF level on the 2nd and 7th days of life to ANCs, we found no correlation between serum 1st day G-CSF levels and ANCs on the 1st, 2nd and 7th days of life in the study group. High serum G-CSF levels and lack of correlation between serum G-CSF levels and ANCs in neutropenic infants of the study group in contrast to the control group support the hypothesis that diminished neutrophil production is possibly caused by diminished response to G-CSF due to an unknown inhibitory factor.

In conclusion, it has been shown that the development of neutropenia in infants of preeclamptic mothers does not seem to be the result of G-CSF deficiency. The high levels of serum G-CSF found in this study might show a decreased or altered response to G-CSF in neutropenic infants of preeclamptic mothers.

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