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The Importance of Fibronectin, Haptoglobin, Ceruloplasmin and Transferrin in the Early Diagnosis of Neonatal Sepsis

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Abstract: Serum fibronectin, haptoglobin, ceruloplasmin, and transferrin concentrations were measured in 44 term neonates with culture proven sepsis and a control group of 37 normal neonates of similar age, sex and weight. The white blood cell count, total neutrophil count, and the ratio of band to neutrophils were also determined. While the serum fibronectin and haptoglobin concentrations were significantly different ($P<0.05$), the serum ceruloplasmin and transferrin levels of the two groups were similar. The total neutrophil count and the band to neutrophil ratio in peripheral differential leukocyte counts were significantly higher in the sepsis group than in the control group ($P<0.01$ and $P<0.001$, respectively). The combination of a hematological marker and fibronectin or haptoglobin seems to be more particularly predictive of sepsis than either of the parameters alone. The combination of hematological and acute phase protein tests may provide a more rapid diagnosis of neonatal sepsis than conventional microbiological methods.

Key Words: Neonatal sepsis, fibronectin, haptoglobin, ceruloplasmin, transferrin.

Introduction

Sepsis is the major cause of morbidity and mortality during the neonatal period in spite of the use of potent antibiotics and intensive supportive care[1,2]. Any sign suggesting sepsis should alert the physician and every effort should be made for the early diagnosis of the condition. Until the results of blood cultures become available, some tests are usually considered helpful to support a preliminary diagnosis of sepsis. Whole blood cell count (WBC)[3], C-reactive protein (CRP)[4], erythrocyte sedimentation rate (ESR)[5] and nitroblue tetrazolium test (NTT)[6] have been used in the diagnosis of sepsis, but there is evidence that these tests are not specific for the diagnosis of sepsis. Even though, cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6)7, and intercellular adhesion molecule-1 (ICAM-1)[8] are currently considered to be specific tests, these procedures are difficult, time-consuming and expensive. Therefore, we need cheaper and more easily performed tests for early diagnosis of neonatal sepsis. In our study, we investigated the role of hematologic parameters with fibronectin, haptoglobin, ceruloplasmin and transferrin in the early diagnosis of newborn sepsis.

Materials and Methods

Twenty-four neonates with sepsis (25 males, 19 females; mean age 10.9±5.8 [range 3-22] days, mean gestational age 36.2±3.0 [range 32-41] weeks, and mean weight 2964±499.0 [range 2500-3800] grams) and seventeen term neonates without sepsis (19 males, 18 females; mean age 14.4±9.4 (range 3-28] days, mean gestational age 36.5±3.8 [range 35-4] weeks, and mean weight 3102.9±304.9 [range 2600-4000] grams) of similar age, weight and gestational age were included in the study. Three patients with sepsis were born of healthy mothers by caesarean and the others by normal delivery at the Gynecology and Obstetrics Clinic at the Medical Faculty at Ondokuz Mayıs University, Medical Faculty. In three patients (one normal delivery and two with premature rupture of the membranes), sepsis developed before discharge from the hospital, whereas in the other patients it developed afterwards. The babies in the control group were delivered normally after an uncomplicated pregnancy of healthy mothers in our hospital. Gestational age was estimated from physical and neurologic assessment at birth with routine pediatric examination[9]. Signs suggesting sepsis were temperature instability, lethargy, apneic spells, poor peripheral

circulation, poor feeding and increased oxygen requirements[10,11]. Sepsis was defined as a clinical suspicion, a positive blood and/or cerebrospinal fluid (CSF) culture, and at least three of the following hematological findings:[1,3,12].

I. Total leukocyte counts <5000/mm^3 or >20000/mm^3

II. Total neutrophil counts <175/mm^3 or >5400/mm^3

III. Immature neutrophil counts >600/mm^3

IV. Immature/total neutrophil ratio >0.20

V. Platelet counts <150000/mm^3

The blood and/or CSF cultures in the sepsis group revealed Escherichia coli in 19 cases, Staphylococcus aureus in 14 cases, Klebsiella pneumonia in 8 cases, Enterobacter spp in 2 cases, and alpha hemolytic streptococcus in one case. The treatment of the patients was planned either empirically (ampicillin+cefotaxime) or according to the culture results.

In both groups, informed parental consent was obtained for the laboratory tests, and blood specimens were drawn from a peripheral vein. In the control group, blood specimens were obtained during the routine complete blood counter test. In the patients with sepsis, blood samples for fibronectin, haptoglobin, ceruloplasmin ad transferrin estimation were drawn on during the other routine diagnostic procedures on the first and tenth days of the hospitalization. In four cases the second sample could not be taken because of the patients' death within five days of hospitalization. In the control group, blood samples were taken only once during the routine examination at the admission.

The total leukocyte counts and platelet counts were measured on a Stakes counter. Differential counts were performed manually on wright-stained blood smears through examination of at least 200 cells. Blood samples for serum fibronectin, haptoglobin, ceruloplasmin and transferrin were collected in tubes without any anticoagulant and allowed to coagulate. The blood samples were then centrifuged for 10 min at 1000xg and the serum was removed for storage at -70°C until analysis. Serum fibronectin, haptoglobin, ceruloplasmin and transferrin levels were measured nephelometrically by Behring Nephelometer using corresponding kits from Behring Diagnostics Inc. (USA). Values of smaller than 14.5 mg/dl (145 mg/L) for fibronectin[13,14] and greater than 250 mg/dl (2500 mg/L) for haptoglobin[12,15] were accepted as sepsis.

Statistical analysis: Wilcoxon Rank Sum test and Mann-Whitney U test were used to calculate the statistical significance between the two groups, two partners and values. Diagnostic values of hematological and biochemical markers were determined by the Bayesian method.

Results

The lobaratory data for the patients with sepsis and the control group are shown in Table I. The WBC, total neutrophil, band/total neutrophil and band/mature neutrophil counts were significantly different between to two groups (Table I). The serum fibronectin and haptoglobin values were different between both groups ($p<0.05$), but serum ceruloplasmin and transferrin levels

Table 1. Hematological markers and acute phase proteins in sepsis and control groups (mean±SD)

		Sepsis group	Control group
WBC (/mm3)*	Day 1	13429.2±7926.5	8035.7±1535.5
	Day 10	13370.8±13840.8	
Total neutrophil (/mm3)**	Day 1	8295.8±5689.9	4310.7±1356.0
	Day 10	5763.3±2998.8	
Band/total neutrophil***	Day 1	0.27±0.16	0.12±0.14
	Day 10	0.19±0.14	
Band/mature neutrophil***	Day 1	0.47±0.45	0.09±0.03
	Day 10	0.22±0.38	
Fibronectin (mg/dl)*	Day 1	14.5±9.0	28.5±11.6
	Day 10	16.8±7.0	
Haptoglobin (mg/dl)*	Day 1	284.7±56.2	112.3±22.4
	Day 10	164.4±31.5	
Ceruloplasmin (mg/dl)n.s.	Day 1	44.1±23.7	37.8±10.7
	Day 10	34.2±15.8	
Transferrin (mg/dl)n.s.	Day 1	256.6±73.9	235.9±47.4
	Day 10	211.0±65.9	

n.s. not significant (P>0.05), *P<0.05, **P<0.01, ***P<0.001

were similar in both groups (p>0.05) (Table I). The sensitivity, specificity and positive and negative predictive values for WBC, total neutrophil, band/total neutrophil, band/mature neutrophil, fibronectin and haptoglobin are shown in Table II. Sensitivity and specificity of the fibronectin or haptoglobin values increased when taken into account with total neutrophil count (Table III). Similar increases were also found when fibronectin with band/mature neutrophil or band/total neutrophil ratios were evaluated altogether. The addition of a third parameter to any of these combinations led to the increment rather than the decrement of specificity (Table III).

In the sepsis group, four patients died within five days of hospitalization. Forty cases with sepsis were divided into two groups: early-onset sepsis (<7 days of age; 16 cases), and late-onset sepsis (>7 days of age; 24 cases). According to the statistical analysis, there was no difference between the two groups for fibronectin, haptoglobin, ceruloplasmin and transferrin levels both on the first and the 10th day (P>0.05).

In the patients with sepsis, hematologic parameters and acute phase proteins were not statistically significant between the first and tenth days (Table I) (p>0.05).

Discussion

Neonatal sepsis is clinically similar to diseases such as perinatal asphyxia, hypoglycemia, hyponatremia, prematurity, and intracranial bleeding. Because culture results are available in 24 hours at the earliest and positive culture ratios change between 30-70% of the

Table 2. Sensitivity, specificity, and positive and negative predicitive values for individual tests in newborn sepsis.

Criteria (cut-off)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
WBC (<5000 or >21000/mm3)	29	100	100	50	59
Total neutrophil counts (<1750 or >5500/mm3)	67	76	80	65	71
B/T (>0.12)	88	88	91	83	88
B/T (>0.14)	79	94	95	76	85
B/T (>0.16)	58	94	93	62	73
B/T (>0.20)	50	94	92	57	68
B/M (>0.30)	50	100	100	59	71
Fibronectin (<14.5 mg/dl)	65	62	69	53	63
Haptoglobin (>250 mg/dl)	53	92	90	60	70

B/T: Band/total neutrophil ratio, B/M: Band/mature neutrophil ratio, PPV: positive predictive values, NPV: negative predictive values.

Table 3. Sensitivity, specificity, and positive and negative predictive values considering the combined tests in newborn sepsis.

Criteria	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Total neutrophil+fibronectin	100	53	75	100	80
Total neutrophil+haptoglobin	83	76	83	76	80
B/T+fibronectin	96	65	79	92	83
B/T+haptoglobin	79	88	90	75	83
B/M+fibronectin	96	71	82	92	85
B/M+haptoglobin	79	94	95	76	85
Total neutrophil+B/T+fibronectin	100	47	73	100	78
Total neutrophil+B/T+haptoglobin	88	71	81	80	80
Total neutrophil+B/M+fibronectin	100	53	75	100	80
Total neutrophil+B/M+haptoglobin	88	76	84	81	83

B/T: Band/total neutrophil ratio, B/M: Band/mature neutrophil ratio, PPV: positive predictive values, NPV: negative predictive values.

cases, tests for early diagnosis are needed[16]. Hematologic parameters and acute phase proteins measurements are common methods used in the diagnosis of neonatal sepsis.

In hematologic research, total WBC is not very important because it changes dramatically in the newborn period and it is affected by many factors other than infection. Nonetheless, the total neutrophil count and the ratio of band to total neutrophil is useful in more than 80% of the cases in the diagnosis[17]. In this study, total WBC was different between the two groups, but its sensitivity was low.

The ratio of band to total neutrophil has been shown to be a good hematological marker in the early diagnosis of neonatal sepsis[1,3,9], with the exception of one report[18]. On the other hand, in our study the sensitivity of the ratio of band to total neutrophil was determined to be low (50%). Philip[19] showed that the ratio of band to total neutrophil is less sensitive after the first week following the birth. The fact that the babies in our study group were 3 days older may be an explanation of the low sensitivity. Furthermore, another study about normal newborns showed that the ratio of band/total neutrophil had to be 0.16 during the first 24 hours following birth, and 0.12 on the 5th day, remaining stable at this level until the 28th day[20,21]. We observed that when we chose lower cutoff values for the ratio of band to total neutrophil, its sensitivity and specificity in newborn sepsis increased (Table II). Therefore, lower levels of the ratio of band to total neutrophil such as 0.12 after the first week may be useful in the diagnosis of neonatal sepsis.

The serum fibronectin and haptoglobin concentrations were significantly different between the two groups ($P<0.05$). Furthermore, serum ceruloplasmin and transferrin levels were similar in infected and noninfected neonates. Plasma fibronectin is a multifunctional and heavy molecular weight protein, and it exists in most cell surface and extracellular fluid. Plasma fibronectin augments neutrophil and macrophage phagocytosis and acts as a nonspecific opsonin for the reticuloendothelial system[14]. Normal newborn infants have lower plasma concentrations of fibronectin than adults, and this situation may contribute to the hypofunction of the neonatal reticuloendothelial system, predisposing infants to the development of sepsis[22]. Gerdes[13,14] and Polin[23] noted serum fibronectin levels to be low in neonatal sepsis, but low serum fibronectin levels can not be used alone in the diagnosis of neonatal sepsis. In our study, low serum fibronectin levels were determined in neonatal sepsis. On the other hand, the sensitivity and specificity of serum fibronectin levels were determined to be under 70% (Table II). When total neutrophil count and fibronectin were evaluated together, the sensitivity rose to 100%, while specificity fell to 53%. When serum fibronectin levels were evaluated with the ratio of band to neutrophil, sensitivity and specificity rose to acceptable values, and this may be useful in the diagnosis of sepsis (Table III). These results are compatible with other studies[13,23].

Haptoglobin is an acute phase protein having alpha 2-glucoprotein structure. Plasma haptoglobin levels are used in the diagnosis of hemolytic events in addition to acute and chronic infections[24]. Most of the previous studies have shown higher levels of serum haptoglobin in neonatal sepsis, but this increment can not be used in the diagnosis because of low sensitivity and specificity. Furthermore, Speer et al.[15] stated that serum haptoglobin levels did not vary between neonates with and without sepsis, the reason for which may have been the inclusion of preterm babies in their study. It is known that in preterm infants, haptoglobin levles should be low[24]. In our study, serum haptoglobin levels were higher in babies with sepsis than in normal ones. At the end of the therapy, high serum haptoglobin values decreased to normal levels.

In our study, serum haptoglobin levels had low sensitivity and specificity in the diagnosis of sepsis (Table II). But when it was evaluated with total neutrophil count or the ratio of band to neutrophil, it was considered to be a good marker for the diagnosis of neonatal sepsis (Table III).

Serum ceruloplasmin and transferrin concentrations in neonatal sepsis have not previously been studied. In adults with sepsis, serum ceruloplasmin and transferrin decrease but rise to normal levels after recovery[25]. We found no difference in serum ceruloplasmin and transferrin levels before and after therapy in patients with sepsis.

In conclusion, the combination of a hematological marker and fibronectin or haptoglobin seems to be more particularly predictive of sepsis than either of the parameters alone. The combination of hematological and acute phase protein tests may provide a more rapid diagnosis of neonatal sepsis than conventional microbiological methods.

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