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CIRCULATING MONOCYTES;
HLA-DR EXPRESSION IN HEALTHY AND SICK NEONATES

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ABSTRACT: **Objective:** This study was conducted to measure Monocytes HLA-DR expression in neonatal sepsis in comparison to other diseases with systemic inflammation and high risk of infection (respiratory distress syndrome "RDS" and prenatal asphyxia) and this may be helpful in early diagnosis of infection and therapeutic intervention. **Methods:** This study was carried out on 2007 and it conducted on 38 sick neonates, 22 with proven sepsis diagnosed clinically and by positive blood culture and 16 (8 had RDS and 8 had prenatal asphyxia) with possible infection i.e. they had 2 or fewer clinical signs of sepsis and negative blood culture. Those Patients with possible infection were followed up (for 48 h). Seven out of the 8 RDS Babies developed sepsis as evidenced clinically and by positive blood culture and they considered as patients with early sepsis at the time of admission. Forty healthy age and sex matched newborns were studied as controls. All Babies were subjected to complete blood count (CBC), C-reactive protein (CRP) and flow cytometeric determination of HLA-DR expression on monocytes. **Results:** Neonates with proven sepsis and those with early sepsis (7/8 of RDS) had significantly lower HLA-DR% (15.9±7.8 and 11.4±5.9 respectively) than controls (61.0±20.6). HLA-DR% was reduced below the lowest cut off values in all septicemic neonates (neonates with proven and those with early sepsis). At the time of admission CRP was positive in 91% of neonates with proven sepsis and in only 57% of the neonates of early sepsis. In addition, there was no significant difference between HLA-DR percent in neonates with prenatal asphyxia when compared to control group. Monocytes HLA-DR% had higher sensitivity, specificity, positive and negative predictive values (100%, 85%, 87.5% and 100% respectively) compared to CRP (57.1%, 77.8%, 66.7%, 70% respectively) for neonatal sepsis at its early stages before evident clinical and laboratory diagnosis. **Conclusion:** HLA-DR % expression on monocytes is a sensitive test for both diagnosis of neonatal sepsis and its early stage and exclusion of neonatal infection in high risk neonates to reduce the unnecessary antibiotic use and the costs of neonatal intensive care units.

INTRODUCTION

The first clinical signs of neonatal sepsis are non specific and laboratory indicators, such as CBC, ratio of mature to total neutrophils and CRP, do not have high sensitivity, especially if measures early in the course of sepsis. In addition isolation of causative organisms from microbiological cultures takes up to 72h and does not identify some infected infants[1]. Since outcome and prognosis depend on early and efficient antibiotics therapy, there is a need for sensitive and specific indicators of sepsis at the earliest stage of disease[2]. Studies of neonates with RDS demonstrated the major role of acute inflammatory reaction in its pathogenesis and its progression to bronchopulmonary dysplasia (BPD)[3]. Similarly, in prenatal asphyxia, the brain ischemia elicits an acute inflammatory reaction with monocytes activation[4]. Neonates with RDS and prenatal asphyxia are at high risk to develop infection[5].

Human leucocytes antigens (HLA) are located on the surfaces of most human cells. Loci determining HLA

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antigens are located on the sixth chromosome and they include A, B, C, D, DR, DP, DQ[6]. HLA-DR molecules play a central role in the specific immune response to infection. The reduced HLA-DR expression on monocytes is considered to correlate with infectious complications and the development of sepsis[7]. It is a useful indicator of the immunological status of the patient with sepsis and a guide for treatment as patients with low HLA-DR expression might benefit from immunostimulants while those with normal HLA-DR expression should receive treatment directed to interfere with pro inflammatory pathway[8].

This study was conducted to measure monocyte HLA-DR expression in neonatal sepsis in comparison to other diseases with acute inflammatory reaction and high risk of infection (RDS and prenatal asphyxia) as this may be helpful in early diagnosis of infection as well as the initiation of appropriate therapy.

PATIENTS AND METHODS

This study included 38 sick neonates (19 preterm and 19 full terms) recruited from neonatal unit of Makkah Alnoor Hospital and 40 healthy age and sex matched newborns. The patients were divided into 2 groups as follow:

Group I: It comprised 22 newborns admitted to neonatal intensive care unit (NICU) with proven neonatal sepsis. All had positive blood culture. They were 14 (63.6%) males and 8 (36.4%) females. Their ages ranged from 1to 7 days (mean4.9±3.7 days). They were subdivided into 2 subgroups according to their gestational age as follows:

- Group IA (50%): It included 11 full term neonates (> 37ws).
- Group IB (50%): It included 11 pre term neonates (< 37ws).

Group II: It comprised 16 newborns with suspected infection i.e. with 2 or less of the following clinical signs of sepsis:

- Respiratory dysfunction (apnea, cyanosis, grunting and intercostals retraction)
- Circulatory dysfunction (tachycardia, bradycardia, poor perfusion and shock).
- Gastrointestinal dysfunction (feeding intolerance, abdominal distension, hepatomegally and jaundice).

All had negative blood culture at time of initial evaluation and one or more of the risk factors for infection namely premature rupture of membranes > 18h, chorioamnionitis, intrapartum maternal fever > 38OC, maternal history of urinary tract infection, vaginal bleeding and complicated traumatic delivery. They were subdivided into 2 subgroups according to their clinical diagnosis and gestational age as follows:

Group IIA (50%): It comprised 8 newborns admitted to NICU with provisional clinical diagnosis of RDS. They were 5 (62.5%) males and 3 (37.5%) females. All were preterm. Their ages ranged from 1 to 6 days (mean 2±1.3 days).

Group IIB (50%): It comprised 8 newborns admitted to NICU with provisional clinical diagnosis of prenatal asphyxia. They were 5 (62.5%) males and 3 (37.5%) females. All were full terms. Their ages ranged from 1 to 7 days (mean 4.1±1.9 days).

These patients were followed up clinically and laboratory (CRP and blood culture were reported after 48h) to detect the clinical manifestations and laboratory findings denoting the development of sepsis later on. Seven premature neonates with RDS developed clinical evidence of infection, positive CRP and positive blood culture after 48h. Thus, they were considered as patients with early sepsis at the time of admission i.e. when the condition could not be diagnosed clinically and by laboratory investigations.

Forty healthy age and sex matched newborns were included as control (group III). They were subdivided into 2 subgroups according to their gestational age as follows:
Group IIIA (50%): It comprised 20 full term neonates.
Group IIIB (50%): It comprised 20 preterm neonates.

They were 25 (62.5%) males and 15 (37.5%) females.

Their ages ranged from 1 to 7 days (mean 5.2±3 days).

Patients and controls were subjected to

1. Clinical history taking laying stress on gestational age, mode of delivery, presence of risk factors of sepsis , irritability, weak suckling and bleeding tendency.
2. Clinical examination laying stress on determination of gestational age, birth weight, body temperature, pallor's, neonatal reflexes and signs of sepsis (previously mentioned).
3. Laboratory investigations which included:

- Complete blood count using Coulter (T-540 cell counter) with peripheral blood film examination stained with Leishman stain especially for band and mature form of neutrophils, and monocytes counts.
- C reactive protein by latex agglutination.
- Blood culture and sensitivity.
- Estimation of percentage of HLA-DR expression on monocytes. The cell surface marker of HLA-DR was assessed by flow cytometry (9) on Coulter EPIXS XL (Coulter electronics, Hielaeh Fl, USA). The monoclonal antibodies were purchased from IQ products (Zernikeparm 66, 9747 AN Groningen the Netherland). Neonates were considered to have reduced HLA-DR% if its level were below the lowest cutoff value, which was 30.1 (mean value of the HLA-DR%-1.5 standard deviation (SD) of the control neonates).

STATISTICAL ANALYSIS

Data were analyzed with statistical software package V5 (Statsoft Tulsa,OK,USA). All numeric data were expressed as mean ±SD. Data were analyzed using student t test, Mann Whitney U test and Pearson r correlation coefficient. For all tests, the probability of less than 0.05 was considered as significant. Sensitivity, specificity, positive and negative predictive rate were calculated.

RESULTS

HLA-DR% and CRP in septicemic neonates

Neonates with proven sepsis showed highly significant decrease of HLA-DR % and CRP respectively when compared to the control group ($p < 0.01$). Similarly, neonates with early sepsis (7 preterm with RDS) had highly significantly lower HLA-DR% and significantly higher CRP when compared to the preterm control group ($p < 0.01$) (Table-I). The HLA-DR% was reduced below the lowest cut off value (30.1) in all septic neonates (proven and early sepsis). On the other hand CRP was positive in 90.9% of neonates with proven sepsis and in only 57.0% of the neonates with early sepsis.

Table-I. Comparison between different group studied regarding HLA-DR% and CRP levels

	HLA-DR%		CRP (mg/1)	
	Mean ± SD	P	Mean ± SD	P
Neonates with proven sepsis (n = 22) Healthy controls (n = 40)	15.9 ± 7.8 61.1 ± 20.6	P<0.001	58.8 ± 46.9 2.1 ± 2.2	P<0.001
Neonates with early sepsis (n = 7) preterm controls (n = 20)	11.4 ± 5.9 62.5 ± 20.5	P<0.001	28.9 ± 33.9 1.6 ± 1.3	P<0.001
Neonates with RDS (n = 8) preterm controls (n = 20)	13.3 ± 7.7 62.6 ± 20.5	P<0.001	25.8 ± 32.6 1.6 ± 1.3	P<0.001
Neonates with prenatal. asphyxia (n = 8) Full term controls (n = 20)	60.5 ± 20.4 61.03 ± 21.4	P<0.05	11.1 ± 16.7 2.4 ± 2.6	P<0.05

HLA-DR% and CRP in neonates with RDS and prenatal asphyxia:

Neonates with RDS had highly significantly lower HLA-DR% and significantly higher CRP when compared to the preterm control group ($p < 0.01$) (Table-I). In contrast, there was no significant difference between HLA-DR% and CRP levels of full term neonates with prenatal asphyxia and full term healthy controls. ($p > 0.05$) (Table-I). HLA-DR% did not show any significant correlations with other parameters studied (data not shown).

Effect of prematurity on HLA-DR%

Prematurity had no influence on HLA-DR% levels as there was no significant difference between values of HLA-DR% of septicemic full term and preterm neonates($p > 0.05$) on one hand and healthy full term and preterm control neonates on the other hand ($p > 0.05$) (Figure 1).

Sensitivity, specificity and predictive values of Monocytes HLA-DR% in comparison to CRP for neonatal sepsis at its early stage:

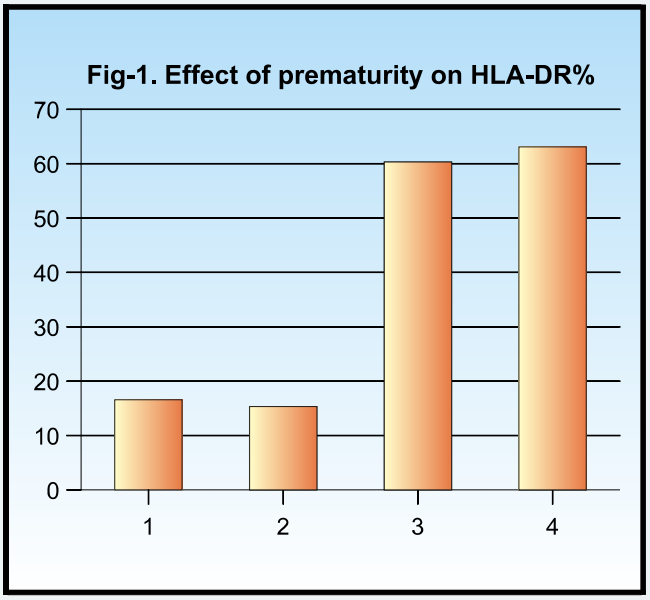
These values were calculated from neonates with possible infection (n=16) and they included:

- Patients who develop sepsis with low HLA-DR % (n=7) i.e. true positive for HLA-DR%.
- Patients who did not develop sepsis with high HLA-DR% (n=8) i.e. true negative for HLA-DR%.
- Patients who did not develop sepsis with low HLA-DR% (n=1) i.e. false positive for HLA-DR%.
- Patients who develop sepsis with high HLA-DR% (n=0) i.e. false negative for HLA-DR%.

For CRP, 4 patients were true positive, 7 were true negative, 2 were false positive and 3 were false negative. HLA-DR% had higher sensitivity, specificity and predictive values than CRP for sepsis at its very early stage before evident clinical and laboratory diagnosis (Table-II).

Table-II. Sensitivity, specificity and predictive values of monocytes HLA - DR% and CRP for neonatal sepsis at early stage:

	Sensitivity %	Specificity %	Positive predictive values %	Negative predictive values %
HLA - DR%	100	88.9	87.5	100
CRP	57.1	77.8	66.7	70



Group: IA (1): Septicemic full term neonates.
Group: IB (2): Septicemic preterm neonates.
Group: IIA (3): Control full term neonates.
Group: IIB (4): Control pre term neonates.

DISCUSSION

HLA-DR monocytes expression is an immunological marker considered to correlate with infections complications and developments of sepsis[2].

Adult's studies showed a significant depression of monocytes HLA-DR expression in trauma and surgical patients who was more severe in those developing septic complications[10,11]. Data regarding the response of neonatal monocytes to sepsis in term of HLA-DR expression are very limited.

In our study, HLA-DR% was significantly reduced in neonates with proven sepsis as compared to the control neonates. In addition, HLA-DR% was significantly low in the 7 premature neonates with early sepsis as compared to the preterm controls. Similarly, Kankoudi et al[5], Perry et al[6] and Sedlácková et al[12] reported that HLA-DR expression was significantly depressed in neonates with sepsis. This is explained by the fact that this immunologic marker allows antigen presentation to T cells and is crucial for initiating the immune cascade during sepsis and thus it is consumed.

In contrast to our results, Ng et al[13] reported that there were no significant differences in monocyte HLA-DR expression between the infected, non-infected and control groups. Also, they did not support the use of monocyte HLA-DR alone or in combination with other infection markers in the diagnosis of early-onset clinical infection in term newborns. The contradictory between our results and their work is contributed to the method used as we estimate the percentage of HLA-DR while they measured antigen molecules bound cell.

Moreover, El- Mohades et al[14] found that in vitro stimulation of neonatal monocytes with lipopolysaccharides resulted in enhancement of HLA-DR antigen expression. These results seem to contrast with the decreased HLA-DR expression found in septicemic neonates of our study. The discrepancy between in vitro and in vivo response of neonatal monocytes to septic stimuli could be attributed to the release of anti-inflammatory cytokines such as IL-10 during sepsis [15,16,17,18], or more probable to extravasations and migration of the activated monocytes into the inflamed tissues[5].

HLA-DR was reduced below the lowest cut off value in all patients with proven sepsis and those with early sepsis with a sensitivity of 100%. In early sepsis, total leucocytes count not elevated and CRP not positive in all cases as proven in our study by the sensitivity of CRP of 57%. These results indicate that HLA-DR can be used to diagnose neonates with early sepsis. Kouranta et al[19] mentioned that HLA-DR monocytes expression is an immunological marker for early diagnosis of neonatal sepsis which is easily assessed using flow cytometry and the results can be obtained within 2 hours and requires only small amount of blood. In contrast, the bacterial culture results which are the gold standard for diagnosis of infection may not be available for 48h after treatment decisions must have been made[20].

HLA-DR% had higher specificity, positive and negative predictive values (88.9%, 87.5%, and 100% respectively) than CRP (77.8%, 66.7% and 70% respectively) in early diagnosis of sepsis among neonates with possible infection before evident clinical manifestations of sepsis and positive blood culture. Its excellent negative predictive value (100%) can reduce the unnecessary use of antibiotics therapy and early discharge from hospital to reduce the nosocomial infection.

In our study, the 8 premature neonates with RDS had significantly lower values of HLA-DR% than control preterm neonates. Studies of neonates with RDS demonstrated the major role of the acute inflammatory reaction in the pathogenesis of the syndrome itself and its progression to bronchopulmonary dysplasia[3].

In bronchoalveolar lavage from neonates with RDS increased numbers of activated macrophages and increased concentrations of the macrophages inflammatory protein -1 alpha, tumor necrosis factor –alpha and IL-8 were found soon after birth and correlated well with subsequent development of bronchopulmonary dysplasia[21, 22]. In addition, increased expression of HLA-DR was found on pulmonary macrophages from infants dying of bronchopulmonary dysplasia[21]. On the basis of these data, an increased expression of HLA-DR antigens on circulating monocytes of neonates with RDS would be expected, considering the fact that these cells virtually constitutes the tissues resident macrophages replacement pool. However, in our study, the percentage of circulating monocytes expressing the HLA-DR antigen in the group of neonates with RDS was decreased in comparison with healthy preterm neonates. Therefore, it appears that the activation state of peripheral blood monocytes does not reflect the status of the tissue resident macrophages. This could be explained by the fact that 7 out of these 8

neonates developed sepsis as evidenced clinically by positive blood culture after 2 days. So, this marker was reduced at the very early stage of sepsis before its full blown picture. So, further studies should be considered to study HLA-DR% in RDS without infections complications to determine the effect of disease per se on the immunologic marker.

The relationship between prenatal asphyxia and monocyte activation is supported by experimental animal studies suggesting that brain ischemia elicits an acute inflammatory reaction in the injured brain involving monocytes, neutrophils and inflammatory mediators and may be causally related to brain damage[4]. But results of HLA-DR expression in neonates with prenatal asphyxia and healthy full term neonates were comparable. Similarly, Kanakoudi et al[5] reported that expression of HLA-DR is not influenced by prenatal asphyxia.

In our study, prematurity did not influence the level of HLA-DR expression as there was no significant difference between its level in preterm and full term healthy neonates. Also, there was no significant difference between HLA-DR% of preterm and full term neonates with proven sepsis. In agreement with our results, Kanakoudi et al[5] reported that monocytes HLA-DR expression was not influenced by prematurity.

No significant correlations were detected between HLA-DR% and other laboratory indices of neonatal sepsis among septic neonates. This could be explained by consumption of this marker in the septic process prior to the effect of the infectious process on other laboratory indices of sepsis. This denoted that monocytes HLA-DR% expression was an earlier marker for diagnosing neonatal sepsis than both white blood cells and CRP.

In conclusion, the assay of HLA-DR expression on monocytes is a promising rapid and sensitive test for both diagnosis of neonatal sepsis at its early stage and exclusion of neonatal infection. This is equally important to early identify and discharge of at risk but healthy neonates. Thus NICU costs as well as unnecessary antibiotics use can be reduced.

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