Original Article

Monocyte HLA-DR Expression in Children with Acute Bacterial Meningitis and Meningococcemia: A Predictor of Outcome and Prognosis

C AYDIN, F GENEL, I DEVrim, S GOZMEN, O OLUKMAN

Abstract

Introduction: Acute bacterial meningitis (ABM) and meningococcemia are among the most serious causes of mortality and morbidity in children. Faster tests with high specificity and sensitivity are required to provide early diagnosis and better prognosis. This study aimed to get serial measurements of monocyte HLA-DR expression rate to evaluate the outcome and prognostic value in children with ABM and meningococcemia. Population and Methods: This prospective case-control study was carried out on 18 paediatric patients diagnosed as ABM and meningococcemia and 36 healthy controls between 2011-2017 at Dr. Behcet Uz Children's Hospital, Izmir, Turkey. Monocyte HLA-DR expression was determined by flow cytometry on admission and the third day of treatment. Results: In the study group, HLA-DR expression was significantly low both on admission and on day 3, as well as HLA-DR mean fluorescence intensity (p<0.001, p=0.001, p=0.001, respectively). When compared to the third day of treatment, monocyte HLA-DR expression was significantly lower on admission (p=0.003). Five patients suffered neurological complications. On day 3, monocyte HLA-DR expression was found significantly lower in patients with neurological complications than the ones with a normal neurological examination (p=0.043). Six patients had used antibiotics before admission. Patients without prior antibiotic usage showed significantly lower monocyte HLA-DR expression (p=0.007). Conclusions: Monocyte HLA-DR expression is down-regulated in patients with ABM and meningococcemia. Higher percentages of monocytes expressing HLA-DR in patients with prior antibiotic treatment supports the importance of early treatment. Low monocyte HLA-DR expression on day 3 also seems to be a valuable predictive marker for neurological complications.

Key words Acute bacterial meningitis; Meningococcemia; Monocyte HLA-DR expression; Paediatric population

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Introduction

The morbidity and mortality caused by acute bacterial meningitis (ABM) in children remains significant worldwide, despite advances in vaccines, chemoprophylaxis, antimicrobial therapy and supportive care. The overall annual attack rate for ABM is 2-5 cases for 100,000 population and the mortality rate is 6-16% in the United States. In developing countries incidence is up to 10 folds and the mortality rate is 2-4 folds higher than the United States. Severe neurological sequelae due to ABM have been reported in 10-20% of the patients. Meningococcemia is a life-threatening bloodstream infection caused by Neisseria meningitidis. Septic shock due to this organism is unique and requires early aggressive management to improve outcome. It is most common in childhood and mortality rates for meningococcemia ranges from 10% in adolescents and 20% in infants.

In children, early diagnosis is very important to improve prognosis both in ABM and meningococcemia. The identification of prognostic factors on admission could alert clinicians and could decrease the occurrence of undesirable events, so faster tests with high specificity and sensitivity are required to predict the outcome and prognosis.

Monocytes are a specific type of leukocytes that have functions in phagocytosis, cytokine production and presentation of antigen to lymphocytes for initiating both cellular and humoral immune responses. Monocytes play an important role in immune regulation and host defence against foreign organisms. HLA-DR molecules are expressed on the majority of monocytes and reflect the activation state of these cells. HLA-DR molecules are important for presenting antigen to the CD4+ cells.

In the paediatric population, significant diminished HLA-DR expression on monocytes in preterm and full-term neonates has been reported. Low monocyte HLA-DR expression was also found to be correlated with lower gestational ages in very low birth weight infants. Downregulation of HLA-DR expression on monocytes has been reported in neonatal sepsis, adult sepsis, different groups of surgical patients, pancreatitis and trauma. It has also been associated with septic complications and increased risk of mortality. Recent studies suggest that decreased monocyte HLA-DR expression is a reliable marker of excessive anti-inflammatory response and immune paralysis. In a study by Shankar-Hari et al, it has been suggested that the optimum biomarker combination associated with subsequent sepsis in emergency department patients with suspected acute infection is the combination of increased neutrophil CD24 and neutrophil CD279 with reduced monocyte HLA-DR expression.

This study aimed to get a serial measurement of monocyte HLA-DR expression rate to predict the outcome and prognosis in children with ABM and meningococcemia.

Population and Methods

Date and Time of Study Conduction / Study Design

This prospective case-control study was carried out between 1st August 2011 and 31st July 2017 at the department of paediatric infectious diseases of Dr. Behçet Uz Children's Hospital which is a 400-bed paediatric training hospital in Izmir, Turkey. In the study period, 18 children hospitalised with the diagnosis of ABM or meningococcemia were enrolled. The control group consisted of 36 healthy age and gender-matched children that applied to the outpatient departments for routine paediatric follow-up. Neonates were excluded from the study.

Hypothesis

Monocyte HLA-DR expression rate can predict the outcome and prognosis in children with ABM and meningococcemia.

Diagnosis of ABM and Meningococcal Disease

Patients were accepted as ABM according to the following criteria: increased cerebrospinal fluid (CSF) protein >100 mg/dL or decreased CSF glucose <40 mg/dL or CSF leukocyte count >100 white blood cell/mm³ with at least 80% neutrophils, identification of bacterial agents in gram staining, or isolation of bacteria from the CSF samples.

The clinical diagnosis of meningococcemia or meningococcal septic shock was made (in the absence of bacterial isolation) if the ill child had a fever and a petechial or purpuric rash and/or signs of meningitis as described before.

Determination of Cell Surface Markers by Flow Cytometry

A study designed by Wu et al showed that a single measurement of mHLA-DR within the first week after patient admission had no predictive value regarding mortality. In contrast, results expressed as dynamic parameters (i.e., differences between two-time points)
provided excellent predictive values, especially the difference in mHLA-DR expression between days 0 and 3 or days 0 and 7. Similarly, Zhuang et al found that dynamic monitoring of monocyte HLA-DR expression was more valuable for the diagnosis, prognosis, and prediction of sepsis. In this respect, we also investigated the monocyte HLA-DR expression on admission and the third day of treatment. Measurements were performed via three-colour flow cytometry by one of the authors who knew neither clinical nor laboratory findings of the patients. In the patient group, samples of peripheral blood were collected in EDTA anticoagulant tubes. In the control group, the plasma of the peripheral blood drawn for routine complete blood count was separated and used. Freshly collected blood samples (100 mL) were stained within 15 min after arrival at the laboratory with 10 mL of each conjugated monoclonal antibodies (mAb). The following commercial mAbs were used: phycoerythrin-cyanin 5 (PC5)-labeled CD45 (clone J.33; Beckman Coulter), fluorescein isothiocyanate (FITC)-labeled HLA-DR (clone Immu-357; Beckman Coulter), and phycoerythrin-labelled CD14 (clone RMO52; Beckman Coulter). After completion of the incubation, the erythrocytes were lysed and leucocytes were stabilised and fixed by TQ-Prep (Coulter). The appropriate isotype controls were used. At least 10,000 cells from each sample were analysed on the Cytomics FC500 (Beckman Coulter) flow cytometer, and the data were processed with CXP cytometer software. The cytometer was routinely optimised using the Flow-Check Fluorospheres (Coulter, Fullerton, California, USA). Monocytes were identified by gating on forwarding/side scatter in combination with CD45 and CD14. Results were expressed as the percentage of monocytes expressing HLA-DR and as mean fluorescence intensity (MFI) of monocytes showing expression.

**Ethical Considerations**

The study was approved by the "Local Research Ethics Committee of Dr. Behcet Uz Children's Hospital, Izmir, Turkey" (Protocol number: 21; Date: 17.04.2013) and adhered to the Declaration of Helsinki for Medical Research involving Human Subjects. The parents of the patients gave their informed consent for participation in the study.

**Statistical Analysis**

Data were expressed as the mean, allowing for the Standard deviation of the mean (mean±SD). All data were tested for normality using the Kolmogorov-Smirnov test. Quantitative values were assessed through analysis of variance (ANOVA) when normally distributed, and by the Kruskal-Wallis test in other cases. Comparisons between two groups were made using the Mann-Whitney U-test or t-test, as appropriate. Categorical data were analysed with the chi-squared test ($\chi^2$). Statistical analyses were performed with SPSS software, version 15.0 (SPSS Inc., Chicago, IL). Statistical significance was defined as p<0.05.

**Results**

Out of the 18 patients in the study group, 10 were male and 8 were female. The mean age was 55.2 months (4.6 years), ranging from 1 month to 15 years. In the control group, male to female ratio was 1.57 (22/14) and the mean age was 54.1 months (4.5 years) ranging from 1 month to 15 years. Comparison of patients and controls in terms of age and gender did not reveal any statistical significance (p=0.944 and p=0.695, respectively) (Table 1).

### Demographical Features, Complications and Outcome of Patients

All of the 18 patients in the study group had fever and meningeal irritations at admission (100%) followed by confusion (n=14, 77.8%), hypotension (n=14, 77.8%), coagulopathy (n=14, 77.8%), vomiting (n=12, 66.7%), headache (n=12, 66.7%) and rash (n=12, 66.7%). Laboratory features of the study group on admission are reviewed in Table 2. Thirteen out of 18 patients had leukocytosis, 1 had leucopenia and 1 had thrombocytopenia. CRP levels of all 18 patients were remarkably high. In 5 patients, the lumbar puncture could not be performed because of haemodynamic instability due to septic shock. Twelve of the patients were diagnosed as meningococcemia with petechial and purpuric skin lesions, confusion, hypotension, prolonged capillary filling time and coagulopathy. CSF analysis of the 13 patients was compatible with ABM. Twelve patients had negative CSF culture and six of these had the history of beforehand

<table>
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<tr>
<th>Table 1</th>
<th>Comparison of age and gender between groups</th>
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<td>Patient group</td>
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<tr>
<td>n=18</td>
<td>n=18</td>
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<tr>
<td>Age, (months)</td>
<td>55.2±60.2</td>
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<tr>
<td>(mean±SD)</td>
<td>(1-180)</td>
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<tr>
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<td>Male (n)</td>
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administration of empirical antibiotics that can penetrate through the blood-brain barrier (5 intravenous ceftriaxone, 1 gentamicin+ceftriaxone). While CSF culture of one patient yielded isolation of *N. meningitidis*, another one resulted in *S. pneumoniae*.

Among 18 patients, 5 had neurologic sequelae including seizures, papillary stasis and strabismus, left hemiparesis, ptosis in the left eye and central facial palsy. No death occurred and all patients were discharged after medical treatment.

**HLA-DR Expression**

Mean percentage of monocytes expressing HLA-DR in the study group on admission was significantly lower than the control group (30.1±23.4 vs 93±5.1, p<0.001). In the study group, monocyte HLA-DR mean fluorescence intensity (MFI) on admission was also significantly lower compared to the control group (12±6.1 vs 18±6.1, p=0.001) (Table 3). Representative flow cytometry plots of an ABM patient a healthy control are presented in Figure 1 and Figure 2, respectively. When compared to initial values, the percentage of monocytes expressing HLA-DR significantly increased (49.9±24 versus 30.1±23.4, p=0.003) on the third day of the treatment, but monocyte HLA-DR MFI did not differ significantly (12±6.1 vs 11.3±1.8, p=0.466) (Table 4). On admission, the percentage of monocytes expressing HLA-DR and monocyte HLA-DR MFI were lower in patients with neurological complications compared to patients with normal neurological examination but these differences were not statistically significant (p=0.104 and p=0.217, respectively). The percentage of monocytes expressing HLA-DR was found to be significantly lower in patients with neurological complications on the third day of treatment (p=0.043), however, monocyte HLA-DR MFI values did not differ significantly (p=0.084) (Table 5). Six patients had used empirical antibiotics before admission. On admission when compared to patients who used antibiotics prior to admission, the percentage of monocytes expressing HLA-DR was significantly lower in patients who did not use antibiotics before hospitalisation (19.11±12.8 vs 52.33±25.1, p=0.007), but monocyte HLA-DR MFI values were similar (11.61±6.92 vs 12.86±4.45, p=0.482). Neither the percentage of monocytes expressing HLA-DR nor the monocyte HLA-DR MFI values differed significantly on the third day of treatment between patients with and without antibiotic usage prior to admission (p=0.349, p=0.373 respectively) (Table 6). In neither the patient nor the control groups we observed any additional MHC class 2 expression defects that could be interpreted as a possible genetic variation.
Figure 1  Monocyte HLA-DR expression percentage of an ABM patient on admission (flow cytometry).

Figure 2  Monocyte HLA-DR expression percentage of a healthy control on admission (flow cytometry).
Discussion

In the pathogenesis of sepsis and serious infectious diseases such as meningitis and meningococcemia, the immune system plays a major role in host defence. In the initial phase of the immune response, increased release of pro-inflammatory cytokines leads to an effective immune response against infection. However, excessive production of these cytokines may be associated with excessive cellular injury, multiple organ failure and death. Following this phase, anti-inflammatory mediators are produced to modulate the inflammatory response. Excessive anti-inflammatory stimulation results with a state termed immune paralysis. The percentage of monocytes expressing HLA-DR is found normal or increased in the inflammatory phase. In the anti-inflammatory phase, damaged monocyte functions and activations, inadequate oxidative burst and antigen presentation result in a decreased percentage of monocytes expressing HLA-DR. Decreased monocyte HLA-DR expression is considered as a reliable marker of immune paralysis.

In our study, we found the percentage of monocytes expressing HLA-DR and monocyte HLA-DR MFI significantly lower on admission in the study group. On the third day of treatment, the percentage of monocytes expressing HLA-DR was significantly higher than the initial values. Fortunately, no mortality occurred. However, 5 patients developed neurological complications. Patients with neurological complications had a lower percentage of monocytes expressing HLA-DR and lower monocyte HLA-DR MFI on admission, but these differences were not statistically significant. On the other hand on the third day of treatment, the percentage of monocytes expressing HLA-DR was significantly lower in patients with neurological complications while the monocyte HLA-DR MFI values remained similar.

Percentage of monocytes expressing HLA-DR is considered to be one of the best reflectors of immune functions in adults with serious diseases treated in intensive care units. A low percentage of HLA-DR expression on monocytes has been reported as a poor prognostic factor in septic adults, different groups of surgical patients, pancreatitis and trauma. In adult sepsis, percentage of monocyte HLA-DR expression less than 20% and its persistence on consecutive 5 days are associated with mortality rates as high as 90%. In a study involving adult patients with cryptococcal meningitis, authors defined an immune signature associated with early mortality which was characterised by monocyte deactivation (reduced HLA-DR expression and tumour necrosis factor α response

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<th>Parameters</th>
<th>On admission</th>
<th>The third day of treatment</th>
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<tr>
<td>Percentage of monocytes expressing HLA-DR (%)</td>
<td>30.1±23.4</td>
<td>49.9±24</td>
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<tr>
<td>Monocyte HLA-DR MFI</td>
<td>11.3±1.8</td>
<td>12±6.1</td>
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MFI: mean fluorescence intensity

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<tr>
<th>Parameters</th>
<th>Patients with neurological complications (n=5)</th>
<th>Patients without neurological complications (n=13)</th>
<th>p</th>
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<tbody>
<tr>
<td>Percentage of monocytes expressing HLA-DR on admission (%)</td>
<td>14.88±6.39</td>
<td>36.07 ±25.14</td>
<td>0.104</td>
</tr>
<tr>
<td>Percentage of monocytes expressing HLA-DR on the third day of treatment (%)</td>
<td>25.76±20.11</td>
<td>59.31±29.99</td>
<td>0.043</td>
</tr>
<tr>
<td>Monocyte HLA-DR MFI on admission</td>
<td>14.75±7.11</td>
<td>10.98±5.62</td>
<td>0.217</td>
</tr>
<tr>
<td>Monocyte HLA-DR MFI on the third day of treatment</td>
<td>7.69±2.80</td>
<td>13.38±8.42</td>
<td>0.084</td>
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MFI: mean fluorescence intensity
to lipopolysaccharide); increased serum interleukin 6, CXCL10 and interleukin 10 levels; as well as increased neutrophil counts; and decreased T-helper cell type 1 responses. Published reports on the relationship between monocyte HLA-DR expression and outcome in children are limited. A study including children after cardiac bypass surgery reported that decreased HLA-DR in the early postoperative period predicts sepsis/systemic inflammatory response syndrome and prolonged stay in the intensive care unit. Hoffman et al, showed the association between persistent low monocyte HLA-DR expression and the risk of post lung transplant pneumonia in children. Döring et al investigated children and young adults after haematopoietic stem cell transplantation. In this study, prior to and during sepsis or bacterial infection, a significant decrease in human leukocyte antigen DR expression occurred. Wakiguchi et al studied the relationship between T-cell HLA-DR expression and intravenous immunoglobulin (IVIG) treatment response in Kawasaki disease (KD). They found increased T-cell HLA-DR expression associated with IVIG resistance in KD patients, indicating that T-cell activation could be a contributing mechanism underlying this phenomenon. A previous study in our centre demonstrated that the percentage of HLA-DR expressing monocytes was significantly lower in the non-survivor late-onset neonatal sepsis group (16.6%), compared with the survivor group (45.2%), and patients with monocyte HLA-DR expression ≤30% had lower survival rate with a 30-fold higher risk of mortality. These results may indicate monocyte HLA-DR expression as an early predictive marker for prognosis in critically ill children and late-onset neonatal sepsis. In the present study, children with ABM and meningococcemia had down-regulated monocyte HLA-DR expression compared with healthy children reflecting compensatory anti-inflammatory response and immune paralysis. The increase on the third day of antibiotic treatment demonstrated improved immune functions. Our study also showed that the percentage of HLA-DR expressing monocytes on the third day may be a predictive marker for the development of neurological complications. In the present study, we found that a mean monocyte HLA-DR expression ≤50% on admission was strongly associated with the diagnosis of ABM. Moreover, we also demonstrated that the patients with monocyte HLA-DR expression still ≤40% on the third day of treatment were more likely to have poor neurological outcome. HLA-DR MFI did not differ significantly either on the first or the third day of follow up. Similarly, we could not find any significant relation between neurological complications and first or third-day monocyte HLA-DR MFI values. In a previous study, Wu et al reported that in survivors of sepsis monocyte HLA-DR MFI values increased significantly after 6 days. In our study, we did not obtain any values further than the third day of treatment.

In ABM and meningococcemia, it is vital to begin antimicrobial treatment as early as possible. Bacterial load must be reduced immediately in order to prevent the complications of uncontrolled infection. Large studies, especially in adults with meningitis indicate that delay in antimicrobial treatment is a strong and independent risk factor for mortality and morbidity. Recent data in children suggest early antibiotics are independently associated with improved outcomes in septic shock. In our study, six patients had used empirical blood-brain barrier penetrating antibiotics (ceftriaxone) before admission. The percentage of monocytes expressing HLA-DR was significantly lower in patients without prior antibiotic usage than the patients who used antibiotics before admission, supporting the importance of early treatment. This result also suggests that early antibiotic treatment may have favourable effects on immune response and regulation in serious infections.

The strength of the current study comes from its being the first study in the current literature that introduces decreased monocyte HLA-DR expression as a reliable predictive marker of outcome and prognosis in paediatric patients with ABM and meningococcemia. On the other hand, the main drawback of the study is the small sample size. Larger scaled prospective studies are required to confirm our findings.

**Conclusion**

According to our findings, monocyte HLA-DR expression is downregulated in paediatric patients with ABM and meningococcemia. Higher percentages of monocytes expressing HLA-DR in patients referred with prior antibiotic treatment supports the importance of early treatment of ABM and meningococcemia. The percentage of monocyte HLA-DR expression on the third day of treatment also seems to be a valuable predictive marker for complications during follow up. Using monocyte HLA-DR expression may help clinicians to save time and effort in the differential diagnosis of critically ill children, lead them in decision making of early antibiotic treatment and prediction of possible complications.
Conflicts of Interest

None of the authors declares any conflict of interest.

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