ORIGINAL ARTICLE

Diagnostic Value of Serum Adenosine Deaminase in Myelodysplastic Syndromes: an Observational Study

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SUMMARY

Background: This study aimed to explore the diagnostic value of serum adenosine deaminase (ADA) in patients with myelodysplastic syndromes (MDS) and identify potential risk factors for MDS.

Methods: Eighty patients with MDS and 80 healthy individuals were included. The serum ADA level was found to be significantly higher in patients with MDS compared with that of healthy controls (p = 0.014).

Results: The receiver operator characteristic curve (ROC) for ADA had an area under curve (AUC) of 0.807 (p = 0.0018). Serum ADA level of 4.5 U/L had a sensitivity of 71.43% and specificity of 80% for MDS diagnosis. The multivariate analysis showed hemoglobin (Hb, OR = 1.322, 95% CI: 1.035 - 2.323, p = 0.039), prothrombin time (PT, OR = 1.524, 95% CI: 1.156 - 3.280, p = 0.042), fibrinogen (OR = 1.335, 95% CI: 1.022 - 2.775, p = 0.027), calculated international normalized ration (INR, OR = 2.212, 95% CI: 1.320 - 3.085, p = 0.038), D-dimer (OR = 2.043, 95% CI: 1.623 - 4.293, p = 0.038), fibrin degradation product (FDP, OR = 2.525, 95% CI: 1.129 - 3.400, p = 0.029), and serum ADA (OR = 2.057, 95% CI: 1.248 - 3.572, p = 0.033) were independently associated with MDS.

Conclusions: Serum ADA might be a potential biomarker in the diagnosis of MDS. Serum ADA level, Hb level, PT, fibrinogen level, INR, D-dimer, and FDPs were independent risk factors of MDS.


KEYWORDS

adenosine deaminase, myelodysplastic syndrome, diagnosis, clinical enzyme tests, case control

INTRODUCTION

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal myeloid neoplasms with variable clinical manifestations, and overall outcomes [1]. The incidence rate of MDS is about 5/100,000 in population, the incidence of MDS has a gendered tendency, occurring more often in males than in females [2]. MDS is a disease of the elderly with a median age of 70 - 75 years and cytopenia of different severity exists, but it usually continues without treatment [3]. The clinical course of MDS is variable, although some patients have a long life span with minimal therapy, others rapidly progress to AML. Characterized by a compromised hematopoietic stem cell function and excessive apoptosis within the marrow [4], these hematopoiesis disorders
have an increased predisposition to acute myeloid leukemia [5-7]. At least one cytopenia must occur when MDS is diagnosed. Standard laboratory value of hemocytopenia is hemoglobin < 13 g/dL, < 12 g/dL, absolute value of neutrophil count < 1.8 × 10⁹, or below each individual laboratory’s reference range. Further, MDS has been shown to be associated with immune dysregulation and impaired adaptive immune response [8]. Although MDS may occur in children [9], it primarily affects the elderly, wherein the mean age at onset is 70 - 75 years [10]. Several prognostic models using clinical and laboratory variables, such as the International Prognostic Scoring System (IPSS) and revised IPSS (IPSS-R) are used to predict outcomes. Both the IPSS and IPSS-R stratify newly diagnosed, untreated patients based on the number and degree of anemia, thrombocytopenia, or neutropenia, myeloblast percentage, and karyotypic abnormalities. Currently, the diagnosis of MDS is based on the World Health Organization (WHO) classification criteria — presence of one or more cytopenias, morphological dysplasia of ≥ 10% of nucleated cells in at least one lineage, < 20% blasts forms in blood and bone marrow, and/or characteristic cyto genetic or molecular aberrations, excluding other causes of dysplasia and/or cytopenia(s) [11,12]. However, these features are neither pathognomonic nor indispensable clues for the diagnosis of MDS [13]. Moreover, the clinical features of many other conditions mimic dysplasia, cytopenias, and/or clonal abnormalities; therefore, it is imperative to perform an extensive differential diagnosis which excludes non-MDS entities prior to a definitive diagnosis of MDS. Consequently, this necessitates the need for an analytic factor that aids in a definitive diagnosis of MDS.

Adenosine deaminase (ADA), a key immunoregulatory enzyme in the physiological microenvironment, catalyzes the deamination of adenosine and 2’ deoxyadenosine to inosine and 2’ deoxyinosine. The two principal isoenzymes are ADA1 and ADA2 [14]. It plays a critical role in regulating the human immune balance [15]. ADA is probably the most widely used and the simplest biomarker in evaluating for the diagnosis of tuberculous pleural effusion (TPE). A meta-analysis showed that the sensitivity and specificity of ADA in the diagnosis of pleural TB were 92% and 90%, respectively [34]. Several studies have demonstrated associations between serum ADA levels and disease activity in various autoimmune disorders, such as systemic lupus erythematosus [15], rheumatoid arthritis [17], juvenile idiopathic arthritis [18], autoimmune hepatitis. Interestingly, a study by Fargo et al. reported that erythrocyte adenosine deaminase levels were elevated in a patient with Fanconi anemia associated with MDS [19]. Despite its quintessential role in immune regulation, the role of ADA in the pathophysiology of MDS remains inconclusive. Owing to a paucity of research on the significance of serum ADA in MDS, this study aims to explore the diagnostic value of serum ADA in patients with MDS and risk factors for MDS.

Some methods used to diagnose MDS patients, particularly cytogenetic and morphological assessment, depend on individual experience. Many studies should be done to identify novel diagnostic tools to make MDS diagnosis more convenient.

MATERIALS AND METHODS

Subjects

This prospective case-control study enrolled patients with MDS and healthy individuals who underwent physical examination at the Department of Hematology, Chengde Second People’s Hospital between June 2020 and June 2021. A straightforward diagnosis of MDS was made on the basis of WHO criteria [11,12] based on clinical characteristics, cytogenetic data from BM aspirates, and histological results from BM smears and H&E-stained sections of BM biopsies. The inclusion criteria for patients were: 1) newly diagnosed with MDS, 2) no treatment. The exclusion criteria for patients were: 1) hematological disease other than MDS, 2) autoimmune disease. Healthy individuals were selected from individuals who underwent physical examination without abnormal result of physical examination or chronic disease. This study was reviewed and approved by the Ethics Committee of the Chengdu Fifth People’s Hospital and written informed consent was obtained from all participants prior to enrollment. All research protocols were in accordance with the Declaration of Helsinki and were approved by the review board of Chengdu Fifth People’s Hospital.

Data collection

Venous blood samples were collected in tubes containing EDTA, from subjects in the fasting state. Serum was separated after centrifugation at 3,000 rpm for 10 minutes at room temperature and was subsequently used for assessing various laboratory parameters. Serum ADA levels were measured by using LABOSPECT 008 α Hitachi Automatic Analyzer (Hitachi High-Technologies, Tokyo, Japan), according to the manufacturer’s instructions. Demographic data, including age and gender, and hematological indices, including white blood cell count (WBC), red blood cell count (RBC), hemoglobin levels (Hb), platelet count, prothrombin time (PT), thrombin time, activated partial thromboplastin time, fibrinogen levels, International Normalized Ratio (INR) of PT, D-dimer, fibrin degradation products (FDPs), and serum ADA levels were assessed.

ADA genotypes

The genotype of ADA gene was identified by PCR. For TaqMan genotyping technology, DNA was extracted using a DNA Extraction kit (Beijing Tiangen Biotechnology Co., Ltd., China). PCR amplification was performed as described by Fontana L. et al. [20]. Sense and
Table 1. Demographic and clinical characteristics of study population.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients (n = 80)</th>
<th>Controls (n = 80)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, median (range)</td>
<td>69.3 ± 12.53</td>
<td>65.23 ± 11.13</td>
<td>0.9004</td>
</tr>
<tr>
<td>Male/female</td>
<td>48/32</td>
<td>40/40</td>
<td></td>
</tr>
<tr>
<td>White blood cell count (*10^9/L)</td>
<td>4.1 ± 58.63</td>
<td>5.40 ± 1.14</td>
<td>0.6548</td>
</tr>
<tr>
<td>Red blood cell count (*10^12/L)</td>
<td>2.26 ± 0.22</td>
<td>4.72 ± 0.50</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>70.1 ± 9.37</td>
<td>145.4 ± 15.0</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Platelet count (*10^9/L)</td>
<td>39.5 ± 41.89</td>
<td>180.4 ± 36.4</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Prothrombin time(s)</td>
<td>13.4 ± 1.94</td>
<td>11.24 ± 0.27</td>
<td>0.0027</td>
</tr>
<tr>
<td>Thrombin time(s)</td>
<td>17.33 ± 1.28</td>
<td>19.13 ± 0.78</td>
<td>0.0014</td>
</tr>
<tr>
<td>Activated partial thromboplastin time(s)</td>
<td>27.8 ± 3.05</td>
<td>27.21 ± 1.24</td>
<td>0.5797</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>4.11 ± 1.68</td>
<td>2.84 ± 0.46</td>
<td>0.0332</td>
</tr>
<tr>
<td>Calculated international normalized ratio</td>
<td>1.25 ± 0.19</td>
<td>1.03 ± 0.02</td>
<td>0.0027</td>
</tr>
<tr>
<td>D-dimer (µg/mL)</td>
<td>1.49 ± 1.41</td>
<td>0.26 ± 0.09</td>
<td>0.013</td>
</tr>
<tr>
<td>Fibrin degradation product (µg/mL)</td>
<td>5.04 ± 2.51</td>
<td>2.50 ± 0.49</td>
<td>0.0051</td>
</tr>
<tr>
<td>Serum adenosine deaminase (U/L)</td>
<td>14.7 ± 13.19</td>
<td>3.3 ± 1.15</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Table 2. Multivariate analysis of risk factors associated with myelodysplastic syndromes.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>1.322</td>
<td>1.035 - 2.323</td>
<td>0.039</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>1.524</td>
<td>1.156 - 3.280</td>
<td>0.042</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>1.335</td>
<td>0.381 - 2.053</td>
<td>0.56</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>1.864</td>
<td>1.022 - 2.775</td>
<td>0.027</td>
</tr>
<tr>
<td>Calculated International Normalized Ratio</td>
<td>2.212</td>
<td>1.320 - 3.085</td>
<td>0.038</td>
</tr>
<tr>
<td>D-dimer</td>
<td>2.043</td>
<td>1.623 - 4.293</td>
<td>0.038</td>
</tr>
<tr>
<td>Fibrin degradation products</td>
<td>2.525</td>
<td>1.129 - 3.340</td>
<td>0.029</td>
</tr>
<tr>
<td>Serum adenosine deaminase</td>
<td>2.057</td>
<td>1.248 - 3.572</td>
<td>0.033</td>
</tr>
</tbody>
</table>

antisense primer for ADA1 genotype were: 5’-ACCGA GCCGCGAGAGCCACTCCAA-3’ and 5’-ACTTGACAGA CAGCGAAACTGAGACCCAGA-3’. The positive and negative primers of ADA2 genotype were respectively: 5’-GAGCACAAGCTTTGGAATTGGGCTTGGGTT-3’ and 5’-ACACCAGGAGCAGACACTCAGAGGC CCAGAA-3’.

Statistical analysis
Data were analyzed by using the GraphPad Prism version 8.0.2 for Windows (GraphPad Software, San Diego, CA, USA). Continuous variables with normal distribution are presented as mean ± standard deviation (SD) and were compared by Student’s t test. Non-normal variables are presented as median (interquartile range [IQR]) and were compared by Wilcoxon signed-rank test. Categorical data are expressed as numbers (percentages) and were compared using Pearson’s χ² test. The best cutoff value of serum ADA for diagnosis of MDS was selected by the receiver operating characteristic (ROC) curve. A multivariate regression model was used to identify variables independently associated
Table 3. Distribution of adenosine deaminase genotypes in patients with myelodysplastic syndromes.

<table>
<thead>
<tr>
<th>Locus</th>
<th>ADA1</th>
<th>ADA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td>*1/*1% (n)</td>
<td>*1/*2% (n)</td>
</tr>
<tr>
<td>Serum ADA level &lt; 4.5U/L MDS patients (n = 24)</td>
<td>70.84 (17)</td>
<td>25 (6)</td>
</tr>
<tr>
<td>4.5 &lt; serum ADA level &lt; 9.5U/L MDS patients (n = 16)</td>
<td>68.75 (11)</td>
<td>31.25 (5)</td>
</tr>
<tr>
<td>Serum ADA level &gt; 9.5U/L MDS patients (n = 40)</td>
<td>52.63 (21)</td>
<td>42.37 (19)</td>
</tr>
<tr>
<td>Chi-squared</td>
<td>3.985</td>
<td>1.212</td>
</tr>
<tr>
<td>df</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>p-value</td>
<td>0.408</td>
<td>0.8761</td>
</tr>
</tbody>
</table>

Figure 1. Receiver-operating curve for serum adenosine deaminase.

The area under curve (AUC) was 0.807 (95% CI: 1.208 - 4.672, p = 0.0018). Adenosine deaminase level of 4.5 U/L would have a sensitivity of 71.43% and specificity of 80% for the diagnosis of myelodysplastic syndromes.

with MDS. A two-sided p < 0.05 was considered significant. A chi-squared test was used to analyze the genotype of ADA inpatients with MDS.

RESULTS

A total of 80 patients with MDS (48 males and 32 females; mean age: 69.31 ± 2.53 years) and 80 healthy individuals (40 males and 40 females; mean age: 65.23 ± 11.13) were included. RBC count (2.26 ± 0.22 vs. 4.72 ± 0.50, p < 0.0001), Hb (70.1 ± 9.37 vs. 145.4 ± 15.0,
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p < 0.0001), platelet count (39.5 ± 41.89 vs. 180.4 ± 36.4, p < 0.0001), and thrombin time (17.33 ± 1.28 vs. 19.13 ± 0.78, p = 0.0014) were significantly lower in patients with MDS compared with those of healthy individuals. On the contrary, PT (13.4 ± 1.94 vs. 11.24 ± 0.27, p = 0.0027), fibrinogen (4.11 ± 1.68 vs. 2.84 ± 0.46, p = 0.0332), INR (1.25 ± 0.19 vs. 1.03 ± 0.02, p = 0.0027), D-dimer (1.49 ± 1.41 vs. 0.26 ± 0.09, p = 0.0130), and FDPs (5.04 ± 2.51 vs. 2.50 ± 0.49, p = 0.0051) were significantly higher in patients with MDS compared with those of healthy individuals. Notably, the mean serum ADA level was found to be significantly higher in patients with MDS compared with that of healthy controls (14.7 ± 13.19 vs. 3.3 ± 1.15, p = 0.0140) (Table 1).

The AUC of ROC was 0.807 (95% CI: 1.208 - 4.672, p = 0.0018) (Figure 1). ADA level of 4.5 U/L would have a sensitivity of 71.43% and specificity of 80% for the diagnosis of MDS.

Multivariate regression analysis revealed that Hb level (OR = 1.322, 95% CI: 1.035 - 2.323, p = 0.039), PT (OR = 1.524, 95% CI: 1.156 - 3.280, p = 0.042), fibrinogen level (OR = 1.864, 95% CI: 1.022 - 2.775, p = 0.027), INR (OR = 2.212, 95% CI: 1.320 - 3.085, p = 0.038), D-dimer (OR = 2.043, 95% CI: 1.623 - 4.293, p = 0.038), FDPs (OR = 2.525, 95% CI: 1.129 - 3.340, p = 0.029), and serum ADA level (OR = 2.057, 95% CI: 1.248 - 3.572, p = 0.033) were independent risk factors for MDS (Table 2).

Table 3 showed the proportion of MDS subjects associated with ADA1 and ADA2 genotypes. We divided 80 MDS patients into three groups based on their ADA levels: serum ADA level < 4.5 U/L (n = 24); 4.5 < serum ADA level < 9.5 U/L (n = 16); serum ADA level > 9.5 U/L (n = 40). Comparing ADA1 and ADA2 genotypes among the three groups showed no significant difference between the two groups (p > 0.05).

**DISCUSSION**

This study shows that serum ADA might be a relatively accurate biomarker in the diagnosis of MDS. Further, this study highlighted that serum ADA level, Hb level, PT, fibrinogen level, INR, D-dimer, and FDPs were independent risk factors for MDS. The results might provide a reference for the diagnosis of MDS.

Over recent years, medical workers have gained much insight into the mechanisms underlying the pathogenesis of MDS, owing to the rapid advances in molecular diagnostics. Although prompt treatment is crucial, successful outcome and improvement in overall survival is often impeded by a delay in diagnosis due to the heterogeneity of this syndrome, variable clinical presentation, and lack of specificity in the clinical and laboratory findings. As most of the clinical features for MDS do not represent sine qua non for diagnosis, there is an imperative need for a diagnostic tool with definitive value. Recently, an increasing amount of evidence has highlighted the role of ADA in the function, maturation, and regulation of immune responses [20-24]. Further, it was shown that ADA provides an indispensable clue for cellular immunity. This led many researchers to focus exclusively on the associations between ADA and disease activity in various autoimmune diseases [16,18]. A few studies evaluated the significance of elevated erythrocyte adenosine deaminase (eADA) in MDS [26,27]. However, the diagnostic value of serum ADA was constrained to studies on tuberculosis [25,28,29]. To our knowledge, no prior studies have examined its diagnostic value in MDS. A notable finding of this study is that the mean serum ADA level was significantly higher in patients with MDS compared with that of control. Moreover, ROC analysis for ADA revealed that serum ADA level of 4.5 U/L had a sensitivity of 71.43% and specificity of 80% for the diagnosis of MDS. The diagnostic value of serum ADA for MDS is further strengthened by our finding that there was no association of mean serum ADA level with either age or gender. Therefore, these results suggest the importance of utilizing serum ADA in the diagnosis of MDS, regardless of age or gender of an individual. In addition, the sensitivity of serum ADA in distinguishing patients with MDS from non-MDS individuals indicates that serum ADA could remain a minor criterion in the diagnosis of MDS.

Further, significantly lower mean values of RBC count, Hb, platelet count, and thrombin time were observed, in contrast to significantly higher mean values of PT, fibrinogen, INR, D-dimer, and FDPs in patients with MDS compared with those of healthy individuals. Increased levels of D-dimer and FDPs indicate the presence of hypercoagulable state and secondary fibrinolysis following a higher fibrinolytic activity in the body. Further, elevated levels of INR imply a decline in the synthesis of clotting factors. In addition, a higher fibrinogen level is an important indicator for many thrombotic diseases [30-32]. Collectively, the findings suggest that PT, fibrinogen, INR, D-dimer, and FDPs may be used as sensitive indicators that could reflect the impairment of coagulation in patients with MDS. Further corroborating these findings, multivariate logistic regression showed hemoglobin (Hb, OR = 1.322, 95% CI: 1.035 - 2.323, p = 0.039), prothrombin time (PT, OR = 1.524, 95% CI: 1.156 - 3.280, p = 0.042), fibrinogen (OR = 1.864, 95% CI: 1.022 - 2.775, p = 0.027), calculated international normalized ratio (INR, OR = 2.212, 95% CI: 1.320 - 3.085, p = 0.038), D-dimer (OR = 2.043, 95% CI: 1.623 - 4.293, p = 0.038), FDPs (OR = 2.525, 95% CI: 1.129 - 3.340, p = 0.029), and serum ADA level (OR = 2.057, 95% CI: 1.248 - 3.572, p = 0.033) were independently associated with MDS. Serum ADA independently predicted the risk of MDS. Therefore, abnormal coagulation might be strongly related to the risk of MDS.

This study has some limitations. The small overall sample size of this study strongly suggests avenues for future research. The possibility of recall bias and misper-

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CONCLUSION

In conclusion, serum ADA might be a relatively accurate biomarker in the diagnosis of MDS. Further, serum ADA level, Hb level, PT, fibrinogen level, INR, D-dimer, and FDPs were independently risk factors of MDS.

Declaration of Interest:
The authors report no potential conflict of interests.

References:


