


**Lactate Dehydrogenase and Its Isoenzymes in Serum from Patients with Multiple Myeloma**

Sıtkı Çopur,1,4 Sazali Kus,9 Ayse Kara,2 Nurten Renda,3 Gülten Tekuzman,7 and Dinçer Fırat2

Concentrations of total lactate dehydrogenase (LDH; EC 1.1.1.27) and LDH isoenzyme patterns were studied in serum of 19 patients with multiple myeloma and in 19 healthy controls. Patients were divided into three groups (pretreatment, nonresponders, and responders to treatment), based on their clinical status at the time of blood sampling for LDH. The LDH values were found to be significantly higher (P < 0.05) in the pretreatment group and in the nonresponders than in the responders and the control group, the mean ± SE values being 445 ± 35 and 532 ± 75 units/mL vs 349 ± 75 and 190 ± 7.1 units/mL, respectively. Compared with responders and healthy controls, newly diagnosed patients and nonresponders had slight diminutions in LDH-1 and LDH-2, but increased LDH-3. We conclude that determination of LDH and its isoenzymes in serum can be of value as prognostic factors in patients with multiple myeloma.

Lactate dehydrogenase (LDH; EC 1.1.1.27), a glycolytic enzyme, is present in various tissues and neoplasms of the human body in multiple molecular forms. This heterogeneity allows electrophoretic fractionation of the enzyme into at least five isoenzymes (1-2). In recent years the relationship between neoplasia and LDH has been studied with increasing intensity (3-4). Serum LDH is known to be of prognostic significance in hematological malignancies such as leukemia and malignant lymphoma (5-7). Moreover, in some patients with malignancy, changes in specific LDH isoenzyme patterns in serum correlate with tumor growth or regression; such changes have been used as tumor markers for monitoring therapy or detecting recurrent disease (8-9). Although the prognostic value of serum LDH in multiple myeloma has been reported (10), LDH isoenzyme patterns have been studied in very few patients with this disorder (11). Thus the distribution of LDH isoenzymes and their possible significance in prognosis of multiple myeloma patients have not yet been established. We undertook this study to determine the value of determining LDH activity and the isoenzyme pattern in serum of patients with multiple myeloma.

**Patients and Methods**

Serum LDH and its isoenzymes were studied in 19 patients with multiple myeloma: nine men, 10 women, ages 52 ± 3 years (mean ± SE; range 43–73). At the time of serum sampling, 10 patients were newly diagnosed and had received no treatment (pretreatment group). Of the nine patients who had been given treatment, five had responded to therapy (responders), and four had not (nonresponders). Patients with concomitant malignancies were excluded from the study. Venous blood samples obtained from 19 healthy normal people (11 men, eight women, ages 49 ± 3 years; range 28–75) served as controls. Blood samples, allowed to clot for 30 min at room temperature, were centrifuged to separate the serum, and total LDH activity and LDH isoenzyme concentrations were determined that same day. Serum samples with any signs of hemolysis were discarded.

**Diagnosis and classification of the patients:** The diagnosis of multiple myeloma was based on the fulfillment of at least two of the three following criteria: demonstration of focal or generalized increase in abnormal plasma cells in the bone marrow or other tissues; presence of serum or urinary myeloma proteins, often with an associated reduction of immunoglobulin concentration; and typical roentgenographic changes.

Of the 19 patients with multiple myeloma, 18 had IgG and one had IgA myeloma. The patients were staged according to the method of Durie and Salmon (12): three
were in Stage I, five were in Stage II, and 11 were in Stage III.

Treatment protocol: The nine patients being treated were receiving continuous melphalan (one responder); vincristine, adriamycin, and dexamethasone (VAD) combination (four of the responders); intermittent melphalan and prednisolone, and later VAD (three of the nonresponders); and continuous melphalan, later followed by VAD (one nonresponder). Clinical response was defined mainly according to the criteria proposed by the Committee of Chronic Leukemia-Myeloma Task Force (13).

Enzyme and isoenzyme analysis: Total LDH activity in serum was determined by measuring the rate of decrease in absorbance at 340 nm in the method described by Wroblewski and La Due (14). All determinations were made with a Shimadzu (Kyoto, Japan) spectrophotometer (Model UV-120-02) at pH 7.4 and 32 °C. One unit of LDH was defined as the amount of enzyme required to produce a decrease in absorbance of 0.001 per minute. We previously had determined a normal reference interval of 165–280 units/mL for total LDH activity in the sera of normal healthy controls.

LDH isoenzymes in serum were separated by electrophoresis on cellulose acetate and detected by use of staining solution containing NAD+, lithium L+ lactate, nitro blue tetrazolium, and phenazine methosulfate (Sigma Technical Bulletin No. 705-EP, Sigma Chemical Co., November 1978). The relative activity of each isoenzyme on the cellulose acetate strips was determined by scanning with a Quick-scan densitometer (Helena Labs, Beaumont, TX) at 550 nm, and expressing the results as percentages of the total electrophoretically determined LDH activity. Our normal reference intervals for serum LDH isoenzymes in the control group were: 26–35% for LDH-1, 33–44% for LDH-2, 15–22% for LDH-3, 2–8% for LDH-4, and 3–8% for LDH-5. The statistical significance of differences between groups was estimated by use of the Mann-Whitney U test and Fisher's exact chi-square analysis.

Results

Total serum LDH activity: Total serum LDH activity of the patients and control group is shown in Table 1. Total LDH was increased in six of the 10 in the pretreatment group. All four nonresponders and three of the five responders also had increased total LDH. This increase was significant (P < 0.05) in newly diagnosed patients and in nonresponders, as compared with normal healthy controls. Table 2 summarizes the significance of differences in total serum LDH in the different patient groups and healthy controls. Total serum LDH values had no relation to the clinical stage, being increased in two of three patients in Stage I, two of five patients in Stage II, and nine of 11 patients in Stage III, respectively. Also the differences in serum LDH among these three groups were not significant (P > 0.05, Fisher's exact chi-square analysis).

Determination of serum LDH isoenzymes: Table 3 shows the LDH isoenzyme patterns of all patients and the control group. In all serum samples obtained from patients at presentation (n = 10) and from the four patients who were nonresponders, isoenzyme LDH-3 was more prominent, whereas LDH-1 and LDH-2 were slightly diminished, than in responders (n = 5) and the control group (n = 19). In responders, the distribution of serum LDH isoenzymes was similar to that of normal healthy controls.

Discussion

Serum LDH activity has been shown to be increased in various malignant conditions, but with a great variability in activity (7, 8, 15–20). Vezzoni et al. (21) suggested that the increase of serum LDH in patients with malignant lymphoma may be due to a release from the tumor cells. Although serum LDH activity is known to be of prognostic value in other hematological malignancies such as leukemia and malignant lymphoma (5–7, 16), its relation to prognosis in multiple myeloma has been shown only recently (10). Simonsson et al. (10) found increased LDH in 27 of 93 patients with multiple myeloma and showed that the concentration of this enzyme in serum correlated negatively with survival. In our study, 13 of 19 patients with multiple myeloma had increased LDH activity in serum. We also noticed that, although serum LDH activity was increased in all patient groups, it was significantly higher in the nonresponders than in the responders, which suggests a relationship between the disease activity and the concentration of this enzyme in serum. Consistent with the previous report (10), we found that total LDH in serum had no relationship to the clinical stage (a rough estimation of tumor cell mass). Although the difference between the concentrations of serum LDH in nonresponders and responders seems significant, we observed no such difference between low, intermediate, and high tumor-mass groups. Perhaps the activity concentration of this enzyme in serum reflects differences in proliferation of the malignant cells; however, the underlying mechanisms involved are unknown.

Of the aberrant isoenzyme patterns generally observed in malignant diseases, the most frequently encountered is an increase of the middle group of LDH isoenzymes (8). In hematopoietic tissues, maturing cells of the granulocytic and lymphocytic series contain mainly isoenzymes LDH-1, -2, and -3; however, at the blast stage, LDH-3 is the predomin-

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>No. of subjects</th>
<th>Range (units/mL)</th>
<th>Mean ± SE</th>
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</thead>
<tbody>
<tr>
<td>Pretreatment group</td>
<td>10</td>
<td>210–550</td>
<td>445 ± 35</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>4</td>
<td>480–630</td>
<td>532 ± 35</td>
</tr>
<tr>
<td>Responders</td>
<td>5</td>
<td>140–530</td>
<td>349 ± 75</td>
</tr>
<tr>
<td>Control group</td>
<td>19</td>
<td>165–280</td>
<td>190 ± 7</td>
</tr>
</tbody>
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Table 2. Significance of Between-Group Differences in Total Serum LDH Activity

<table>
<thead>
<tr>
<th>Pretreatment group</th>
<th>Nonresponders</th>
<th>Responders</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>U = 28</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &lt; 0.05</td>
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<tr>
<td>U = 50</td>
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* Calculated U value and statistical significance (Mann-Whitney U test).
inant form (22). Isoenzyme LDH-3 predominance has also been shown in lymphoblasts obtained from patients with acute lymphoblastic leukemia (23).

To our knowledge, alterations in the serum LDH isoenzyme pattern in patients with multiple myeloma have not been studied in detail. Ananthanarayanan and Ramakrishnan (11) reported a decrease in isoenzymes LDH-1 and LDH-2 in only three patients with multiple myeloma. In our study, isoenzyme analysis revealed a consistent increase in LDH-3 and a slight diminution of LDH-1 and LDH-2 in the newly diagnosed patients and in the nonresponders in comparison with the findings for the responders and the healthy controls. Although the number of patients in the nonresponder and responder subgroups might seem small, overall there was increased LDH-3 and decreased LDH-1 and LDH-2 in 14 patients (10 newly diagnosed patients and four nonresponders), with a normal isoenzyme pattern in 24 subjects (five responders and 19 controls). In other neoplastic diseases, changes in serum LDH isoenzyme pattern have correlated with either tumor growth or regression (8, 9). Our results suggest that an increase in serum LDH-3 and a decrease in LDH-1 and LDH-2, may indicate progressive disease in patients with multiple myeloma.

We conclude that determination of LDH concentration and isoenzyme distribution in serum may have potential diagnostic and prognostic value in patients with multiple myeloma. Obviously, other clinical and laboratory studies must be used before arriving at a final interpretation, and studies with more cases are needed to fully evaluate the importance of this subject.

References