Urinary Lactate Dehydrogenase Isoenzyme Analysis in Adult Population*

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ABSTRACT

This investigation was a systemic study on an adult population of urinary lactate dehydrogenase (LDH) isoenzyme analysis for the distinction between upper and lower urinary tract infections. The study included 160 urine samples from patients and healthy individuals. On the basis of clinical symptoms, urinary bacterial colony counts, renal function tests and radiologic findings, the adults were divided into (1) pyelonephritis group, (2) cystitis group, (3) pelvic lesion group, and (4) control group.

This technique correctly identified 23 of 26 patients with pyelonephritis by the presence of elevated LDH-V (over 10 percent) and all of 12 patients with cystitis by the presence of elevated LDH-I (over 60 relative units) but low LDH-V (below 10 percent or lower than LDH-I). In the pelvic group, the results of eight patients were consistent with cystitis and four with pyelonephritis.

Our study confirms the sensitivity and specificity of the LDH isoenzyme technique for the differential diagnosis of urinary tract infection on adult patients and is consistent with previous studies on pediatric patients. However, one should be cautious to interpret the results of LDH isoenzymogram before extra-urinary tract lesions are excluded.

Introduction

The differentiation between upper and lower urinary tract infections has been a clinical problem which needs laboratory assistance. These two clinical entities may not have distinguished clinical symptoms and signs or they may even be asymptomatic. However, their distinction is mandatory as the management and prognosis of these two groups are

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obviously different. For instance, cases of cystitis (lower urinary tract infection) respond well to single-dose antimicrobial therapy, whereas pyelonephritis (upper urinary tract infection) is a more serious clinical problem requiring intensive treatment.8

To help solve this problem, many laboratory procedures have been devised to locate the site of infection.17 Among direct methods, there are culture of kidney tissue,4,16 culture of urethral urine,23 and Fairley's bladder-washout technique.10 These methods, while generally reliable, are time consuming, potentially hazardous, and costly.17 Indirect methods include the assessment of maximal urinary concentrating ability,6,22 urinary enzyme assays,12,20 serum antibody against the infecting organism,2 leukocyte excretion rate,9 and detection of antibody coating bacteria in urine.13,14,24 These tests, while simple, non-invasive and cost-effective, have been questioned as to their accuracy.1,17

A relatively recent development is the analysis of urinary lactate dehydrogenase (LDH) isoenzymes for the differential diagnosis between upper and lower urinary tract infections.5 This inexpensive technique is generally available in clinical laboratories, and its sensitivity and specificity have been well established in children by several clinical studies.1,3,7,17,18 However, the usefulness of this technique has rarely been studied in the adult population.17,18 The present study is to evaluate whether or not the conclusion based on studies of pediatric cases is also valid in adult patients.

Materials and Methods

The present study included 160 urine samples from patients and healthy individuals consisting of 81 males and 79 females between 18 and 89 years of age. The grouping of patients was essentially based on the criteria used by Devaskar and Montgomery7 with the exception of the pelvic lesion group which is an additional group that has not been included in any previous studies of the same nature.

Pyelonephritis Group

There were 26 patients in this group. All patients had urinary bacterial colony counts exceeding 100,000 per ml plus at least one of the following diagnostic criteria: (1) related clinical symptoms: fever, chills, vomiting, costovertebral tenderness, or loin pain; (2) radiologic abnormalities: staghorn calculi, vesicoureteral reflux, cortical scarring, calyceal dilatation or distortion; (3) inability to concentrate urine above 700 Osm per L after prolonged water deprivation; and (4) impairment of renal function: elevated creatinine and blood urea nitrogen levels or abnormal creatinine clearance test.

Cystitis Group

This group was composed of 12 patients, all of whom had urinary bacterial colony counts exceeding 100,000 per ml but who lacked other criteria of kidney infection, as listed in the pyelonephritis group.

Pelvic Lesion Group

This group consisted of 37 patients who had pelvic lesions (prostatic carcinoma, benign prostatic hypertropy, ovarian carcinoma, cervical carcinoma, pregnancy, and pelvic inflammatory disease) but who lacked evidence of urinary tract infection.

Control Group

This group comprised 85 healthy persons. All had no history or present evidence of urinary tract infection or pre-existing renal abnormality.
Random urine samples were collected in morning hours and LDH isoenzyme assays were performed within two hours after collection. During the interim, urine samples were concentrated 50 times by ultrafiltration in a Minicon B 15 concentrator.* Electrophoresis was carried out on agarose films for 30 minutes in 0.065 M barbital buffer at a pH of 8.6. After electrophoresis, a mixture of substrate and coloring reagent† was applied onto the agarose film, and LDH isoenzymes were then demonstrated by the stained bands based on the principle of the formazan method. Quantitation of LDH isoenzymes was accomplished by scanning the pattern with a densitometer‡ utilizing a 520 nm filter. The fraction that migrated fastest to the anode was designated isoenzyme I (LDH-I); the slowest, isoenzyme V (LDH-V).

Comparison of the scans was based on two different measurements. As the presence of LDH isoenzyme V was always accompanied by the other four fractions, the comparisons of LDH-V were based on the percentage of this isoenzyme in the total activity. On the other hand, isoenzyme I was frequently present alone; calculation of its percentage was found to be deceiving. Since all samples were concentrated to the same magnitude and all slides were scanned at the same sensitivity, fraction I curves were, therefore, compared on the basis of the area under the curve. Measurements of dimensions of the curves were taken under a dissecting microscope. Though the curves were obviously not triangles, the formula for area of a triangle (½ height × width of the base) was found to be adequate for relative comparisons. Area units will be referred to as relative units (RU’s).

Results

Pyelonephritis Group

In this group, total urinary LDH activity was generally high as judged by gross inspection of the patterns. All but three of the 26 cases had LDH-V higher than 10 percent, ranging from 11 percent to 39 percent of the total activity (table I and figure 1). The remaining three showed 0 percent, 3 percent, and 4 percent of LDH-V, respectively. LDH-I was also more prominent in this group than in the control group with 17 cases showing a peak greater than 60 RU, a cutoff point derived from the study of the cystitis group (table I).

Cystitis Group

In this group, total urinary LDH activity was also high. All of the 12 cases showed a predominant LDH-I with activity over 60 RU, ranging from 65.5 to 248 RU (table I and figure 2). The median was 141.7 RU. LDH-II was frequently present but usually at a lower level than LDH-I. LDH-V was demonstrated in six cases, but only two were over 10 percent (13 percent and 23 percent).

| Comparison of Lactate Dehydrogenase Isoenzyme Analyses in Four Study Groups |
|------------------------------------------|-----------------|------------------|---------------------|
| Pyelonephritis Group                     | Cystitis Group  | Pelvic Lesion Group | Control Group       |
| LDH-V* Positive                          | 23              | 2                | 4                   | 0                   |
| Negative                                 | 3               | 10               | 33                  | 85                  |
| Total                                    | 26              | 12               | 37                  | 85                  |
| LDH-I† Positive                          | 17              | 12               | 8                   | 0                   |
| Negative                                 | 9               | 0                | 29                  | 85                  |
| Total                                    | 26              | 12               | 37                  | 85                  |

* Activity greater than 10 percent is considered positive.
† Activity greater than 60 relative units (RU’s) is considered positive.
URINARY LDH ISOENZYMES

Figure 1: A case of pyelonephritis showing marked increase in LDH-V and the presence of all five fractions in isoenzymogram and tracing.

percent). Even in these two cases, LDH-I was far more prominent than LDH-V.

Pelvic Lesion Group

In this group, 20 of the 37 cases showed no distinguishable peaks. The remaining 17 cases revealed LDH-I activity with eight cases over 60 RU. Four cases demonstrated a LDH-V level over 10 percent (11 percent, 12 percent, 14 percent, and 24 percent) (table I).

Control Group

In this group, 61 of the 85 cases showed no distinguishable peaks. The remaining 24 cases revealed low LDH-I and LDH-II activities; the highest being 37.5 RU. These cases also demonstrated insignificant levels of LDH-V (table I and figure 1).

The $X^2$ test, including correction for continuity, shows statistical significance of the association between test results and the clinical classification of subjects. The sensitivity of using LDH-I for the diagnosis of cystitis is 100 percent, and the specificity 94 percent when the 17 cases of pyelonephritis are excluded as false-positive. These 17 cases were diagnosed as pyelonephritis and not cystitis because of the presence of five LDH fractions with a prominent LDH-V.

Discussion

Urinary LDH isoenzyme analysis was first used by Carvajal et al for the localization of the site of urinary tract infections. Their study revealed that the elevated LDH-V correctly identified 15 out of 16 children with pyelonephritis, whereas LDH-V was absent in all 22 patients with cystitis. Although this conclusion was challenged by a few reports, most of the subsequent studies confirmed the reliability of LDH analysis. Brouhard and Cunningham, for instance, reported that 97 to 100 percent of the upper urinary tract infections were accurately localized by this analysis.

Carvajal's original study included quantitation of total LDH. However,
Lorentz and Resnick\textsuperscript{18} considered it unnecessary, as did Devaskar and Montgomery.\textsuperscript{7} The former authors maintained that calculation of the percentage of LDH-V was sufficient for the purpose of differential diagnosis, and 10 percent was used as the level which distinguished pyelonephritis from cystitis.\textsuperscript{18}

By utilizing the cutoff point of 10 percent for LDH-V, 23 of our 26 patients with pyelonephritis were accurately identified. At the same time, when 60 RU was used as a cutoff point for LDH-I in combination with the quantitation of LDH-V, all of 12 patients with cystitis were correctly localized. As a matter of fact, a gross inspection of the LDH isoenzymogram is usually adequate to distinguish upper and lower urinary tract infections under most circumstances: a predominant LDH-I is consistent with cystitis, while a prominent LDH-V is indicative of pyelonephritis. The calculation of RU for LDH-I is only helpful when a borderline case between normal and cystitis is encountered.

The kidney tissue contains all five LDH isoenzymes. The cortex is high in LDH-I-III, while the medulla LDH-V.\textsuperscript{25} As pyelonephritis involves mainly the medulla, that explains why LDH-V is predominant in cases of this disease. LDH-V is released from necrotic renal tubules.\textsuperscript{1,5,21} The mechanism of elevations of LDH-I in cystitis is not clear. Normal urinary bladder tissue contains only five percent of LDH-I.\textsuperscript{11} However, in normal urine, LDH-I is the predominant fraction.\textsuperscript{5,17} Therefore, the elevation of LDH-I in cystitis may merely represent exaggeration of the normal pattern owing to the increase of total LDH. When cystitis subsides, urinary LDH returns to normal within two or three weeks.\textsuperscript{5} On the other hand, the inflamed bladder may release disproportionately higher LDH-I than normal tissue. A comparable example can be seen in the elevation of CPK-II isoenzyme in myositis while normal skeletal muscle does not contain CPK-II at all.\textsuperscript{11}

However, one should keep in mind that lysis of leukocytes in urine may also cause elevation of LDH-V,\textsuperscript{1,19} and when urinary pH is below 5.5, LDH-V diminishes rapidly.\textsuperscript{1} These factors may account for the two cases of cystitis with raised LDH-V and the three cases of pyelonephritis with normal LDH-V. When fresh urine sample is examined, the chance of lysis of leukocytes and change in urinary pH is greatly minimized. Several studies showed no clear-cut correlation between pyuria and enzymuria.\textsuperscript{5,7} In the study conducted by Devaskar and Montgomery,\textsuperscript{7} patients from both cystitis and pyelonephritis groups had significant pyuria, but the urinary LDH patterns were distinctly different in these two groups of patients.

Leukocytes contain 5,000 mU of LDH per 10\textsuperscript{8} cells, while erythrocytes contain 240 mU per 10\textsuperscript{8} cells.\textsuperscript{21} Since normal urine contains 13 mU LDH per ml, it requires the complete lysis of 2.5 \times 10\textsuperscript{5} leukocytes per ml (about 50 cells per high power field) or lysis of 5 \times 10\textsuperscript{6} erythrocytes per ml (about 1,000 erythrocytes per high power field) to double the LDH activity in urine.\textsuperscript{21} From this calculation, it seems that the influence of leukocytes and erythrocytes to urinary LDH is very limited, except in unfresh specimens of pyuria and where large numbers of cells are lysed.

Our inclusion of a pelvic lesion group in our study reveals some new problems. The basic pathology in this group is non-urinary, but urine samples are frequently received from patients of this group and the results of LDH isoenzyme analysis are confused with those of the urinary groups. In our study, for instance, the results of eight cases of the pelvic lesion group were consistent with cystitis while four were consistent with pyelonephritis. However, all of the 12 patients had no clinical symptoms of urinary tract infec-
tion, and their urinary bacterial colony counts were below 100,000 per ml. It seems likely that pelvic lesions might have caused obstruction of the urinary tract or disturbances of the blood circulation of the urinary system. In the case of carcinoma, tumor metastasis might have involved the tissues of kidney or urinary bladder directly. As a result, LDH isoenzymes from these organs were released into urine. False positive results could thus be obtained when a pelvic lesion is present. Therefore, when the results of LDH isoenzymogram are not consistent with clinical and microbiological examinations, clinicians should look for pelvic lesions to rule out the possibility of false positive results. Obviously, the release of LDH is not limited to inflammatory condition but also occurs in other destructive processes, such as tumors or obstruction of urinary flow.

No matter what the basic mechanism is, our study indicates that LDH isoenzyme analysis is a reliable test to distinguish upper and lower urinary tract infection when infection is confirmed by clinical and microbiological observations.

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References