THE USE OF CONCOMITANT SERUM PYRUVATE-KINASE (PK) AND CREATINE-PHOSPHOKINASE (CPK) FOR CARRIER DETECTION IN DUCHENNE'S MUSCULAR DYSTROPHY THROUGH DISCRIMINANT ANALYSIS

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SUMMARY

Serum pyruvate-kinase (PK) and creatine-phosphokinase (CPK) determinations have been carried out in a sample of 30 obligate carriers for the DMD gene and 50 normal adult control females. In all the subjects under study blood samples have been collected 3 times on 3 independent occasions and the means of these 3 determinations were considered for both CPK and PK activities in the statistical analysis.

Discriminant analysis has shown that in the group of 30 obligate carriers the estimated proportions of misclassification using either serum CPK, PK or both enzymes were found to be: 10/30 (33.3%) for CPK alone, 6/30 (20%) for PK alone and 5/30 (16.6%) for both enzymes.

It is concluded that although a small proportion of carriers still remain undetected the concomitant use of PK and CPK determinations enhances the capability of detecting carriers for the Duchenne gene mainly when compared with CPK alone.

INTRODUCTION

Serum creatine-phosphokinase (CPK) activity is known to be increased in 60–80% of the obligate carriers for the Duchenne muscular dystrophy (DMD) gene.
(Pearce et al. 1964; Dreyfus et al. 1966; Emery and Walton 1967; Gardner-Medwin et al. 1971; Zatz et al. 1976). Therefore a normal serum CPK level in a female at risk for Duchenne dystrophy is not sufficient to rule out a carrier status. Risks of heterozygosity for suspected carriers (mothers of isolated patients, sisters of probands and other at-risk female relatives) are estimated by combining, through statistical methods (Bayesian calculation) the results from serum CPK with data derived from pedigree analysis (Emery and Morton 1968; Zatz et al. 1975; Emery and Holloway 1977).

More recently it has been suggested that serum pyruvate-kinase (PK) activity is also elevated in a proportion of DMD carriers (Alberts and Samaha 1974; Weinstock et al. 1977) and that the concomitant use of both serum PK and CPK could enhance the capability of discriminating heterozygous females (Zatz et al. 1977; Percy et al. 1979; Zatz et al. 1980).

In the present paper, using the results of serum PK and CPK determinations in a sample of 30 obligate carriers (H) and 50 normal adult females (N) a quadratic discriminant function has been derived. The values obtained from this function correspond to the conditional biochemical probability to be used in Bayesian calculation for the determination of heterozygosity risks for suspected carriers.

MATERIALS AND METHODS

Thirty obligate carriers for the DMD gene and 50 normal adult female controls (with no history of neuromuscular disease in their family) were included in this study. Obligate carriers for the DMD gene were defined as: (a) a woman with one affected brother (or maternal uncle) and at least one affected son; (b) a mother of at least 2 affected sons; (c) a woman with one affected son and one affected grandson through a daughter.

Blood samples were drawn by venipuncture without any anticoagulant and centrifuged shortly after collection. Sera that showed any sign of hemolysis were discarded. None of the individuals had undergone vigorous exercise 24 h prior to collection.

All PK and CPK determinations were performed in fresh sera within 2–4 h of venipuncture. In all subjects the test was repeated 3 times on 3 subsequent weeks for both enzymes. The mean value of 3 separate determinations was assigned to each individual.

Assay of total PK activity was performed at 21°C by measuring the decrease in absorbance of reduced nicotinamide adenine dinucleotide at 340 nm with a Beckmann recording spectrophotometer. The assay was similar to that used by Valentine and Tanaka (1966) as modified by Alberts and Samaha (1974). All enzyme activities are reported as μmoles/ml serum/h. CPK activities were determined with Sigma kits in the same sera as those used for PK determinations. The colorimetric method used is described in Sigma Bulletin 520-C (1976). The results are expressed in Sigma units.

For statistical methods non-linear (quadratic) discriminant functions were
determined since the variances of both traits were nonhomogeneous in the normal female and heterozygote groups. These were performed following the procedures described by Penrose (1947), Karn and Penrose (1951) and Smith (1969).

RESULTS

As shown by Kolmogorov-Smirnov goodness of fit tests, the distributions of both characteristics (CPK and PK) in normal women as well as in the carriers could be treated as Gaussian ones.

The statistical parameters found in the samples are shown in Table 1.

TABLE 1
MEANS, VARIANCES AND STANDARD DEVIATIONS OF CPK AND PK, AS WELL AS OF THE AGES OF THE HETEROZYGOTES (H) AND CONTROL WOMEN (N)

<table>
<thead>
<tr>
<th></th>
<th>x</th>
<th>s^2</th>
<th>s</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>CPK</td>
<td>14.556</td>
<td>86.058</td>
<td>9.277</td>
</tr>
<tr>
<td></td>
<td>PK</td>
<td>2.949</td>
<td>0.485</td>
<td>0.696</td>
</tr>
<tr>
<td></td>
<td>age</td>
<td>42.667</td>
<td>109.126</td>
<td>10.446</td>
</tr>
<tr>
<td>N</td>
<td>CPK</td>
<td>5.581</td>
<td>7.849</td>
<td>2.802</td>
</tr>
<tr>
<td></td>
<td>PK</td>
<td>1.942</td>
<td>0.106</td>
<td>0.327</td>
</tr>
<tr>
<td></td>
<td>age</td>
<td>27.580</td>
<td>48.983</td>
<td>6.999</td>
</tr>
</tbody>
</table>

In the control group, no correlations between age and CPK (r = -0.253; t = 1.811; d.f. = 48; P = 0.076), age and PK (r = -0.120; t = 0.834; d.f. = 48; P = 0.408) and between PK and CPK (r = 0.180; t = 1.271; d.f. = 48; P = 0.210) were found. In the group of carriers no significant correlations have been found between age and CPK (r = 0.177; t = 0.950; d.f. = 28; P = 0.350) and age and PK (r = -0.095; t = 0.507; d.f. = 28; P = 0.616) but a positive significant correlation coefficient was found between PK and CPK levels (r = 0.424; t = 2.476; d.f. = 28; P = 0.020).

Both CPK and PK were significantly higher in the heterozygote group than in normal females (r' = 4.575; d.f. = 49; P = 3 × 10^-5 and r' = 7.444; d.f. = 37; P = 7 × 10^-5, respectively).

The quadratic discriminant function using both the CPK and the PK determinations was found to be

$$L(x_1, x_2) = \ln \left[ \frac{\theta(x_1, x_2|H)}{\theta(x_1, x_2|N)} \right] = \frac{1}{2} (x - x_H)^T V_H^{-1} (x - x_H) - \frac{1}{2} (x - x_N)^T V_N^{-1} (x - x_N) + \frac{1}{2} \ln(\det V_H) - \frac{1}{2} \ln(\det V_N)$$

$$= 0.0588 x_1^2 - 0.1238 x_1 x_2 + 3.5889 x_2^2 - 0.3689 x_1 - 11.4376 x_2 + 7.2528,$$

where \(x_1\) is the CPK value and \(x_2\) the PK value.
Figure 1 shows the ellipses given by the following equations:

\[0.0588x_1^2 - 0.1238x_1x_2 + 3.5889x_2^2 - 0.3689x_1 - 11.4376x_2 + 4.30836 = 0\]  
(curve A),

\[0.0588x_1^2 - 0.1238x_1x_2 + 3.5889x_2^2 - 0.3689x_1 - 11.4376x_2 + 7.2528 = 0\]  
(curve B) and

\[0.0588x_1^2 - 0.1238x_1x_2 + 3.5889x_2^2 - 0.3689x_1 - 11.4376x_2 + 10.1972 = 0\]  
(curve C).

Curve A represents the set of points \(x_1, x_2\) which make \(P(H) : P(N) : 19 : 1\); Curve B is the set of points \(x_1, x_2\) such that \(P(H) = P(N)\); and curve C is the set of points \(x_1, x_2\) such that \(P(H) : P(N) : 1 : 19\), where \(P(H)\) is the probability of heterozygosity and \(P(N)\) of normal homozygosity. In the Bayesian sense, A, B, and C are the boundaries for 95%, 50%, and 5% conditional biochemical probabilities favouring the diagnosis of the carrier state.

The practical use of the discriminant functions derived from serum CPK and PK activities can be illustrated below in a case of genetic counseling:

M.S. is a female who has 2 brothers affected by DMD. Her serum CPK was 6.00 Sigma Units and the PK was 3.25 μmol/ml/h. Both values are means of 3 independent determinations.

Therefore we have \(x_1 = 6.00\) and \(x_2 = 3.25\); applying these values in the formula

\[L(x_1, x_2) = 0.0588x_1^2 - 0.1238x_1x_2 + 3.5889x_2^2 - 0.3689x_1 - 11.4376x_2 + 7.2528,\]

it comes out that \(L(x_1, x_2) = 5.4777\) and

\[e^{L(x_1, x_2)} = e^{5.4777} = 239;\]

thus \(P(H) : P(N) : 239 : 1\) and therefore \(P(H) = 239/240\) and \(P(N) = 1/240\).
Applying this to Bayes’ theorem, we have:

<table>
<thead>
<tr>
<th></th>
<th>Consultant is heterozygote</th>
<th>Consultant is normal homozygote</th>
</tr>
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<tbody>
<tr>
<td>(A) Prior probability</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(B) Conditional biochemical probability</td>
<td>239/240</td>
<td>1/240</td>
</tr>
<tr>
<td>(C) Total probability</td>
<td>$\frac{239 \times 1}{240} = \frac{239}{480}$</td>
<td>$\frac{1 \times 1}{240} = \frac{1}{480}$</td>
</tr>
<tr>
<td>(D) Posterior probability</td>
<td>$\frac{239}{239 + 1} = 0.996$</td>
<td>$\frac{1}{239 + 1} = 0.004$</td>
</tr>
</tbody>
</table>

Therefore, the probability that the consultant M.S. is heterozygote is approximately 99.6% and that she is normal homozygote 0.4%.

If only CPK determinations would have been carried out in this consultant, using the discriminant function $L(x_1)$ derived from CPK levels alone, we would have, from

$L(x_1) = 0.0579x_1^2 - 0.5419x_1 - 0.4439$,
$L(x_1) = -1.6109$ and therefore $e^{L(x_1)} = 0.1997$, that is:
$P(H) : P(N) :: 1 : 5$

Applying these biochemical conditional probabilities to Bayes’ theorem the posterior probability of heterozygosity would be 16.7%.

If, on the other hand, only PK determinations would have been carried out, using the discriminant function $L(x_2)$ derived from PK levels alone, we would have, from

$L(x_2) = 3.6659x_2^2 - 12.1645x_2 + 7.9939$,
$L(x_2) = 7.1803$ and therefore $e^{L(x_2)} = 1313$, that is:
$P(H) : P(N) :: 1313 : 1$

The final posterior probability in this case that the consultant is heterozygote would be 99.9%.

In conclusion, the estimated risk of heterozygosity for the consultant M.S. would be approximately 17% using only CPK determinations and is over 99.5% with the use of either PK alone or both PK and CPK.

DISCUSSION

Alberts and Samaha (1974) were the first to point out that the determination of serum PK could detect more carriers of the DMD gene than serum CPK. This finding was confirmed by us in a previous study (Zatz et al. 1977, 1980) and recently by the group of Percy et al. (1979). It is fundamental, however, that serum PK determinations are performed in fresh samples since there is a progressive loss of enzyme activity in frozen sera (Zatz et al. 1978; Percy et al. 1979).
The results of the present investigation, in which multiple determinations of serum PK and CPK have been carried out in an enlarged sample of obligate carriers and normal female controls, confirm our previous data on the importance of serum PK for carrier detection.

Discriminant analysis has shown that:

Using the discriminant function \( L(x_i) \), derived through the CPK values alone, and which is \( L(x_i) = 0.0579x_1^2 + 0.5419x_1 - 0.4439 \), a total of 16.25% misclassification has been observed in the total sample of normal and heterozygous females; using the discriminant function \( L(x_i) \), derived from the PK determinations alone and which turns out to be \( L(x_i) = 3.4459x_1^2 - 12.1645x_2 + 7.9939 \), a total of 12.5% of misclassification has been observed in the total sample and, using the discriminant function \( L(x_i, x_j) \), this proportion was 10%. If only the group of obligate carriers is considered, the estimated proportions of misclassification among the 30 heterozygous females using either serum CPK, PK or both enzymes are: 10/30 (33.3%) for CPK alone, 6/30 (20%) for PK alone and 5/30 (16.6%) for both enzymes.

In conclusion, although a proportion of carriers still remain undetected, these results indicate that the concomitant use of PK and CPK determinations enhances the capability of detecting carriers for the Duchenne gene, mainly if compared with CPK alone.

It is important to point out that although no positive correlation has been found between serum PK levels and age in the adult normal females or obligate carriers, we have observed that PK activity in normal young children (below 14 years old) is significantly higher than in adults (Zatz et al. 1978; Passos and Zatz, in preparation).

Therefore, before estimating heterozygosity risks in young suspected carriers at risk for Duchenne dystrophy, it is imperative to establish the normal limits of serum PK for young normal girls of comparable age.

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REFERENCES


