Evaluation of maternal plasma creatine kinase activity as a marker of abnormal early pregnancy

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We have tested the value of maternal plasma creatine kinase activity for diagnosing ectopic pregnancies obtained after in-vitro fertilization and embryo transfer. Plasma creatine kinase was assayed in 57 patients: 20 normal, 23 miscarriages and 14 ectopic pregnancies, for a total of 240 samples. All values were in the lower part of the normal range except only one in a miscarrying patient. A statistically significant difference was observed for a cut-off value of 45 IU/l between normal and ectopic pregnancies. However, for this cut-off point, the measurement of plasma creatine kinase activity had a sensitivity of 0.50 and a specificity of 0.76 for the diagnosis of ectopic pregnancy. The positive predictive value was 0.69. Creatine kinase activity measurements are thus of no practical value in this particular population, in which an early and specific marker of ectopic implantation would be of paramount interest. The association of human chorionic gonadotrophin (HCG) determinations and ultrasound scanning of the pelvis still remain the best paraclinical support for an early diagnosis of ectopic implantation.

Key words: creatine kinase/ectopic pregnancy/in-vitro fertilization

Introduction

It was recently postulated by Lavie et al. (1993) that maternal creatine kinase could serve as a predictor of tubal pregnancy. The rationale was the following: (i) the trophoblast is able to invade the muscular layer of the Fallopian tube in the absence of a submucosal layer (Budowick et al., 1980) and (ii) the damaged muscle cells release creatine kinase in the maternal bloodstream through the tubal blood vessels eroded by the growing trophoblast. Therefore, an elevation of maternal creatine kinase activities could be an early biological index of tubal nidation. Their attractive hypothesis was supported by a prospective study comparing three groups of 17 patients each: group A = documented tubal pregnancy, group B = spontaneous abortion, and group C = normal pregnancy. Creatine kinase concentration was >45 IU/l in all patients with tubal pregnancy, significantly higher than the concentration in patients of the two other groups.

We have tested the value of maternal plasma creatine kinase (CK) activities for the diagnosis of ectopic pregnancies obtained after in-vitro fertilization (IVF) and embryo transfer. This population is of particular interest, since the frequency of ectopic pregnancy is high after IVF-embryo transfer (2–5% in our centre), the gestational age is clearly defined, and serial blood samples are routinely performed to evaluate early gestation.

Materials and methods

Patients

Plasma creatine kinase was assayed in 57 patients, for a total of 240 samples collected during 57 early pregnancies (Table I).

Twenty normal pregnancies were obtained after IVF-embryo transfer or intrauterine insemination (IUI). All resulted in normal healthy babies. Twenty-three miscarriages and 14 ectopic pregnancies were either spontaneous or obtained after IVF-embryo transfer or IUI. The diagnosis was ascertained in all cases by pathology, after dilatation and curettage in the case of abortion, or by laparoscopic surgery for all ectopic pregnancies except one treated with methotrexate.

Blood samples

IVF pregnancies were monitored by repeated human chorionic gonadotrophin (HCG) assays, starting on day 12 after embryo transfer and continuing two or three times a week until ultrasonic detection of a fetal cardiac activity. Several aliquots were obtained from each plasma sample, and stored at −20°C until assayed. The blood samples were dated taking the day of IVF as day 1 of pregnancy. The percentiles were determined in our laboratory from 333 singleton pregnancies obtained after IVF.

In spontaneous pregnancies, blood samples were taken in the emergency room for the measurement of HCG. Plasma aliquots were stored at −20°C until assayed. The term was calculated clinically, from the menstrual history (late menstrual period, mean duration of cycles) and/or data from ultrasonic scans, when available.

Biochemical assays

Total plasma creatine kinase activity (CK) was measured by a standard method using a commercial CK-NAC reagent kit (Boehringer-Mannheim) on a Hitachi 717 analyser (Szasz et al., 1976). Activity was expressed as IU/l at 37°C. The interassay coefficient of variation for normal values was 4.4%.

CK-MB activity was also determined in each specimen with the CK-MB (NAC-activated) reagent (Boehringer-Mannheim). Analysis of creatine kinase isoenzymes was performed by electrophoresis and scanned fluorimetrically with the REP system (Helena Laboratories). Possible loss of CK activity during the sample storage time at −20°C (Duncan et al., 1995) was checked for in our series: the drop of CK activity was only 13% in 42 samples for a mean CK activity of 594 IU/l.

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pregnancies (31.5%) exceeded the cut-off value of 45 IU/l either normal or abortive. As shown in Figure 1, only 18 of CK concentrations between extra- and intrauterine pregnancies, women (24-170 IU/l). There were no significant differences in pregnancies, the values chosen were those closest to this term. For normal days for miscarriages, and 32 days for ectopics. For normal Table II. At that time, the mean terms of pregnancy were 31 Table I. Population screened for creatine kinase activity

<table>
<thead>
<tr>
<th>Pregnancies</th>
<th>Patients</th>
<th>Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>IVF</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>IUI</td>
<td>20</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>Spontaneous</td>
<td>14</td>
</tr>
<tr>
<td>Ectopics</td>
<td>Spontaneous</td>
<td>12</td>
</tr>
<tr>
<td>IVF</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>IUI</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>240</td>
</tr>
</tbody>
</table>

Table II. Creatine kinase (CK) concentrations (IU/l) in normal versus abortive or ectopic pregnancies

<table>
<thead>
<tr>
<th>Pregnancies</th>
<th>Term</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20</td>
<td>36.8 ± 5.1</td>
<td>20.3-109.2</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>23</td>
<td>51.7 ± 7.2</td>
<td>10.9-137.3</td>
</tr>
<tr>
<td>Ectopics</td>
<td>14</td>
<td>44.2 ± 3.6</td>
<td>17.2-65.5</td>
</tr>
</tbody>
</table>

Statistical analysis
Comparison among the three groups was carried out with the non-parametric test of Spearman and the χ² test. Probability values <0.05 were considered significant.

Results

CK concentrations in normal versus abortive or ectopic pregnancies

The mean concentrations of creatine kinase in the blood samples obtained closest to the curettage for miscarriages or laparoscopic surgery for ectopic pregnancies are given in Table II. At that time, the mean terms of pregnancy were 31 days for miscarriages, and 32 days for ectopics. For normal pregnancies, the values chosen were those closest to this term.

All CK values but one were in the normal range for adult women (24-170 IU/l). There were no significant differences in CK concentrations between extra- and intrauterine pregnancies, either normal or abortive. As shown in Figure 1, only 18 of these samples (31.5%) exceeded the cut-off value of 45 IU/l described by Lavie et al. (1993) but 10 corresponded to miscarriages, five to ectopics and three to normal pregnancies.

The analysis of the isoenzymes did not show any abnormal fraction. The electrophoretic profiles were similar in each group.

HCG levels in normal versus abortive or ectopic pregnancies

On the same day as CK measurement, HCG concentrations were determined. All the results were above the 10th percentile in normal pregnancies. Out of 14 ectopic pregnancies, 10 were under the 10th percentile (Table IIIa).

Time course of CK levels during early pregnancy

Most patients monitored after IVF-embryo transfer gave several samples during the period of observation (Table IV). Thirteen out of 14 ectopic pregnancies had one to seven assays, one had 14 assays, and one treated with methotrexate had 15 assays.

As shown in Figure 2, most values were below the cut-off of 45 IU/l in each group. More values were over this cut-off in ectopic (42%) than in normal pregnancies (24%) (P < 0.02). There was no significant difference between either normals and miscarriages, or between miscarriages and ectopics. Finally, only one pathological CK value >170 IU/l was observed, in a patient having a miscarriage. For this cut-off point, the measurement of plasma CK activity during the period of observation had a sensitivity of 0.50, and a specificity equal to 0.76 for the diagnosis of ectopic pregnancy. The positive predictive value was 0.69 (Table V).

Figure 3 depicts the time course of individual values in patients who had six assays or more.
Figure 2. Distribution of creatine kinase (CK) levels during normal early pregnancies, miscarriages and ectopics.

Table V. Sensitivity, specificity, predictive values obtained with multiple creatine kinase (CK) assays

<table>
<thead>
<tr>
<th>CK</th>
<th>Ectopics</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 IU/l</td>
<td>37</td>
<td>16</td>
</tr>
<tr>
<td>&lt;45 IU/l</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

CK = creatine kinase.

Discussion
A major feature of our study is the homogeneity of our population: the gestational age was clearly defined, serial blood samples were routinely performed to evaluate early gestation, and the prevalence of ectopic pregnancy after IVF-embryo transfer was high (4.5% in our centre in 1993) during the study period, in agreement with the French IVF register FIVNAT: 4.9% for the years 1986–1992 (FIVNAT, 1994). These conditions are optimal to evaluate the practical value of the marker.

In our series, a statistically significant difference was observed for a cut-off value of 45 IU/l between normal and ectopic pregnancies. For the cut-off point of 45 IU/l, the positive predictive value was 0.69. Our results are therefore different from those described by Lavie et al. (1993) and agree with the findings of Duncan et al. (1995): plasma concentration of creatine kinase is not sufficiently discriminatory to be of clinical value in the diagnosis of tubal ectopic pregnancy.

The suggestion of Duncan et al. (1995) that a transient release of CK might be undetectable because of the short half-life (12–24 h) of this enzyme appears unlikely considering the numerous assays performed in the patients with ectopic pregnancies, showing no increase in CK concentrations.

There is no clear explanation for the statistically different levels of CK observed between normal and pathological pregnancies (miscarriages and ectopics) (Figure 2). Unfortunately, this observation cannot be of great use to the clinician, since only one test is usually performed.

Finally, it must be emphasized that all values were in the normal range, except only one in a miscarriage. Moreover, they were near the lower normal limit. This may be explained by the physiological haemodilution known to be already present in pregnant women at 6 weeks of amenorrhoea (Hellman and Pritchard, 1971), the mean term at which our patients were investigated. Hence, it is not surprising that minor

Figure 3. Evaluation of individual values of creatine kinase (CK) activity in patients with >6 assays during normal early pregnancies, miscarriages and ectopics.
variations inside the normal range were not discriminatory. The attractive hypothesis of Lavie et al. (1993) is thus rebutted in our study, as well as that of Duncan et al. (1995).

In conclusion, CK activity measurements have no practical value in this particular population of IVF–embryo transfer pregnancies, in which an early and specific marker of ectopic implantation would be of paramount interest. The association of HCG determinations (Table IIIa) and ultrasound scanning (Table IIIb) still remain the best paraclinical support for an early diagnosis of ectopic implantation.

References

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