Behavior of Creatine Kinase Isoenzymes in Hepatic Diseases
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Abstract:
Serum creatine kinase isoenzymes were determined in 107 patients with various hepatic disorders (29 with hepatocellular carcinoma; 26 with alcoholic cirrhosis; 33 with primary biliary cirrhosis and 19 with hepatic failure) and compared with that found in 34 control individuals with the same age which ranged between 25–76 years. Elevations in total serum CK activity levels were observed in 69%; 76.9%; 51.5% and 63.2% of the cases studied and the maximum value was reached to 10.9 I.U./ml; 9.4 I.U./ml; 8.2 I.U./ml and 13.4 I.U./ml respectively as compared with that found in control group (4.1 ± 1.2 I.U./ml).

Serum CK isoenzymes have been separated from these types of hepatic patients by mini-column ion-exchange chromatography. CK-MM isoenzyme was found in sera of all cases studied in a variable degrees, whereas CK-BB isoenzyme was separated from 79.3% of hepatocellular carcinoma; 73.1% of alcoholic cirrhosis; 60.1% of primary biliary cirrhosis and 79% of hepatic failure and its activity levels was reached to 25%; 19%; 15% and 24% of total serum CK activity receptively.

CK-MB isoenzyme was also observed and separated only from sera of hepatic disorders when massive myocardium damage is occurring. Therefore the detection and measurement of CK-BB isoenzyme may act as a good biochemical marker and to give some information about the severity of liver diseases and also to differentiate between them.

Column isoelectric focusing was also performed and the isoelectric points of each of CK isoenzyme separated from sera of different types of hepatic disorders have been determined.

Introduction:
Cytosolic creatine kinase (ATP: creatine N-phospho transferase, EC 2.7.3.2,CK) is a dimeric enzyme consisting of two immunologically distinct subunits, muscle (M) and brain (B) origin. Combination of these subunits results in the formation of three cytosolic isoenzyme which are distributed in different human tissues: CK-3 or CK-MM, the predominant isoenzyme in muscle and most tissues that contain CK activity, particularly skeletal muscle. CK-2 or CK-MB (a hybrid form) found almost exclusively in heart muscle tissue and necrosis in the myocardium such as occurs after a myocardial infarction, results in the release of CK-MB isoenzyme into the blood circulation and the most anodic brain type CK-1 or CK-BB isoenzyme found predominantly in brain tissue but also present in the prostate, bladder, spleen, liver, pancreas, breast, kidney and gastrointestinal tract of normal tissues it has been rarely detected in normal serum.
Other than cytosolic CK isoenzymes have generated considerable interest among laboratory scientists because of their unusual physical and immunological properties and their effects on methods for measuring CK-MB isoenzyme activity level. These variant of CK isoenzymes are of high molecular mass, more stable than the cytoplasmic CK isoenzymes and have been characterized as Type 1 complex which migrate between CK-MM and CK-MB and usually contain no immunoglobulin bound to CK, or Type 2 complexes which migrate cathodic to CK-MM isoenzyme and are believed to originate from mitochondria.\(^\text{(10-12)}\)

A recent papers reports the existence of three different iso-forms of CK-MM isoenzyme and two iso-forms of CK-MB isoenzyme in human sera with increased or decreased CK activity by using isoelectric focusing. This phenomenon with the fact that CK has a dimeric structure, suggest the existence of two different M-subunits in molecular structure of CK.\(^\text{(13)}\) Therefore isoelectric focusing in sucrose gradient has been shown to be a powerful technique for isolation of protein and enzymes and have also been proposed for CK isoenzyme characterization.\(^\text{(14)}\) The purpose of the presented work is to identify and to differentiate between the tissue source of serum CK isoenzyme which are purified firstly from sera of different types of liver diseases by mini-column ion-exchange chromatography, isoelectric focusing and then the isoelectric points of each isoenzymes was measured.

Materials and Methods:

Isoelectric focusing of CK isoenzyme fractions in sucrose density gradient was carried out on a 110 ml electro focusing column (LKB-8100) using a power supply (LKB-2103) as described previously. After 36 hours, when the current had reached a minimum value, 100 fractions were collected. The pH, protein concentration and CK isoenzymes activity levels in each fraction were measured. Therefore, the isoelectric point (\(P_I\)) of each CK isoenzyme separated is determined.\(^\text{(13)}\)

Creatine kinase activity in sera of 29 patients of hepatocellular carcinoma, 26 patients with alcoholic cirrhosis; 33 patients with primary biliary cirrhosis and 19 with hepatic failure were compared with that found in 34 healthy control individuals with the same age ranged between 25-76 years and was measured according to Hughes method.\(^\text{(15)}\) All of these patients were admitted in Al-Hakeem Teaching Hospital / Najef-Iraq; AL-Hussein General Hospital / Kerbala – Iraq and Marjan Teaching Hospital/ Hilla-Iraq during March, 2004 to Feb., 2006 which were diagnosed according to the history and all of the clinical and biochemical findings required.

Quantitative analysis of CK activity was performed to separated the isoenzymes by modification of the discontinuous elution from micro-column of DEAE- Sephadex A-50 as described by Yasmineh and Lewis,\(^\text{(4)}\) in which 1.0 ml of pathological serum was loaded on 0.7 x 0.9 cm mini-column of the anion exchanger pre-equilibrated with the starting buffer. The CK-MM, MB, BB isoenzymes were then eluted with 0.5 mmol /L Tris –buffer pH 7.0 containing 0.1, 0.2 and 0.4 mol /L NaCl respectively. Final volumes of the elutes were 8; 6 and 6 ml respectively and the volume of each eluate was 1 ml contained 5 micro liter of 20 mmol/ I mercaptoethanol. CK activity in each eluate was then determined beside to protein concentration which was measured according to biuret reaction.\(^\text{(15)}\)

DEAE-Sephadex A-50 which was used as anionic exchanger and purchased from Pharmacia Fine Chemicals / Sweden, while all other chemicals used were imported from

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Results:

The total serum CK activity was moderately increased in 69% of the hepatocellular carcinoma and the total circulatory levels of CK activity was reached to 10.9 IU/ml (mean 8.9 IU/ml ± 2.4 IU/ml), while 76.9% of alcoholic cirrhosis induced an elevation in the circulatory level of the total enzyme activity and reached in some patients to a maximum levels 9.4 IU/ml (mean 7.9 IU/ml ± S.D. 1.2), whereas primary biliary cirrhosis caused a slightly increased in the total serum CK activity in 51.5% of patients studied and the maximum activity reached in sera of some patients to 8.2 IU/ml (mean 6.8 IU/ml ±S.D. 1.4), while hepatic failure induced a markedly elevation in the total enzyme activity levels in 63.2% of the cases studied and reached to 13.4 IU/ml (mean 10.8 ± 2.4 IU/ml) as compared with the control group (4.1 ± S.D. 1.2 IU/ml). The total serum CK activity levels which was elevated in hepatic diseases included in this study depends upon the type and the severity of the disease as shown in Table -1-.

CK isoenzyme have been separated from sera of hepatocellular carcinoma, alcoholic cirrhosis; primary biliary cirrhosis and hepatic failure patients by mini-column ion exchange chromatography as described previously.(4) The degree of purification of each isoenzyme separated from sera of some type of hepatic diseases was measured as indicated in tables -2, 3, 4 and 5-. As a result, CK-MM isoenzyme was detected and separated from sera of all patients studied in different degrees.

On the other hand, CK-BB isoenzyme was also observed and increased in sera of 79.3%; 73.1%; 60.1% and 79% of hepatocellular carcinoma; alcoholic cirrhosis; primary biliary cirrhosis and hepatic failure respectively as compared with that found in normal control sera which contains almost exclusively CK-MM isoenzyme with only trace amount of each of CK-BB and CK-MB isoenzymes as shown in Table-1. (13) The percentage of CK-BB isoenzyme activity levels in sera of hepatocellular carcinoma, alcoholic cirrhosis, primary biliary cirrhosis and hepatic failure was also measured and reached to 25%; 19%; 15% and 24% of the total serum CK activity respectively as compared with that found in control group.

CK-MB isoenzyme was also detected in sera of 17 patients of some types of hepatic disorders included in this study and reached to 12.7%; 11.8%; 6.7% and 8.5% of total serum CK activity respectively as compared with the control group and shown in the same table.

Isoelectric focusing of serum CK isoenzymes isolated from all types of hepatic patients was also performed. The observed data indicate five active peaks of CK isoenzymes, the first one represent CK- BB isoenzyme with isoelectric point (P_I = 0.48), the second peak with isoelectric point (P_I = 5.7) represent CK-MB isoenzyme, and the last three peaks represents CK-MM isoenzyme isolated from sera of all patients studied indicating that it composed of two different subunits leads to the formation of three different isoforms designated CK-MM\textsubscript{1}, CK-MM\textsubscript{2} and CK-MM\textsubscript{3} with P\textsubscript{I} values = 5.95, 6.6 and 6.95 respectively.

Discussions:

The results obtained indicated that hepatocellular carcinoma; alcoholic and primary biliary cirrhosis induced a slightly to moderate elevations in total serum CK activity levels in 69%; 76.9% and 51.5% of the cases studied respectively, whereas hepatic failure
induced a markedly elevated levels in 63.2% of the cases examined and reached to a maximum total CK activity levels observed in this study which is 13.4 IU/ml. These elevations were due to increased in CK-BB isoenzyme level originating from hepatocytes as a richest source of the isoenzyme activity, in addition to the elevation of CK-MB isoenzyme which is found in 17 cases studied and originated only from myocardium. Its appearance in sera of these cases demonstrated the presence of massive myocardial damage and it considered as a good indicative of myocardial damage when its activity levels exceed 3-5% of the total serum CK activity levels. The percentage of CK-BB isoenzyme activity levels elevated in sera of hepatocellular carcinoma was higher than that found in both alcoholic cirrhosis and primary biliary cirrhosis due to the massive hepatocellular destruction occurring in hepatocellular carcinoma as primary effect of hepatic necrosis, therefore there is a leakage of CK-BB isoenzyme into the circulation, this leakage was dependent in the type and severity of liver diseases, but the degree of hepatocellular destruction observed was in a lesser extent in primary biliary cirrhosis, therefore the percentage of CK-BB isoenzyme activity increased in this type of cirrhosis is lower than that found in other cases studied. On the other hand hepatic failure patients which was due to sever acute and chronic hepatic diseases with encephalopathy and different degrees of coma showed markedly elevation in CK-BB isoenzyme in 79% of the cases studied. A possible explanation in the appearance of brain CK-BB isoenzyme in serum is probably due to the leakage from its respective tissues of the brain and the toxic effect and surface activity properties of bile acids and bilirubin which was increased in liver diseases among other factors. The toxic detergent properties of bile acids, bilirubin and fatty acids could be the primary cause of membrane damage at the nerve cell in hepatic diseases with encephalopathy and therefore produce the leakage of CK-BB isoenzyme into serum.

Isoelectric focusing experiment carried out prove the hypothesis about the structure of CK-MM isoenzyme. This can only be explained if two different M subunits exists, which lead to the formation of three different isoforms of CK-MM isoenzyme and their isoelectric points have been determined. The CK-MB isoenzyme appears to have its isoelectric points P_I = 5.70. In the isoelectric focusing described her it was not possible to detect two forms of CK-MB isoenzyme. The difficulty in determining the isoelectric point of the two isoforms of CK-MB isoenzyme is due to the presence of huge amount of albumin in the same part of the PH gradient. Also this experiment prove that CK-BB isoenzyme composed of two similar B subunits and appear to have its isoelectric point P_I = 5.48.

Conclusion:
Hepatocellular carcinoma; alcoholic cirrhosis, primary biliary cirrhosis and hepatic failure induce an elevation in total serum CK activity which is almost exclusively due to the elevation of serum CK-BB isoenzyme present moderately in a high concentration in hepatocytes as a rich source of isoenzyme activity and its activity levels in different type of hepatic disorders studied could be ranked as follows:
Hepatocellular carcinoma > hepatic failure > Alcoholic cirrhosis > Primary biliary cirrhosis.

Therefore, we propose that the detection and measurement of serum CK-BB isoenzyme activity may prove to be an approach to obtain some information about the severity of different types of liver cirrhosis, hepatocellular carcinoma and hepatic failure in conjunction with the other investigations of serum enzyme activities used in the
diagnosis of liver diseases, and also as a good biochemical marker to differentiate between the different types of these diseases which caused hepato-cellular destruction.
Table (3): Purification chart of CK isoenzyme from sera of primary biliary cirrhosis by using mini-column ion-exchange chromatography containing DEAE-Sephadex A-50 as anionic exchanger in Tris – buffer at pH 7.0.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Vol.(ml)</th>
<th>Prot. conc. mg/ml</th>
<th>Total prot. mg</th>
<th>CK activity IU/ml</th>
<th>Total CK I.U.</th>
<th>Specific activity I.U./mg</th>
<th>Degree of purification.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1</td>
<td>74.9</td>
<td>74.9</td>
<td>8.5</td>
<td>8.5</td>
<td>0.1135</td>
<td>1.0</td>
</tr>
<tr>
<td>CK-MM</td>
<td>6</td>
<td>0.483</td>
<td>2.9</td>
<td>1.15</td>
<td>6.9</td>
<td>2.3739</td>
<td>21</td>
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<tr>
<td>CK-MB</td>
<td>3</td>
<td>0.45</td>
<td>1.35</td>
<td>0.1667</td>
<td>0.50</td>
<td>0.3704</td>
<td>3.3</td>
</tr>
<tr>
<td>CK-BB</td>
<td>3</td>
<td>0.366</td>
<td>1.1</td>
<td>0.3333</td>
<td>1.0</td>
<td>0.91</td>
<td>8</td>
</tr>
</tbody>
</table>

Table (4): Purification of CK isoenzymes from sera of hepatocellular carcinoma with massive myocardial damage by using mini-column ion-exchange chromatography containing DEAE- Sephadex A-50 as anionic exchanger at PH 7.0 in Tris – buffer.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Vol.(ml)</th>
<th>Prot. conc. mg/ml</th>
<th>Total prot. mg</th>
<th>CK activity IU/ml</th>
<th>Total CK I.U.</th>
<th>Specific activity I.U./mg</th>
<th>Degree of purification.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1</td>
<td>67.2</td>
<td>67.2</td>
<td>10.2</td>
<td>10.2</td>
<td>0.152</td>
<td>1.0</td>
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<tr>
<td>CK-MM</td>
<td>6</td>
<td>0.43</td>
<td>2.58</td>
<td>1.52</td>
<td>9.12</td>
<td>3.535</td>
<td>23.3</td>
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<tr>
<td>CK-MB</td>
<td>3</td>
<td>0.57</td>
<td>1.71</td>
<td>0.38</td>
<td>1.14</td>
<td>0.67</td>
<td>4.4</td>
</tr>
<tr>
<td>CK-BB</td>
<td>3</td>
<td>0.27</td>
<td>0.81</td>
<td>0.67</td>
<td>2.01</td>
<td>2.48</td>
<td>16.33</td>
</tr>
</tbody>
</table>

Table (5): Purification chart of CK isoenzyme from sera of hepatic failure by using mini-column ion-exchange chromatography containing DEAE-Sephadex A-50 as anionic exchanger in Tris – buffer at pH 7.0.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Vol.(ml)</th>
<th>Prot. conc. mg/ml</th>
<th>Total prot. mg</th>
<th>CK activity IU/ml</th>
<th>Total CK I.U.</th>
<th>Specific activity I.U./mg</th>
<th>Degree of purification.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1</td>
<td>75.7</td>
<td>75.7</td>
<td>12.9</td>
<td>12.9</td>
<td>0.170</td>
<td>1.0</td>
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<tr>
<td>CK-MM</td>
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<td>0.405</td>
<td>2.43</td>
<td>1.098</td>
<td>6.59</td>
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<td>CK-MB</td>
<td>3</td>
<td>0.41</td>
<td>1.23</td>
<td>0.22</td>
<td>0.67</td>
<td>0.545</td>
<td>3.2</td>
</tr>
<tr>
<td>CK-BB</td>
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<td>0.39</td>
<td>1.17</td>
<td>0.45</td>
<td>1.35</td>
<td>1.154</td>
<td>6.8</td>
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</table>

References:


