New, simple and cheap alternative to troponin test for diagnosis of acute myocardial infarction

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Acute myocardial infarction (AMI) is often a fatal disorder in humans seen throughout the world. It was earlier diagnosed with some serum enzymes like aspartate transaminase, creatine phosphokinase and its isoenzyme CPK-MB and lactate dehydrogenase which were shown to be increased in AMI. However, in the last few years importance has been given to measuring serum troponins released from the injured myocardium to confirm an AMI. Troponin estimation involves immunological technique, which is expensive with other associated problems like shelf life of reagents, number of samples to be analysed and availability of the kit itself, used for estimation. Under these circumstances the present work involves the measurement of total salt soluble proteins which are proteins associated with troponins also released from myocardium of a patient with AMI. This new test overrules all the disadvantages of the troponin test but seems equally viable and useful for diagnosis of AMI.

One of the leading causes of death in humans throughout the world is coronary artery disease. The condition often manifests as acute myocardial infarction (AMI) which is often fatal and the number of incidences continues to rise the world over. The first diagnostic tool in biochemistry, for AMI was the estimation of serum aspartate transaminase (AST) which was shown to increase in these patients. Subsequently, a number of serum enzyme parameters like lactate dehydrogenase (LDH) and their specific isoenzyme, creatine phosphokinase (CPK) and its isoenzyme CPK-MB were added to aid diagnosis of AMI. However, further work in this field indicated that these parameters alone could not be relied on for diagnosis of all types of AMI.

This led to the establishment of procedures for estimation of specific cardiac contractile protein called troponins as reliable alternatives for diagnosis of AMI. However, in developing countries of the world, like India with a large economically backward population who are also victims of AMI the high cost of troponin test, which is an immunological method makes it often prohibitive. Moreover, reagents for this assay are also not easily available in a rural set up. Under these circumstances the aim of the present work was to establish a new test which would be cheap and easy but equally sensitive and viable alternative to the troponin test for diagnosis of AMI.

Blood was collected by venepuncture using sterile disposable plastic syringes from patients with confirmed AMI admitted to the ICCU of Kasturba Medical Hospital, Attavar, Mangalore, Government Wenlock Hospital and University Medical Centre, Mangalore. The diagnosis of each patient was confirmed by cardiologists of respective hospital based on ECG changes and clinical investigations. Blood, 8ml, was collected from each patient immediately after admission to ICCU and 5ml poured into a tube without anticoagulant to obtain serum and the balance into another tube containing EDTA (1mg/ml) as anticoagulant to obtain plasma. Blood was also collected and processed similarly from some patients on day two and three after AMI. Normal controls, constituted middle aged workers of our medical college with no known history of cardiac or any other disease.

Serum levels of enzymes LDH, CPK, CPK-MB and AST were estimated without storage of samples, using kits supplied by Boehringer Knoll, Bangalore, India. Troponin T was estimated using a kit from Roche diagnostics. Each kit constituted a standard and blank for respective estimation along with procedural details for use with an autoanalyzer. Technicon RT-XT.

The salt soluble proteins of both serum and plasma were separated using the general principle and procedure reported earlier. These proteins which includes Troponin originate from the myocardium of patients who suffer an AMI leading to partial tissue degradation
and are soluble in solutions of ionic strength greater than 0.6% at around neutral pH\textsuperscript{13} and hence are found dissolved in the ionic strength of 0.9% of blood. A volume of 0.2ml of serum or plasma was diluted to a total volume of 2ml with distilled water and vortexed for 2 min. The tubes were allowed to stand for 5 min and centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the precipitate washed thrice with distilled water before protein estimation by Lowry’s method using bovine serum albumin as standard. The precipitate was solubilised in the alkali used in Lowry method and quantified using Polin-Ciocalteau phenol reagent\textsuperscript{14}. All chemicals used were of reagent grade. Results were analysed using Student’s ‘t’ test and values expressed as mean ± SE\textsuperscript{15}.

Earlier, laboratory diagnosis of AMI was traditionally based on the measurement of enzymes like CPK, CPK-MB, LDH, AST believed to be released into the blood stream from injured myocardium\textsuperscript{7}. Results of these enzymes investigated in the present work indicates statistically significant increase in all cases of AMI (Table 1) compared to normal controls. However, recent focus for diagnosis of cardiac infarction has shifted to measurement of specific cardiac contractile protein (troponin T) released into the blood\textsuperscript{18} due to partial myocardial degeneration after AMI. A number of reasons have been put forward to firmly sustain this shift in diagnostic aid for AMI\textsuperscript{17}, with firm conclusion in its favour. Under these circumstances troponin T was also investigated in AMI cases in the present investigation and showed statistically significant increase in all cases studied compared to values obtained for normal controls (Table 2).

However, the immunosorbant assay used for troponin estimation is costly and induces a lot of economic strain on patients. Alternatively, we measured the total cardiac myofibrillar proteins released into the blood after an AMI. Serum was diluted to precipitate these proteins which was then separated and estimated. Results indicate a statistically significant increase in the level of these proteins in all AMI cases compared to normal controls (Table 2). The increase in these proteins in plasma was much higher than that seen in serum (Table 2). The lower concentration of these proteins in serum compared to plasma could probably be due to significant loss of these proteins during the process of clotting of blood. Moreover it is obvious that more proteins will be lost during coagulation as their concentration increases in blood as seen after AMI.

Further, we observed that the values of salt soluble proteins were 52.1 mg/dl and 53.1 mg/dl for serum and 101.9 mg/dl and 110 mg/dl for plasma on the second and third day after AMI respectively and this elevation remained within the same range seen for the first day after AMI for both serum and plasma (Table 2). Similar results have been reported\textsuperscript{16,17} for troponin levels after AMI suggesting delayed breakdown and clearance of these proteins from blood. This was considered an advantage of the troponin test, not seen with enzyme parameters that attain normal levels within a short time after AMI\textsuperscript{15,17}. Similar elevation of salt soluble protein for three continuous days after AMI without any significant change in their levels is beneficial to developing countries like India with a large rural population where many a times there is delay in hospitalization, diagnosis and treatment of AMI patients.

Finally, the troponin test has many disadvantages like cost factor, shelf life of reagents and availability of reagents, which makes it a tedious preposition. In

### Table 1: Serum enzyme values in normal controls and patients with AMI

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal controls</th>
<th>AMI patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n = 15</td>
<td>n = 15</td>
</tr>
<tr>
<td>Aspartate transaminase (AST)</td>
<td>21 ± 14</td>
<td>*65.8 ± 7.7</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>320 ± 120</td>
<td>*784.7 ± 70.8</td>
</tr>
<tr>
<td>Creatine phosphokinase (CPK)</td>
<td>110 ± 75</td>
<td>*616.1 ± 234.6</td>
</tr>
<tr>
<td>Creatine phosphokinase MB (CPK-MB)</td>
<td>16.5 ± 8.5</td>
<td>*89.3 ± 28.1</td>
</tr>
</tbody>
</table>

*P ≤ 0.005, n = number of cases.

### Table 2: Serum Troponin T and salt soluble protein levels from serum and plasma in normal controls and patients on first day after AMI

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>Normal controls</th>
<th>AMI Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Troponin T (µg/L)</td>
<td>10</td>
<td>0.1 ± 0.05</td>
<td>*1.1 ± 0.02</td>
</tr>
<tr>
<td>Serum salt soluble proteins (mg/dl)</td>
<td>30</td>
<td>20.2 ± 1.5</td>
<td>*58.2 ± 3.5</td>
</tr>
<tr>
<td>Plasma salt soluble proteins (mg/dl)</td>
<td>30</td>
<td>32.2 ± 1.9</td>
<td>*108.6 ± 5.6</td>
</tr>
</tbody>
</table>

*P ≤ 0.005
contrast the estimation of salt soluble proteins have none of these problems and seems equally viable for adaptation and use in the diagnosis of AMI. Further work is being pursued, in this area, in our laboratory to firmly establish this new method as a reliable marker for diagnosis of AMI.

References