Letters

Prognostic utility of luminol (5-amino, 2-3 dihydro 1-4 phthalazinedione)-enhanced neutrophil chemiluminescence in fulminant hepatic failure

A number of variables have been shown to have prognostic significance in fulminant hepatic failure (FHF). Abnormalities of neutrophil number and function are known in FHF. During phagocytosis and oxidative metabolism in neutrophils, reactive oxygen species are released along with generation of light, i.e., chemiluminescence. This can be enhanced by addition of luminol (5-amino, 2-3 dihydro 1-4 phthalazinedione). We hypothesized that neutrophil chemiluminescence in peripheral blood would help in understanding neutrophil function in FHF.

Fifteen consecutive patients, aged 19 to 70 years, with FHF following viral hepatitis were studied. Three of them had history of alcohol ingestion. Two were pregnant and two were less than ten days post-partum. None of them had bleeding manifestations or septicemia. All patients were managed using standard treatment protocol. Eleven patients died; all deaths occurred within 48 hours of admission. Fifteen healthy subjects matched for age and sex were studied as controls. The study was approved by the ethics committee of the institute and informed consent from patients' relatives was taken.

Ten mL heparinized blood was taken from all surviving patients and control subjects on days 0, 2, 7 and 28. The samples were kept at 27°C for 45 minutes. The supernatant leukocyte-rich plasma was separated by dextrin gelatin sedimentation. This plasma was poured on ficole isopaque column and centrifuged at 4°C at 400 X g for 30 minutes in a swing-out bucket centrifuge. The neutrophils along with the RBC settle at the bottom. The upper mononuclear cells were removed and to this 1-2 mL of cold 0.83% NH4Cl was added to remove contaminating erythrocytes. The neutrophils were then suspended in double-strength McCoy's 5 (a medium without calcium and magnesium). The cells were counted in a hemocytometer and were suspended in a concentration of 2 X 10^6 cells/mL. The cells were then incubated with 20 µL of luminol solution and 20 µL of latex particles. The chemiluminescence produced was measured in a luminometer (Becthld; West Germany). Chemiluminescence response was recorded every minute till peak chemiluminescence was obtained. Results were calculated as counts per minute per 10^6 cells.

Significantly higher values were observed in patients as compared to control subjects, and in survivors as compared to those who died. Values dropped to normal at recovery (Table).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>15</td>
<td>74.3 (57-345.6)</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>14.9 (2.6-41.29)</td>
</tr>
<tr>
<td>Survivors</td>
<td>4</td>
<td>104.6 (62.9-436.6)</td>
</tr>
<tr>
<td>Non-survivors</td>
<td>11</td>
<td>39.5 (27-346.2)</td>
</tr>
<tr>
<td>At recovery</td>
<td>4</td>
<td>17.85 (2.0-29.2)</td>
</tr>
</tbody>
</table>

*p<0.001 (t test)

Oxidant free radicals have been implicated in the pathogenesis of many diseases, including FHF. In the present study also the luminol-enhanced neutrophil chemiluminescence response was higher in patients. The higher value in survivors may be because all four of them had initial values more than 3SD above mean of controls, versus 5 of 11 who died. We believe the lower values in the other 6 of 11 may be a preterminal event, when neutrophils fail to respond to stimuli.

This suggests that oxidative stress plays an important role in FHF. The increased oxidative metabolism in FHF may be due to loss of detoxifying function of the liver, or interaction between neutrophils and immune complexes, or activation of neutrophils by a serum factor. In a rat model, administration of hydroxy radical scavengers prevented liver injury and improved survival.

References

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Hepatitis B control: to vaccinate or not

The hepatitis B vaccine has been available for decades now. It has been established that the vaccine reduces the frequency of chronic liver disease, liver cancer and horizontal transmission of the hepatitis B virus (HBV) in
the community. The cost-effectiveness of the vaccine has been proven, and the World Health Organization has recommended its use in developing countries. We are all aware that a majority of Indian doctors have immunized their children with the vaccine.

Then why are we still debating whether and which newborns in India should be vaccinated? The real issues are elsewhere. First, who will pay for vaccinating millions of newborns, year after year for 25-30 years, before hepatitis B ceases to be a problem? Secondly, do we have the infrastructure for such a large-scale vaccination program in the community, and to complete it efficiently so that 90% of all Indian babies are protected?

Two days after this article was published, our institution received a circular from the Ministry of Human Resource Development through the University Grants Commission, forwarded by the University of Mumbai to all its colleges, making vaccination against HBV compulsory for all students, teachers and employees. So, while the academia is still debating the merits and demerits of HBV vaccination of newborns, the Union Government has recommended immunization of all adults!

This reminds us of Blumberg's philosophical essay on the problems with HBV. The Indian HBV vaccination scenario is part of this "Daedalus effect." We talk of preventing HBV infection by vaccination and yet reuse sclerotherapy needles in the new millennium! Isn't it time that academic societies like the ISG played an active role in disseminating sensible advice to our patients, countrymen, and our governments?

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References

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Reply from the authors
The issues involved are not as clear-cut as Drs Peshwe and Mohandas state. In February this year, we published a letter in the Lancet showing how the WHO's figure of 250,000 persons dying each year in India from hepatitis B can be arrived at by wrongly projecting figures from Taiwan. The validity of the WHO recommendation for universal immunization based on such faulty projections is therefore questionable.

Peshwe and Mohandas ask who will pay for the program and who will ensure that 90% babies are covered. This is a very pertinent point, especially as we know the coverage for DPT (a much cheaper vaccine) in some areas is less than 20%.

Finally, they question the wisdom of the Union Government in recommending immunization of adults. This happens in many countries: the clout of vested interests and vaccine manufacturers can subvert national interests.

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Tea or tobacco: etiology of esophageal cancer in India

We read the article by Chitra et al, and were surprised at the finding that drinking three cups of tea daily would increase the risk of esophageal cancer more than with smoking. The authors should have discussed this new finding. Tea is supposed to actually lower the risk for many cancers, except when it is of the salted type (as in Kashmir), which is rich in nitrosamines. We believe the finding in this study is because of confounding or bias, which is common in case-control studies. It is likely that excess tea consumption is either a recall bias or is a confounding factor because of the liquid diets patients with dysphagia consume.

Only 63% of cases in the study used tobacco, which is much lower than what is reported from other parts of India. Does this mean that 37% of southern Indians have other causes for esophageal cancer? In our center, about 84% of patients with esophageal cancer admit to the use of tobacco; when patients with tobacco stains on teeth are asked how they got it, an additional 8% admit to the use of tobacco (unpublished observations). In other words, 92% of patients with esophageal cancer have exposure to tobacco in some form. This is very important from a preventive viewpoint. We must strive to eradicate tobacco usage in India.

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