ANALYSIS OF BLOOD PARAMETERS & RT-PCR RESULTS IN DENGUE SUSPECTED PATIENTS FROM SRI LANKA

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ABSTRACT

Background & Objectives: Early laboratory confirmation of dengue viral infection is vital to minimize its fatal outcomes. There is no information on the relationship between reverse transcriptase polymerase chain reaction (RT-PCR) results and different blood parameters of patients with dengue fever (DF) in Sri Lanka. Therefore the objective was to analyze the blood parameters and RT-PCR results in clinically suspected dengue patients.

Materials and Methods: Blood samples were obtained from 111 in-ward clinically suspected adult male dengue patients during the febrile phase of the infection. RNA was extracted from serum samples and single tube multiplex RT-PCR was performed. Total white cell count (WBC), differential count, platelet count, haemoglobin concentration (Hb), pack cell volume (PCV), aspartate aminotransferase concentration (AST) and alanine aminotransferase concentration (ALT) were quantified.

Results: Of the 111 samples, 48 were positive with RT-PCR. All positive samples were of DEN-2 serotype. WBC, platelet counts, percentage of neutrophils and lymphocytes, Hb, PCV, AST and ALT showed a significant difference (p<0.05) between RT-PCR positive and negative samples. Increased ALT levels was the most significant as more than 50 IU/L of ALT levels was observed in 90% of the RT-PCR positive patients whereas its increase was observed only in 3% of the RT-PCR negative patients.

Conclusions: ALT was the most significantly affected parameter in RT-PCR positive group and appears to be the best serological parameter among the tested, in early identification of patients with DF.

Keywords: Dengue fever, RT-PCR and ALT

INTRODUCTION

Dengue Fever (DF) is now endemic in more than 100 countries and 100 million cases of DF and half a million cases of dengue hemorrhagic fever (DHF) occurs annually in the world¹. Tropical countries are most seriously affected by DF as environmental conditions of the tropics favor the development and proliferation of Aedes Aegypti, the principal vector².

Dengue virus is a single-stranded RNA virus in the genus Flavivirus, family Flaviviridae. There are four closely related serotypes of dengue virus (DEN 1-4) classified according to biological and immunological criteria. Infection with any of these serotypes may result in asymptomatic infection or may cause a range of manifestations from non-specific fever to DHF which is a syndrome characterized by increased vascular permeability and thrombocytopenia¹. In severe cases, the increased vascular permeability may result in life-threatening dengue shock syndrome (DSS). Infection with one
Dengue virus, serotype specific antigen, and antibodies in clinical samples: A multiplex RT-PCR approach to confirm the clinically suspected dengue patients at the acute phase using RT-PCR and secondly to correlate the results of blood parameters to RT-PCR positive and negative groups.

MATERIALS AND METHODS

Ethical clearance and permission from the relevant authorities and informed written consent from the patients were obtained.

Blood samples were collected from clinically suspected male patients between the ages 20-50 y, admitted to three hospitals during a period of one year. Samples were collected within the first 3-5 days of febrile phase, who had at least two of DF related symptoms (skin rash, retro orbital pain, myalgia, arthralgia) and whose platelet count was less than 150x10^9/L. The demographic details of the patients were taken from bed head tickets and through questioning. Blood was collected in to a sterile tube for RNA extraction and to an EDTA coated tube for blood parameter analysis.

DNA was extracted from serum samples as previously described using guanidine thiocyanate method and purified using silica gel and eluted with RNase-free sterile deionized water. Single tube multiplex RT-PCR was carried out according to the method described by Harris et al. The 5' Primer targeted a region conserved in all four dengue virus serotypes and the four 3' primers are targeted sequences unique to each serotype. Reverse transcriptase was used in the same tube. Forty cycles were used to amplify cDNA. Expected sizes of the RT-PCR products were, DEN-1: 482 bp, DEN-2: 119 bp, DEN-3: 290 bp and DEN-4: 389 bp. RT-PCR products were separated using electrophoresis and the serotype was identified based on the size of the product. Positive and negative controls were used in each batch of analysis.

Total white cell counts (WBC), differential count, platelet count, haemoglobin concentration (Hb) and pack cell volume (PCV) were performed using manual methods. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were analyzed using assay kits (Catalog No. 12011 and 12012 Human GmbH Germany). Data are presented as mean ± standard deviation.

Blood parameters of RT-PCR positive and negative groups were compared and were analyzed.
RESULTS

Forty eight samples out of 111 samples tested (43%) were RT-PCR positive whereas the balance 63 samples (57%) were RT-PCR negative. All the positive samples were of DEN-2 serotype.

The results of all the blood parameters tested in RT-PCR positive and negative patient groups are given in Table 1. Reference values of these parameters of healthy males and previously reported reference values are also given in this table.

The lowering of the mean WBC count, neutrophil percentage, platelets and Hb in RT-PCR positive group compared to that of RT-PCR negative group was statistically significant (p=0.000 to p=0.003). These values in total clinically suspected dengue sample were also significantly lower than that of reference values. However, the mean percentage of eosinophils of RT-PCR positive group was not significantly different from that of RT-PCR negative group.

Mean percentage of lymphocytes, PCV, AST and ALT of RT-PCR positive group was significantly higher than that of RT-PCR negative group (p=0.000 to p=0.001). These values in clinically suspected dengue patients was also significantly higher compared to that of reference values.

Percentage of samples showing measurements below the lower limits of the normal ranges and above the upper limits of the normal ranges were analyzed (Table 2). Accordingly, the most significantly affected blood parameter observed in relation to RT-PCR positivity was ALT level among the tested parameters. Approximately 1/3rd of RT-PCR positive samples had ALT > 100 IU/L. Highest ALT level observed was 201 IU/L.

DISCUSSION

In the present study, out of the 111 samples analyzed, 48 (43%) samples were RT-PCR positive. All RT-PCR positive samples were of DEN-2 serotype. Though the predominant serotype prevailed during the study period within the regions was DEN-2, this serotype as well as DEN-3 have been previously identified as major circulating serotypes prevailing in Sri Lanka.

In our study, reduction of several blood parameters such as WBC, platelets, Hb and percentage of neutrophils below the normal range was more prominent in RT-PCR positive samples compared to that of RT-PCR negative samples (Table 1). Elevation of several blood parameters such as ALT, AST, PCV and percentage lymphocytes (however, total lymphocyte count was lower than that of RT-PCR negative group, as the total WBC count was lower) above the normal range was more prevalent in RT-PCR positive samples compared to that of RT-PCR negative samples (Table 1).

According to a study conducted in Sri Lanka using adult patients, mean WBC count and platelet count were started to fall from the 2nd day of fever with the lowest counts observed on the 5th to 7th day. A review on features that differentiate DF from other febrile illnesses indicated that low platelet count and decreases in WBC and neutrophils were independently associated with the presence of dengue, in both adults and children. Our results were compatible with those findings showing significant reduction of these parameters in RT-PCR positive group.

In our study, most significantly affected blood parameter observed in relation to RT-PCR positivity was the ALT level among the tested parameters. This finding is novel to Sri Lanka as the blood parameters were compared after confirming the samples using RT-PCR, with acute phase samples. In a study conducted in Sri Lanka using children with clinical features suggestive of DF, 49% had high ALT and 67% had high AST. However, method of confirmation of DF was based on detection of IgM and IgG after the 7th day. Several other studies have also reported elevations of AST and ALT in majority of DF patients but mostly after the acute phase of the illness. In these studies, confirmation of DF was based on detection of IgM and IgG.
Table 1. Blood parameters from RT-PCR positive and negative groups

<table>
<thead>
<tr>
<th>Blood Parameter</th>
<th>RT-PCR positive Mean values (± SD)</th>
<th>RT-PCR negative Mean values (± SD)</th>
<th>Reference from healthy males Mean values (± SD)</th>
<th>Published reference(^{15})</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10^6 / L)</td>
<td>3161 (542)</td>
<td>4354 (1065)</td>
<td>7255 (1729)</td>
<td>4000-10000</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>45 (4)</td>
<td>48 (5)</td>
<td>51 (10)</td>
<td>40 - 80</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>53 (4)</td>
<td>50 (5)</td>
<td>46 (9)</td>
<td>20 - 40</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>1.8 (1.3)</td>
<td>1.8 (1.6)</td>
<td>4 (2.7)</td>
<td>1 – 6</td>
</tr>
<tr>
<td>Platelet (x10^9/L)</td>
<td>102 (25)</td>
<td>138 (32)</td>
<td>258 (57)</td>
<td>280 (130)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.7 (2)</td>
<td>14.7 (1.6)</td>
<td>14.6 (1.4)</td>
<td>15 (2)</td>
</tr>
<tr>
<td>PCV %</td>
<td>49 (5)</td>
<td>44 (5)</td>
<td>45 (5)</td>
<td>45 (5)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>44 (13)</td>
<td>32 (9)</td>
<td>25 (6)</td>
<td>0 - 50</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>88 (36)</td>
<td>34 (10)</td>
<td>25 (7)</td>
<td>0 - 50</td>
</tr>
</tbody>
</table>

Reference values are given for comparison.

One study used virus isolation method for acute serum and detection of IgM and IgG for samples collected between 10ths to 14ths days after the onset of illness\(^{22}\). Number of patients seen with elevated AST level in DF in these studies was greater than that of ALT level. However, in our study, number of patients with elevation of ALT was greater than that of AST. Aminotransferase levels increases from 1st to 3rd day after the onset of illness and peak during the second week of illness\(^{23}\).

In our study, we collected the samples during 3rd to 5th day of febrile phase and therefore, before the appearance of peak aminotransferase. We have observed elevations of <201 IU/L. Higher ALT concentrations may have been observed during the second week of illness. The predominant elevation of ALT (compared to that of AST) seen in our study may also indicate more acute nature of the liver damage releasing mostly the cytosolic markers. Another feature revealed was that samples which had elevated AST in RT-PCR positive group had elevated ALT too.
Table 2. Percentage of RT-PCR positive and negative samples showing abnormal blood parameters

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Cut off value</th>
<th>RT-PCR Positive</th>
<th>RT-PCR Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10⁶ / L)</td>
<td>&lt; 4000</td>
<td>93.8</td>
<td>41.3</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>&lt; 41</td>
<td>20.8</td>
<td>11.1</td>
</tr>
<tr>
<td>Platelet (×10⁹/L)</td>
<td>&lt; 150</td>
<td>95.8</td>
<td>63.5</td>
</tr>
<tr>
<td>Platelet (×10⁹/L)</td>
<td>&lt; 100</td>
<td>48</td>
<td>1.6</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>&lt; 13</td>
<td>37.5</td>
<td>11.1</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>&gt; 55</td>
<td>31.3</td>
<td>12.7</td>
</tr>
<tr>
<td>PCV %</td>
<td>&gt; 50</td>
<td>31.3</td>
<td>4.8</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>&gt; 50</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>&gt; 50</td>
<td>90</td>
<td>3</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>&gt; 100</td>
<td>31.25</td>
<td>0</td>
</tr>
</tbody>
</table>

This feature was not seen in RT-PCR negative group. This finding suggests that the liver is likely to be compromised in DF as ALT is more specific to liver where as AST observed in RT-PCR negative samples may have been originated from extra hepatic sources such as skeletal muscle.

Liver is one of the major target organs involved in acute DF. Involvement of the liver in the pathogenesis of dengue virus has been indicated by mild-to moderate increases in aminotransferase levels. The pathogenesis of hepatic involvement during DF is poorly understood. Potential mechanisms include direct effects of the virus or host immune response on hepatocytes, ischemia due to circulatory collapse, hepatotoxic effects of drugs and tissue tropism of viral serotypes or genotypes. DEN-2 virus has been shown to replicate actively and cause severe cytopathic effects in liver cell lines. Antibodies against non structural proteins such as NS1 of dengue virus have been shown to play a role in liver damage. In our study, immune response may not have contributed to the elevation of ALT as a major cause, as the blood samples were collected at a stage where antibodies against the virus could not have been detected unless the DF was due to a secondary infection. There is experimental evidence to suggest that liver damage could be serotype-
dependent and the damage has been found to correlate with DEN-2, 1 and 3 in that order\(^1\). Hence, whether ALT changes seen in our study are more specific to DEN-2 or common to other serotypes, need to be addressed.

Based on our findings, only 43% of the clinically suspected dengue patients were confirmed to have DF. Previous study conducted in Sri Lanka\(^17\), suggested that a combination of clinical picture, thrombocytopenia, leukopenia and elevated liver enzymes could be used as markers for early diagnosis of dengue infection. Our findings support those findings and further, identify ALT as the most distinctly affected blood parameter in these patients. Therefore, our findings could be considered as an early diagnostic method to be implemented in clinically suspected DF patients.

**CONCLUSION**

All the positive DF samples were of DEN-2 serotype. Compared to the RT-PCR negative group, RT-PCR positive group showed a decline in WBC count, platelets, Hb and percentage of neutrophils and an increase in ALT, AST, PCV and percentage of lymphocytes. ALT was the most significantly affected parameter in RT-PCR positive group and appears to be the best parameter among the tested, in early identification of patients with DF.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


