To the Editors:

# Dengue fever and ionized calcium levels: significance of detecting hypocalcaemia to predict severity of dengue

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Low total serum calcium is unknown to occur in more than 80% of patients with dengue fever (DF) [1]. Unlike serum total calcium, serum ionized calcium (SIC) is not affected by other factors. A recent study revealed that SIC levels were significantly lower and hypocalcaemia was more prevalent in patients with dengue haemorrhagic fever (DHF) than in patients with DF [2].

Most of the complications in DHF are due to plasma leakage leading to third space fluid accumulation (TSFA) in pleural and peritoneal cavities. Ultrasound Scan provides a quick assessment of TSFA. In previous studies TSFA was not objectively measured and ionized calcium levels were not serially done during hospital stay [1, 2].

We carried out a cross sectional study at University Medical Unit (UMU), Teaching Hospital Anuradhapura (THA) from January to May 2014 to assess the distribution of SIC during DF and to assess the association between hypocalcaemia with TSFA and other organ involvement. Consecutive probable DF patients who were admitted to UMU, THA were recruited to the study after obtaining informed written consent. Ethical approval was obtained from Ethics Review Committee, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka.

Case confirmation was done using dengue NS1 antigen test [Standard Diagnostics BIOLINE, Kyonggi, Korea (immunochromatographic method)] or dengue IgM antibodies [Standard Diagnostics BIOLINE, Kyonggi, Korea (immunochromatographic method)]. A SIC level of <1.1mmol/l was defined as hypocalcaemia.

Blood samples were obtained on admission and every morning till the patient recovered. All investigations were done in a single laboratory. Ion electrode selective method was used to determine SIC levels. All patients underwent ultrasound scans on admission and daily in the morning. It was carried out by two MBBS qualified medical officers. The consultant radiologist at THA interpreted the saved scan images.

Of the 36 probable cases of dengue recruited for this study, 31 (86.1%) were confirmed by detecting NS1 antigen (n=07), dengue antibodies (IgM) (n=05) or both (n=19). Majority of confirmed cases (n=26) were males and mean age was 32.6±11.2 years. Among confirmed cases, SIC levels from fever day 3 to 10 were available (Table 1). These values ranged from 0.79 to 1.34 mmol/l. Seventeen (54.8%) of the confirmed cases either had hypocalcaemia on admission (n=13) or developed it during hospital stay (n=4). Most of them were first detected as having hypocalcaemia (n=10) on day 6 and 7 of fever. None of the patients with hypocalcaemia had clinical features suggestive of hypocalcaemia or received calcium supplementation. In all serum calcium levels became normal within a day or two.

Five patients developed TSFA. Three of them had TSFA and hypocalcaemia on admission (Table 2). Two more patients developed TSFA after admission and they too developed hypocalcaemia before or at the time of first detection of TSFA. None of the patients with normocalcaemia developed TSFA. Positive and negative predictive values of hypocalcaemia predicting TSFA was 29% and 100% respectively with a positive likelihood ratio of 2.17 (95% CI 1.84-2.551). Mean SIC (lowest) among patients with TSFA was 0.97 (SD 0.1) mmol/l compared to 1.12 (SD 0.14) mmol/l among those without TSFA (*p*=0.035).

Patients with hypocalcaemia had significantly lower platelet count and serum albumin levels (Table 3). All five patients with platelet count <20 000 × 109/L had

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hypocalcaemia. However, there was no significant difference in lowest mean white cell count and highest mean AST/ALT levels (Table 3). None of the patients developed features suggestive of myocarditis or encephalitis.

These findings raise several unanswered questions on the role of calcium in dengue. In in-vitro studies, presence of calcium ion is obligatory for cytotoxic activity of the dengue virus and the cell death is associated with increased intracellular calcium ion concentration [3]. Therefore, one can postulate that hypocalcaemia due in DF could be due to influx of calcium and calcium replacement could enhance the dengue virus activity by increasing intracellular calcium ion concentration. This can be supported by in vitro studies showing calcium channel blockers inhibiting the activity of the influx of calcium ion to macrophages and T cells reducing the disease activity of dengue [4]. However, there is only very limited evidence on calcium supplementation in dengue fever [5].

### Table 1. Detection and recovery of hypocalcaemia in confirmed cases of dengue patients according to day of fever

| Duration of fever (days) | Number of patients whose calcium values are available | Number of patients with hypocalcaemia | Number of patients first detected with hypocalcaemia | Number of patients whose calcium level normalized from low levels | Serum ionized calcium (mmol/L) Mean (SD) |
|---|---|---|---|---|---|
| 3 | 2 | 1 | 1 | 0 | 1.10 (0.01) |
| 4 | 7 | 3 | 3 | 1 | 1.15 (0.11) |
| 5 | 14 | 4 | 1 | 0 | 1.15 (0.11) |
| 6 | 20 | 7 | 5 | 2 | 1.14 (0.12) |
| 7 | 24 | 9 | 5 | 3 | 1.16 (0.12) |
| 8 | 20 | 3 | 1 | 7 | 1.21 (0.10) |
| 9 | 6 | 2 | 1 | 2 | 1.16 (0.21) |
| 10 | 2 | 0 | 0 | 2 | 1.25 (0.06) |

### Table 2. Serum ionized calcium (mmol/l) levels among five confirmed cases of dengue fever with third spaced fluid accumulation

| | Serum ionized calcium (mmol/l) | | | | | | |
|---|---|---|---|---|---|---|---|
| Patient ID | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |
| 1 | 1.10 | 1.25 | 1.23 | 1.30* | 1.20** | | |
| 2 | | | | | 1.06* | 1.30** | 1.36 |
| 3 | | | 1.19 | 0.98* | 1.08** | 1.20 | |
| 4 | | 1.09* | 0.90 | 1.23** | 1.28 | 1.20 | |
| 5 | | 0.99* | 1.02 | 1.11** | 0.85 | 1.15 | |

*indicates the date of first detection of fluid leak   ** indicates the day of maximum fluid leak low serum calcium levels are in bold

### Table 3. TSFA, platelet count, white cell count and albumin level according to serum ionized calcium level

| | Hypocalcaemia | | |
|---|---|---|---|
| | Yes (17) | No (14) | P value |
| Lowest mean platelet count (109/L) | 40,529 (±25809) | 61,357 (±30457) | 0.048* |
| Platelet count <20000×109/L | 5 (29.4%) | 0 | 0.027* |
| Lowest mean WCC (×10⁹/L) | 3.35 (±1.79) | 3.2 (±1.74) | 0.856 |
| Lowest mean albumin level (g/L) | 32.7 (±0.34) | 36.9 (±0.31) | 0.001* |
| Highest mean AST (IU/L) | 309.6 (±306.6) | 238.5 (±141.9) | 0.431 |
| Highest mean ALT (IU/L) | 189 (±184.9) | 183 (±118) | 0.916 |
| Albumin level <35 g/L | 10 (58.8%) | 3 (21.4%) | 0.036* |

*significant at p<.05

WCC white cell count, AST aspartate amino transferase, ALT alanine amino transferase

We recommend carrying out prospective studies to examine use of serum calcium level as predictor of TSFA and carrying out randomised controlled studies on oral and intravenous calcium replacement for dengue fever patients to assess its role in prevention of complications and clinical recovery.

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## Conflicts of interest

There are no conflicts of interest.

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