Endotoxaemia in *Vibrio* El Tor cholera


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**Summary**

Endotoxaemia and endotoxin-induced changes were sought in Nigerian patients presenting with cholera diarrhoea. The organism was *Vibrio cholerae*, bio-type El Tor, serotype Hikojima. The limulus amoebocyte lysate gelation test was used qualitatively by the clot method, whilst a spectrophotometric method was used quantitatively to measure endotoxin levels. 25 acutely ill patients tested had detectable endotoxaemia by the Escherichia coli endotoxin standard. The highest endotoxin level was found in a patient with subconjunctival haemorrhage. Changes in platelet counts, the detection of complement breakdown product C3d in plasma, the elevation of fibrin degradation products, the finding of elevated, normal or depressed C3 levels and alteration of prothrombin time and Kaolin Cephalin coagulation time (KCCT) were determined on acid citrate dextrose blood specimens.

**Introduction**

*Vibrio cholerae* diarrhoea results from activation of the adenyl cyclase enzyme in intestinal epithelial cells by cholera enterotoxin, with consequent increase in intracellular cyclic AMP levels and alteration of intestinal and water movements (FIELD, 1971). Subunit A of the enterotoxin is the active component whilst the B subunit is the binding component to the GM ganglioside receptors of the intestinal epithelial target cells (VAN HEYNINGEN, 1976). Bacterial adherence to intestinal surface may also be an important pathogenetic mechanism (SCHWARTZMAN REACTION, 1979; FRETER et al., 1978, 1979).

*V. cholerae* strains contain endotoxin which is a lipopolysaccharide (LPS), differing from LPS of *Escherichia coli* endotoxin standard. *V. cholerae* El Tor LPS may be less potent than LPS of other enterobacteriaceae in lacking 2-keto-3-deoxyoctonate (KDO) which links the toxic lipid A moiety to the polysaccharide moieties (HISATSUNE et al., 1976).

**Materials and Methods**

**Patients**

An outbreak of cholera diarrhoea occurred during the heavy rains of July to September 1978 in villages within Zaria emirate in Northern Nigeria. Over 500 patients were treated at Ahmadu Bello University Hospital, Zaria. 92 patients were studied closely and their clinical and laboratory features documented. Most patients were clustered in families who depended on water drawn from shallow wells for domestic use. Their ages ranged from 10 to 70 years.

**Complications**

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe ECF depletion</td>
<td>34</td>
</tr>
<tr>
<td>Moderate ECF depletion</td>
<td>38</td>
</tr>
<tr>
<td>Mild ECF depletion</td>
<td>20</td>
</tr>
<tr>
<td>Skin petechiae with or without itching</td>
<td>3</td>
</tr>
<tr>
<td>Subconjunctival haemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>Subconjunctival injection</td>
<td>10</td>
</tr>
<tr>
<td>Tintins</td>
<td>5</td>
</tr>
<tr>
<td>Confusion and restlessness</td>
<td>10</td>
</tr>
<tr>
<td>Toxic psychosis with grandiose delusion</td>
<td>1</td>
</tr>
<tr>
<td>Convulsion</td>
<td>1</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>1</td>
</tr>
<tr>
<td>Pregnancy aborted</td>
<td>2 out of 6 pregnant women</td>
</tr>
</tbody>
</table>

**Bacteriological diagnosis**

Rectal swabs were taken from each patient, immediately inserted into universal bottles containing alkaline peptone water and then cultured in thiosulfate citrate bile salt sucrose (TCBS) medium (Difco). Acid production was indicated by a yellow colour with bromothymol blue indicator. Serological identification was achieved using commercial antisera and confirmed as Hikojima serotype. Rectal swabs were again taken at discharge of most patients and found to be negative for *Vibrio cholerae*.

**Haematological investigations**

Venous blood was taken into sequestrene bottles during the first day of admission and one week later for full blood count and platelet determination using a Coulter Counter model ZF. Fibrin degradation products (FDP) were determined using a haemagglutination inhibition system (Burroughs Wellcome) and prothrombin time and Kaolin Cephalin coagulation time (KCCT) were determined on acid citrate dextrose blood specimens.

**Immunological investigations**

Serum C3 levels were determined on samples taken during acute illness, and one week later by standard
radial immunodiffusion technique (Mancini et al., 1965). The complement breakdown product C3d, was detected by counter-immunoelectrophoresis (Arroyave et al., 1978) in EDTA plasma samples. Sera from 25 acutely ill patients were screened for circulating immune complexes by the anti-complementary method using 32Cr-labelled sheep red blood cells (SRBC) (Mowbray et al., 1973). Positive control sera were obtained from children with post-streptococcal glomerulonephritis with known circulating immune complexes (Onyewotu & Mee, 1978).

### Endotoxin determination

2 ml venous blood was aseptically drawn from each patient on admission into plastic pyrogen-free syringes containing heparin. Plasma was obtained and stored at -20°C until analysed. All glassware used was autoclaved and baked for four hours at 180°C to destroy endotoxin; otherwise endotoxin-free tubes, pipettes and glassware obtained from Cape Cod Associates (USA) were used. Pyrotell (Limulus amoeocyte lysate) Lot 26-57-184 from Cape Cod Associates Inc., USA, was used in the qualitative clot method to detect endotoxaemia at 0-23 ng per ml concentration. Endotoxin was quantitatively determined in eight patients by a spectrophotometric method using Escherichia coli endotoxin standard (Lot 0113, PPE-434, Cape Cod Associates, USA), the absorbance being read at 360 nm with a CE 505 Deuterium-Tungsten Double Beam UV spectrophotometer. A standard curve was plotted and used to read out the concentration of test specimens diluted at least ten times with endotoxin-free sterile water supplied by Cape Cod Associates. The procedure outlined in Cape Cod Associates brochure on endotoxin determination was used for both qualitative and quantitative determinations.

### Results

Table I shows the clinical features in 92 closely observed patients and the recorded complications. Petechial skin rash was observed in three patients, two males and one female; subconjunctival haemorrhage (Fig. 1) was seen in a 25-year-old male with severe extracellular fluid (ECF) depletion. A 47-year-old University lecturer had delusions of grandeur though he only had mild ECF depletion. A psychiatrist diagnosed toxic psychosis from which he made a complete recovery one week after successful treatment of the primary disease. He was subsequently discharged from psychiatric care without medication. The temperature was normal in all but 10 patients in whom it was subnormal. Bradycardia of 45 per minute was found in one patient and this reverted to normal following intravenous rehydration. A 10-year-old child without a previous history of epilepsy had grand mal seizures. The mortality amongst 92 patients was 6.5%.

### Haematological Results

Marked leucocytosis with polymorphonuclear cell predominance and toxic granulation in peripheral blood films was observed in over 85% of the patients. The mean WBC count was 14.9 ± 7.2 (range 6.0 to 44.2) × 10³/litre with a mean WBC of 4.9 ± 1.6 × 10³/litre in normal controls (p<001). Neutrophilia ranged from 80 to 90%, lymphocytes 10 to
Fig. 1. Subconjunctival haemorrhage in a patient with vibrio El Tor cholera.

Fig. 2. Platelet counts in 84 patients with acute cholera diarrhea as compared with 18 patients one week after recovery.

Fig. 3. Serum C3 levels in 83 patients with vibrio cholera El Tor diarrhea.
20% and monocytes, 1 to 3%; eosinophilia was not a feature. Abnormally high levels of fibrin degradation products were found in 42% of 31 patients in whom it was measured (range 10 to 40 μg per decilitre). Prothrombin time was prolonged in five of 11 patients and KCCT was prolonged in four of six patients examined. Platelet counts below 100 × 10^9 per litre was found in 32.54% of 84 patients examined (Fig. 2).

**Immunological Results**

Serum C3 was below 100% in 21-6% of the patients using pooled sera of normal Nigerians as standard but was elevated in most patients (Fig. 3). Mean serum C3 in controls was 115.59 ± 18.26 range 71 to 141%. The difference between the means of patients and controls was statistically significant (p < 0.01). Free C3d was detected in 42-86% of 21 patients in whom it was measured. Serum C3 measured in seven follow-up patients one week after acute illness showed that the levels were lower than at admission. The paired values at admission and follow-up were 115, 83; 94, 83; 207, 143; 231, 101; 152, 117; 131, 85; and 94, 85 (expressed as percentage of pooled normal sera).

Immune complex screening in 25 patients using the anti-complementary assay did not reveal the presence of circulating immune complexes when compared with the post-streptococcal nephritis sera and normal sera.

**Qualitative and quantitative endotoxin results**

Pyrotell (Limulus amoebocyte) clotted at 0.23 ng per ml in all 25 acute sera tested but in none of the five available follow-up sera. The quantitative spectrophotometric values are shown in Table II. There was evidence of progressive coagulopathy in the patient with 4.0 μg endotoxin level.

**Discussion**

The pathogenesis of *Vibrio cholerae* diarrhoea is generally attributed to stimulation of intestinal membrane adenyl cyclase by enterotoxin, thereby increasing intestinal fluid and electrolyte secretion (Field, 1971). The role of endotoxin is surprisingly not stressed despite the presence of lipopolysaccharide (LPS) in the Vibrio organism (Hisatsune et al., 1976). Anti-LPS antibodies are even elicited following immunization with cholera vaccines (Merson et al., 1980). Animal experiments demonstrate the lesser potency of Vibrio LPS compared with other Gram-negative bacterial LPS (Raziuddin, 1978), the effects of hypovolemic shock and other yet unidentified factors in man, may explain the temperature pattern of cholera diarrhoea in man, although fever has been induced in rabbits (Raziuddin, 1978). Pyrogenicity and other effects of LPS are related to the fatty acid content and type of linkage in lipid A moiety, as well as the nature of polysaccharide attached to lipid A (McIntire et al., 1976). Further experiments using choleral LPS and particularly Hikojima serotype LPS which needs characterization, may help to elucidate this point, and provide more direct evidence for the lack of its pyrogenic effect in man.

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**References**


Freter, R., O’Brien, P. C. & Maccall, M. S. (1979). Effect of


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