Complement component C5 and C6 mutation screening indicated in meningococcal disease in South Africa


Background. Invasive meningococcal disease (MD), caused by Neisseria meningitidis infection, is endemic in South Africa, with a seasonal peak in winter and spring. There were 2,432 laboratory-confirmed cases between 2006 and 2010. Human deficiency of the fifth complement component (C5D) or complete absence of the sixth component (C6Q0) leads to increased risk of MD, which is often recurrent. All attacks are serious and can lead to death or severe long-term consequences.

Objective. To determine the frequency of specific disease-associated C5 and C6 gene mutations in patients presenting with MD in the Western Cape.

Results. In 109 patients with confirmed invasive MD investigated for local mutations known to cause C5D and C6Q0, 3 were C5D and 11 were C6Q0. In 46 black patients tested, 3 were C5D and 7 were C6Q0. In 63 coloured patients, none were C5D and 4 were C6Q0. All deficient patients were followed up and offered prophylaxis.

Conclusion. C5D and C6Q0 are not rare genetic diseases in South Africa and affected patients are susceptible to repeated MD; 12.8% of MD patients tested were C5D or C6Q0. Blacks were at greatest risk with 21.7% being either C5D or C6Q0. We strongly recommend diagnostic testing for complement C5 and C6 deficiency in the routine work-up of all MD cases in South Africa. Prophylactic treatment should be started in susceptible individuals.
personal contact or information recorded by a health professional. Five white patients were investigated but excluded from the study.

Ethylendiaminetetraacetic acid (EDTA) blood samples were taken from each patient; deoxyribonucleic acid (DNA) was extracted from buffy coat or blood spots. DNA samples were tested for the 2 known C5 gene mutations (Q19X, R1476X) and a third (A252T) that we found associated with C5D.\(^5\)\(^,\)\(^6\) Four C6 gene mutations (821delA, 828delG, 1138delC and 1879delG), all known to be responsible for C6Q0 in the Western Cape, were examined. The regions of interest were amplified by polymerase chain reaction (PCR) and the mutations detected by allele-specific primers or restriction enzyme digestion. All positive C5 or C6Q0 results were confirmed by DNA sequencing.\(^5\)\(^,\)\(^6\)\(^,\)\(^12\)

Frequency controls: 1 500 newborn cord bloods were tested for C5 mutation A252T (750 black, 750 coloured); 400 cord bloods were tested for C5 mutations Q19X and R1476X (200 black, 200 coloured); and 180 white patient samples were tested for all 7 mutations.

**C5 and C6 protein levels**

C5 protein levels were detected with a specific C5 enzyme-linked immunosorbent assay (ELISA) employing the native restricted C5 protein levels were not determined because earlier studies showed that homozygosity, or compound heterozygosity, of any 2 of the 4 specific C6 defects resulted in a complete lack of functional C6 activity.\(^5\)\(^,\)\(^11\)

**Results**

**Mutation frequency of C5 and C6 genes**

A total of 109 patients (or the parents in the case of one infant) with culture-positive MD agreed to participate in the study. C5D or C6Q0 was detected in 21.7% of black (N=46) and 6.3% of coloured (N=63) patients (Table 1).

<table>
<thead>
<tr>
<th>Race</th>
<th>Patients</th>
<th>C5D</th>
<th>C6Q0</th>
<th>Total C5D or C6Q0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>46</td>
<td>3 (6.5)</td>
<td>7 (15.2)</td>
<td>10 (21.7)</td>
</tr>
<tr>
<td>Coloured</td>
<td>63</td>
<td>0 (0)</td>
<td>4 (6.3)</td>
<td>4 (6.3)</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>3 (2.7)</td>
<td>11 (10.1)</td>
<td>14 (12.8)</td>
</tr>
</tbody>
</table>

Three black patients were homozygous for C5 mutation A252T. No patient tested positive for C5 mutations Q19X and R1476X. No coloured patient tested positive for C5D. All 4 previously reported C6 mutations (821delA, 828delG, 1138delC and 1879delG) were observed. One patient was homozygous for 828delG and another for 1138delC. Nine patients were compound heterozygous for all 4 mutations in various combinations; 7 of these patients were coloured and 4 were black.

Controls: C5 mutation A252T was found in 55 (45 black and 10 coloured) of 1 500 (750 black, 750 coloured) newborn cord bloods tested (Table 2); no mutant alleles were identified in 400 controls (200 black, 200 coloured) tested for Q19X and R1476X; none of the 7 defects were detected in 180 white patient controls tested. These mutations appear to be rare in whites (the control sample size was relatively small).

**C5 protein levels**

C5 levels were tested in 2 of the 3 homozygous C5D-A252T patients (the third died of MD and only DNA was available). ELISA-detected C5 levels were very low (approximately 1 - 2% of normal).
Acknowledgements. This work was supported by the Medical Research Council of South Africa, UCT and the NHLS. AO received support from the Department of Infection, Immunity & Biochemistry, Cardiff University, UK. We thank all MD patients and their families who enabled participation in this study. We also thank the NHLS in the Western Cape for assistance in obtaining blood samples.

References

Accepted 23 March 2012.

Table 2. Mutation frequency of C5 defects in black and coloured patient controls

<table>
<thead>
<tr>
<th>C5 mutation</th>
<th>Black</th>
<th>Coloured</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q19X</td>
<td>A252T</td>
</tr>
<tr>
<td>Alleles tested (N)</td>
<td>400</td>
<td>1 500</td>
</tr>
<tr>
<td>Positive alleles (n)</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Frequency (%)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Estimated homozygosity</td>
<td>1/1 111</td>
<td>1/22 500</td>
</tr>
</tbody>
</table>