High Incidence of Complement C9 Deficiency in Koreans

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Abstract. Complement C9 deficiency is the most common complement deficiency in Japan, but it is rare in western countries. Because of Korea's geographical proximity to Japan, C9 deficiency in Korea has also been assumed to be common although this has never before been proven. We investigated complement deficiency in the serum samples of 6,159 Korean hospital outpatients. The deficiency was screened by a sensitive hemolytic assay and was confirmed by immunoassay of each complement component. Three C9-deficient individuals were found, giving an incidence of 0.049%, which is lower than that in Japan but still a considerable figure. Complement deficiencies other than that of C9 were not detected in this study. It is therefore necessary to consider the possibility of C9 deficiency in the interpretation of unexpectedly low complement-mediated hemolytic activity in East Asians. (received 2 November 2004; accepted 31 January 2005)

Keywords: complement C9 deficiency, Korean subjects

Introduction

Complement component 9 (C9) is one of the components of the membrane attack complex (MAC), which is assembled by the sequential binding of complement components C5b, C6, C7, C8, and variable numbers of C9 molecules onto a target cell membrane. Up to 18 molecules of C9 can bind to each C5b-8 complex, forming stable ion pores or channels in membranes and leading to lysis and death of the target cell upon complement activation [1].

C9 deficiency is inherited as an autosomal recessive trait and it results in inability to assemble the MAC with a subsequent increased susceptibility to infection. Serum from C9-deficient individuals is able to kill meningococci but at a slower rate than normal control serum [2]. The risk of developing meningococcal disease among C9-deficient individuals is 700-fold greater than that in Japanese individuals with no complement deficiency [3]. C9 deficiency is the most common complement deficiency in Japan. The incidence of homozygous C9 deficiency among blood donors in Japan is 1/2783-1/1055 [4,5] and hospital-based studies in Japan revealed an incidence of 1/1159, compared to the few cases reported in other countries.

Koreans are thought to share some genetic characteristics with the Japanese because of their geographical proximity. A study of HLA class II allele and haplotype frequencies in Koreans revealed that the HLA class II haplotypes of Koreans are closest to those of the Japanese people [6]. Considering the high incidence of C9 deficiency in Japan, C9 deficiency should also be common in Korea, but proven cases of C9 deficiency have yet to be reported in Korea. To assess the incidence of C9 deficiency in Korea, we screened and assayed complement levels in a large group of hospital outpatients.

Material and Methods

Specimens. In this hospital-based study, we obtained 6,595 serum samples from 6,159 outpatients who visited clinics in Hallym University Sacred Heart Hospital, Anyang, Korea. Some of the subjects were healthy, visiting for an annual health check program. Multiple samples from the same patient were identified by the computerized hospital information system.
system and excluded from the study. The serum specimens were sent for clinical serologic tests and the remaining samples were used after the requested tests had been completed. After serum separation, each serum was kept at 4°C and screened for complement activity within 24 hr of blood collection. Sera with low complement activity on screening were stored at -70°C in aliquots for further testing. Sera from more than 20 healthy individuals were pooled and used as normal human serum (NHS). NHS was also stored in aliquots at -70°C.

**Screening test.** Specimen screening to detect low complement activity was done by hemolytic assay using 96-well, U-bottomed, microtiter plates as described previously [4]. To reduce the hemolytic activity of C9-deficient sera, sucrose gelatin veronal buffer (SGVB++), ionic strength 0.075, containing 0.1% gelatin, 0.15 mM Ca++, and 1.0 mM Mg++ was used in the screening test. To each well, 10 µl of undiluted serum, 50 µl of SGVB++ and 25 µl of sensitized sheep erythrocytes (EA) at 5 x 10⁸/ml were added. Zero and complete lysis controls prepared with adding 25 µl EA to each 60 µl of SGVB++ and 100 µl of distilled water were tested in parallel. After gentle shaking, the plate was incubated for 30 min at 37°C. After centrifugation, 50 µl of supernatant from each reaction well was transferred to the corresponding well of a flat bottom plate and the extent of hemolysis was determined by measuring the absorbance at 405 nm. The percent lysis was calculated from absorbances of zero and complete lysis controls. Sera that gave 10% lysis were stored at -70°C in aliquots and used for secondary tests.

**Secondary evaluation.** In sera that demonstrated low hemolytic activity in the screening test, we measured C9 levels and total complement hemolytic activities (CH50). If the sera showed undetectable C9 protein on radial immunodiffusion (RID) or 20% of the CH50 of NHS, measurements of C1q, C2, C3, C4, C5, C6, C7, and C8 levels and alternative pathway complement hemolytic activity were next performed (Fig. 1). To ascertain whether

![Diagram](image-url)
the sera showing no detectable C9 precipitation on RID lacked C9 protein completely, C9 protein in these sera was measured by a sensitive ELISA using capture by anti-C9 polyclonal antibodies (Advanced Research Laboratories, San Diego, CA) and detection by anti-C9 monoclonal antibodies (Quidel Corp, San Diego, CA). This method can detect approximately 2.5 ng/ml of native C9 in serum.

The measurements of C1q, C2, C5, C6, C7, C8, and C9 were performed by RID on 1% agarose gel containing the relevant polyclonal antibody (Advanced Research Laboratories, San Diego, CA) in pre-optimized concentrations. Briefly, melted 2% agarose in Mancini buffer was added to same volume of antibody solution diluted in same buffer to pre-optimized concentration, and then poured onto GelBond film (FMC, Rockland, ME) in an amount of 1 ml of mixture per square inch of GelBond. After the gel hardened, a 3 mm hole was punched in the center of each gel square and 7 µl of each test serum was added into the hole. The NHS in serial dilutions was tested in parallel and used as a calibration standard. The gel film was incubated in humidified chamber at 48°C for 72 hr and then soaked in saline for 24 hr and in distilled water for 2 hr. After drying, the gel was stained with 1% amido black B solution. The diameter of the precipitating ring was measured. The % concentration of each serum was calculated from the plot of area of the precipitating ring versus each dilution of NHS. The normal range of each complement component was determined as the mean ± 2SD of values in 36 healthy volunteers.

C3 and C4 levels were measured by nephelometry (Immage, Beckman, Fullerton, CA, USA) and the manufacturer’s normal ranges were used.

CH50 was determined using Meyer’s 50% hemolysis method [7]. In the reaction solution, sensitized sheep erythrocytes were reacted with sequential dilutions of serum in gelatin veronal buffer (ionic strength 0.147) enriched with Ca++ and Mg++ (GVB++). After incubation, the extent of hemolysis was determined by measuring the absorbance of supernatant at 512 nm.

Activity of the alternative pathway (AH50) was measured by a hemolytic assay [8]. Rabbit red cells were incubated with sequential dilutions of serum in gelatin veronal buffer containing ethylene glycol tetraacetic acid and Mg++. The extent of hemolysis was determined after the incubation.

The normal ranges of CH50 and AH50 were determined as the means ± 2SD of values from 36 healthy volunteers.

Results

Of 6,595 screened sera, 48 showed low or no hemolytic activity on screening. Of these, 3 sera showed no detectable C9 protein on RID and their C9 deficiency was confirmed by ELISA. The overall incidence of C9 deficiency was calculated as 0.049%. Fourteen sera, including 2 of the C9-deficient sera, showed <20% of the CH50 levels of NHS. The causes of the low CH50 were found to be cold activation or systemic disease including SLE, immune complex disease, or malignancy (Table 1). There was no complete deficiency of complement components other than C9.

Of the 3 C9-deficient individuals, none had an accompanying serious deficiency in the other

<table>
<thead>
<tr>
<th>No. of Sera</th>
<th>C9 deficiency</th>
<th>Low complement activity caused by cold activation</th>
<th>Other diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>5</td>
<td>40</td>
<td></td>
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</tbody>
</table>

Table 1. Causes of low complement hemolytic activity observed in 48 serums by the screening test.

<table>
<thead>
<tr>
<th>No. of Sera</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Normal Range</th>
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</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>48</td>
<td>12</td>
<td>7</td>
<td>77-170</td>
</tr>
<tr>
<td>AH50 (unit/ml)</td>
<td>59</td>
<td>4</td>
<td>2</td>
<td>55-106</td>
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<tr>
<td>C1q level (%)</td>
<td>70</td>
<td>81</td>
<td>108</td>
<td>72-135</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td>17</td>
<td>29</td>
<td>25</td>
<td>16-38</td>
</tr>
<tr>
<td>C2 (%)</td>
<td>96</td>
<td>88</td>
<td>56</td>
<td>74-180</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>85</td>
<td>143</td>
<td>127</td>
<td>79-152</td>
</tr>
<tr>
<td>C5 (%)</td>
<td>77</td>
<td>127</td>
<td>97</td>
<td>68-141</td>
</tr>
<tr>
<td>C6 (%)</td>
<td>91</td>
<td>76</td>
<td>40</td>
<td>46-124</td>
</tr>
<tr>
<td>C7 (%)</td>
<td>76</td>
<td>62</td>
<td>67</td>
<td>62-158</td>
</tr>
<tr>
<td>C8 (%)</td>
<td>66</td>
<td>85</td>
<td>58</td>
<td>55-129</td>
</tr>
<tr>
<td>C9 (%)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>64-126</td>
</tr>
</tbody>
</table>

ND, not detected.

Table 2. Profiles of the complement components in the sera from 3 C9-deficient Korean subjects.

ND, not detected.
Complement C9 deficiency in Koreans

Discussion

In this study, we found 3 cases of C9 deficiency among 6,595 sera collected from 6,159 subjects. The incidence was 0.049% which is a considerable figure. In Japanese subjects, the incidence of C9 deficiency was reported as 0.096% in Osaka and 0.043% in Fukuoka blood donors, and a nation-wide study reported 0.086% incidence in hospital-based patients. The incidence in Korea is lower than in Japan but much higher than in western countries.

Complement deficiency is usually screened with the CH50 test. However, without C9 protein, MAC consisting of C5b through C8 on erythrocytes can lyse red cells, although its efficiency is lower than that of the C5b-9 MAC. Because the CH50 of C9 deficient individuals was approximately 30-40% of NHS, C9 deficiency was not identified by CH50 assay. A Japanese research group developed a simple screening test using SGVB++, a low ionic strength buffer [4]. They reported buffer-dependent variations in the CH50 of C9-deficient serum to the extent that levels known to be about 15 U/ml in GVB++ decreased to lower than 5 U/ml in SGVB++ [9]. We adopted this method for the mass screening of C9-deficient sera.

Because we performed tests after routine testing was completed, the cases of low complement hemolytic activity might be over-counted with possible in-vitro loss of complement activity during storage. However, each complement deficiency was confirmed by immunochemical methods in which one day storage at 4°C would not affect test results significantly. Therefore, the estimated incidence of complement deficiency in our study seems to be valid despite a short delay of test performance.

Previously C9 deficiency was usually found among healthy subjects [4,10]. C9-deficient patients with recurrent infections are very rare although such C9-deficient individuals are more susceptible than normal individuals to meningococcal infection. In this study, we used sera that were collected for clinical laboratory tests. These were from patients with a variety of diseases or healthy individuals who were visiting the hospital as part of an annual health care program. Two of 3 C9-deficient subjects in this study had concomitant diseases, which are not likely to be associated with C9 deficiency. The incidence of C9 deficiency is unlikely to be significantly different in blood donors vs hospital outpatients.

Several molecular defects are reported to be responsible for C9 deficiency [11]. Among them, R95X, a nonsense mutation in exon 4 of the human complement C9 gene, is the major cause of Japanese C9 deficiency [12-14]. A molecular epidemiologic study reported that the carrier frequency in Japan, Korea, and China is 6.7%, 2%, and 1%, respectively [12]. It suggested that the high prevalence of C9 deficiency in Japan results from a founder effect of the R95X mutations in Orientals rather than a mutational hot spot. It also suggested that homozygous C9 deficiency is present in Korea and China with estimated incidences of 1/10,000 and 1/40,000, respectively. The incidence of C9 deficiency found in this study was higher than that calculated from the incidence of R95X heterozygosity. Limited numbers of subjects in the molecular study and the possibility of molecular defects other than R95X may explain the difference between incidences in the two studies. We were unable to clarify the molecular defects in the C9 deficiencies in this study, but studies are in progress to define them.

This is the first reported study of C9 deficiency in the Korean population. The incidence is less than that of Japan but it still has considerable prevalence. It is therefore necessary to consider the possibility of C9 deficiency when interpreting an unexpectedly low CH50 test result in members of East Asian ethnic groups.

Acknowledgements

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References