

SOUTH AFRICAN PATIENTS WITH GENETIC DEFICIENCIES OF COMPLEMENT COMPONENTS C5 AND C6 SUFFER A MARKED INCREASE IN SUSCEPTIBILITY TO MENINGOCOCCAL DISEASE

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ABSTRACT

South African Black African and Cape Coloured patients who had suffered culture positive meningococcal disease (MD) were investigated for mutations in genes coding for the terminal complement pathway proteins C5 and C6. C5 mutations p.Q19X, p.R1476X and p.A252T are known to cause absent or very low serum C5 levels and thus increase susceptibility to meningococcal disease. In addition, four C6 deficiency genes are also responsible for increased susceptibility to the meningococcus and they too segregate in the Western Cape.

We tested admission samples from the 81 black patients and 84 coloured patients for the three C5 mutations and four C6 mutations responsible for the deficiencies. Among the 81 black MD patients we found six C5 deficient p.A252T homozygous individuals and 17 C6 deficient individuals. Thus 28% of black patients who had suffered MD were complement deficient and vulnerable to further MD infections. We found no homozygous C5 deficient coloured MD patients, however, 7 out of 84 (8%) coloured patients were C6 deficient. Thus the risk of recurrent MD infections is present for both groups but highest among black individuals. We strongly recommend antibiotic prophylaxis against further infections for terminal complement component deficient patients.

Keywords: Complement deficiency, meningococcal disease

ABBREVIATIONS

C5D - Complement C5 deficiency, C6D - Complement C6 deficiency, MD - Meningococcal disease, TCC - Terminal complement complex, MAC - Membrane attack complex

INTRODUCTION

The terminal complement pathway comprises the final five components of the complement cascade, C5 through to C9.¹ Complement activation results in the splitting of C5 into the small (74 amino acids) C5a molecule, and the larger C5b molecule. C5a has an immunostimulatory role and also participates in pathological

processes including the response to sepsis.² C5b is the initial component of the complement system's membrane attack complex (MAC). C6 is the second component of the MAC and C6 deficiency has been a problem in the Cape for many years.

The MAC has a major role in defending the host from

Neisserial infections, however, it cannot form if any of the five final complement components are absent.

PATIENTS AND INVESTIGATIONS

COMPLEMENT DEFICIENCY GENES SEGREGATING IN THE WESTERN CAPE

In the past, patients with genetically determined deficiency of any terminal complement component, C5 through to C9, were diagnosed because of recurrent invasive *Neisseria meningitidis* infections. We had cared for two adult cases with C5 deficiency who had presented this way to the Immunology Clinic at Groote Schuur Hospital in Cape Town. Patient P1 is a woman who had one C5 mutation, p.Q19X. It was first described by Wang et al.³ in a Black family in the United States. P1 had a second C5 mutation, p.A252T. Although it was not known to be pathogenic we decided to investigate this molecular change.

Another Cape Town adult coloured man, P2, had no detectable C5 in his serum and had suffered several episodes of MD. Unfortunately he died of his last MD episode. Nevertheless, we were able to test his mother and his two children and they were all heterozygous for another C5 defect, p.A1476X, that had also been described by Wang et al.³ Therefore the three C5 defects p.Q19X, p.A252T and p.A1476X were part of our screen as were four C6 defects - c.1086delA, c.1087delG, c.1403delC and c.2144delG. Results are shown in Table I.

SCREENING MD PATIENTS FOR GENETIC DEFECTS RESPONSIBLE FOR C5D OR C6D

Patients presented at hospitals or clinics. We were contacted when they were confirmed *N. meningitidis* culture positive. Patients included were either Black African (predominantly Xhosa speaking) or Cape Coloured. The later included individuals with ancestors from regions in the South Western areas of Southern Africa, Malaysia, Europe and a few Bantu speaking Africans. White South Africans were excluded because there was no evidence that they carry the pathological complement genes.

Each new MD patient was investigated for frequencies of three C5 genetic mutations, and four C6 genetic mutations known to occur in South Africa. The genetic testing was for C5 deficiency genes p.Q19X, p.R1476X and p.A252T and C6 deficiency genes p.821delA, p.828delG, p.1138delC and p.1879delG.^{6,8}

Serum C5 protein levels were determined using the C5 Eliza assay described by Würzner.^{7,9} Serum C6 levels in Cape patients with these defects have been found too low to measure⁴ and diagnosis of C6D depended on genetic studies. Both genetic studies and C5 levels were available for the C5 deficiency studies. Thus if genetic studies were not available C5 deficiency could be diagnosed because of the very low or absent C5 levels presented in C5 deficient individuals.⁹

The Immunology Clinic at the University of Cape Town Allergy Diagnostic and Clinical Research Unit (ADCRU) provided patient clinical care and management including penicillin or alternative antibiotic prophylaxis.

RESULTS

Patients comprised 165 individuals who had presented with culture confirmed MD, either at the time or within the previous five years. Patients included were either Black African (predominantly Xhosa speaking) or Cape Coloured. The latter included individuals with ancestors from regions in the South Western areas of Southern Africa, Malaysia, Europe and a few Bantu speaking Africans. White South Africans were excluded because there was no evidence that they carried the pathological complement genes.

Patients diagnosed C5 or C6 complement deficient were asked to attend the Immunology Clinic. Immediate family members were also interviewed and tested for the three C5 mutations and four C6 mutations detailed above. C6 or C5 deficient patients identified were prescribed antibiotic prophylaxis. We used the collective data from all 165 patients for this study.

GENE FREQUENCIES OF C5 MUTATIONS

Among the 165 MD patients, we identified six independent cases of homozygous C5 deficiency (C5D). They were all Black affected p.A252T homozygous and required prophylaxis. One index case had died in hospital, however, he had a C5D homozygous sister who we investigated and treated in the study. She had not suffered any episodes of MD. The six C5D cases and one affected mother had very low serum C5 protein levels (0.1- 4% of normal) and correspondingly low *in vitro* functional activity.⁹ Allele frequencies of p.A252T in samples from control Black Africans and Cape Coloureds were 3% and 0.66% respectively. Results of genetic testing are presented in Table I.

DISCUSSION

Deficiencies of any of the components of the membrane attack complex are usually identified clinically after patients have presented with recurrent invasive *Neisseria meningitidis* infections.^{6,7} In South Africa, meningococcal disease is endemic with 1668 cases having been reported over a five year period (2008 to 2012).

Our earlier studies^{4,5} confirmed very strong associations between complement deficiencies and meningococcal disease. Molecular modelling has demonstrated the possible reason that p.A252T is pathological.⁹

There are a number of factors that influence susceptibility to MD.¹⁰ At present, the ones we recognise are those important for the spread of the disease in complement sufficient individuals.

TABLE I: RESULTS OF TESTING FOR C5 AND C6 GENETIC DEFECTS IN 165 SOUTH AFRICAN MENINGOCOCCAL DISEASE PATIENTS

	BLACK		COLOURED	
Total number of MD patients	81		84	
Number and percentage p.A252T homozygous C5D	6	7.4%	0	0
Number and percentage C6 deficiency (C6Q0)	17	21%	7	8.3%
Total	23	28.4%	7	8.3%

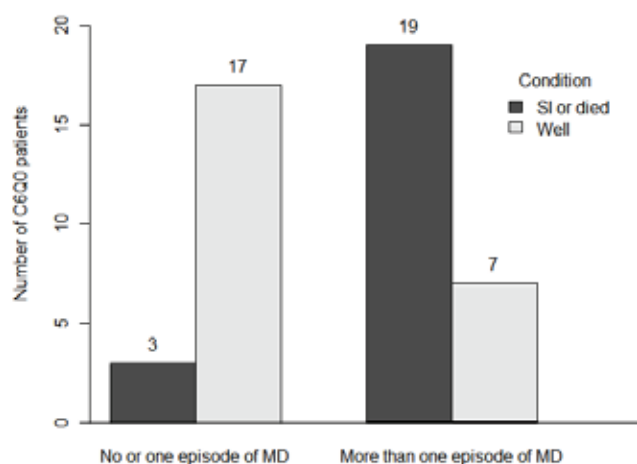


Figure 1: Bar-graph demonstrating that C6 deficient patients who had suffered more than one episode of meningococcal disease (MD) were much more likely to have a serious illness or to have died than those C6Q0 patients who had suffered no or one episode of MD ($P = 0,008$)

However, there may be additional clinical factors that influence susceptibility in complement deficient individuals and we need to understand these in order to provide support for these patients. We have found that antibiotic prophylaxis is beneficial in preventing further infections. Hopefully vaccination will eventually be available for our patients.

The long term condition of complement deficient patients, particularly those who suffered recurrent MD episodes, can be very serious. Also we have seen families in the Cape with more than one sibling who is complement deficient but only one sibling has a history of MD. Recently C5 and C6 deficient patients have been ascertained through genetic screening and we hope that this can help reduce the incidence of recurrent disease. In any case, we have diagnosed patients as complement deficient at the age of twenty years or beyond who have no history of disease. It may be that there are additional immunological differences accountable for outcome. Many complement deficient patients have very serious sequelae from any episode of MD but long term pathology becomes more likely with more than one episode. We have followed up C6 deficient patients, some of them for many years, and the problems they have that result from the multiple infections can be very serious.⁷ In 2005 we recalled approximately 48 C6 deficient patients and assessed their clinical state. They were assessed as to how they were currently and we analysed the results according to their number of episodes of MD. We compared C6 deficient patients with “no or one episodes of MD” to those “who had suffered multiple (2 or more) episodes”. We assessed them as being “well” or suffering a “serious illness” by grouping them according to whether they could live normally. Those who could not were grouped as having a “serious illness”. As can be seen from Figure 1, there were a total of 22 patients who were in the group “Serious illness” and of these 19 had suffered more than one episode of MD. Medical support and the provision of prophylaxis is very important for these patients.

C5 deficiency was not found in any of the 84 coloured patients we tested, however, C6 deficiency is a problem among the Coloureds and Blacks. There are four C6Q0 defects segregating in the Cape⁷ and the particular C6 genetic defects differ somewhat between Coloureds and Blacks. It seems likely that different mutations arose in different areas of Africa. We sincerely hope that diagnosing and treating C5 and C6 mutations will be available in other parts of South Africa and beyond so that help can be provided to aid for these complement deficient patients.

There is also the question of whether boosting opsonophagocytosis could help the complement deficient children. Certainly we hope that meningococcal vaccination will be able to boost the production of antibodies that promote opsonophagocytosis.¹⁰ Hopefully meningococcal vaccines will eventually be provided by the Health Services in South Africa especially for vulnerable children.

ETHICS

The work was approved by the University of Cape Town Ethics Committee.

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