Indian J Med Res 140, December 2014, pp 766-769

Antigen sequence typing of outer membrane protein (*fetA*) gene of *Neisseria meningitidis* serogroup A from Delhi & adjoining areas

S. Dwivedi, B.K. Das, A. Kapil, S. Sood, R. Chaudhry, S. Gupta[*], M. Deb[**], D. Nair[**] & S. Aneja[†]

Department of Microbiology, All India Institute of Medical Science, [*]Department of Microbiology, National Center for Disease Control, [**]Department of Microbiology, Vardhman Mahavir Medical College & Safdarjung Hospital & [†]Department of Paediatrics, Lady Hardinge Medical College & Kalawati Saran Children's Hospital, New Delhi, India

Received March 20, 2012

***Background & objectives*: Meningitis caused by *Neisseria meningitidis* is a fatal disease. Meningococcal meningitis is an endemic disease in Delhi and irregular pattern of outbreaks has been reported in India. All these outbreaks were associated with serogroup A. Detailed molecular characterization of *N. meningitidis* is required for the management of this fatal disease. In this study, we characterized antigenic diversity of surface exposed outer membrane protein (OMP) FetA antigen of *N. meningitidis* serogroup A isolates obtained from cases of invasive meningococcal meningitis in Delhi, India.**

***Methods*: Eight isolates of *N. meningitidis* were collected from cerebrospinal fluid during October 2008 to May 2011 from occasional cases of meningococcal meningitis. Seven isolates were from outbreaks of meningococcal meningitis in 2005-2006 in Delhi and its adjoining areas. These were subjected to molecular typing of *fet*A gene, an outer membrane protein gene.**

***Results*: All 15 *N. meningitides* isolates studied were serogroup A. This surface exposed porin is putatively under immune pressure. Hence as a part of molecular characterization, genotyping was carried out to find out the diversity in outer membrane protein (*Fet*A) gene among the circulating isolates of *N. meningitidis*. All 15 isolates proved to be of the same existing allele type of *FetA* variable region (VR) when matched with global database. The allele found was F3-1 for all the isolates.**

***Interpretation & conclusions*: There was no diversity reported in the outer membrane protein FetA in the present study and hence this protein appeared to be a stable molecule. More studies on molecular characterization of FetA antigen are required from different serogroups circulating in different parts of the world.**

Key words Genotyping - *fet*A3-1 allele - meningococcal meningitis - *Neisseria meningitides* - outer membrane protein

Neisseria meningitidis is a human pathogen capable of causing meningitis, bacteraemia and some less common syndrome[1]. Meningococcal meningitis occurs as outbreaks in certain part of the world such as Asia and Africa. Meningococcaemia may occur with or without meningitis, and is highly lethal even when treated[2,3].

Approximately 100,000 cases of acute bacterial meningitis occur worldwide each year. The incidence rate for meningococcal disease is < 1-3/100,000 and 10-25/100,000 in the developed and developing countries, respectively[3]. In India, the epidemic of cerebrospinal fever (earliest term for meningococcal meningitis) was reported in 1883-1884[4] and the earliest confirmed outbreak was in 1961-1962, followed by 1966-1967, 1985-1986, 2005-2006 in New Delhi and 2008-2009 in Meghalaya and Tripura[5]. Serogroup A has been reported repeatedly associated with all the outbreaks in India.

FetA is a member of the TonB-dependent class of outer membrane protein (OMP) of Gram-negative bacteria[6]. A protein model for FetA proposed 26 membrane spanning beta-sheet structures and 13 surface exposed loop structures[7]. Of these, loop 7, corresponded to a region of variable amino acid sequence which included the epitopes for several anti-FetA mouse monoclonal antibodies. Other shorter polymorphic regions were also located within the surface-exposed loops of the proposed structure[8]. This loop was designated as FetA variable region (VR).

FetA is a potential vaccine candidate against meningococci as it induces bactericidal activity. It has been observed in the serum of convalescent patients[9], and experimental studies with monoclonal antibodies raised against FetA have demonstrated to have similar activity[10]. The antigenic change in outer membrane proteins can occur due to the host immune response or evolutionary pressure. Further, presence of highly variable FetA VR regions and the reports of occasional FetA deletions in invasive meningococcal[11] and pharyngeal isolates appear to lessen its appeal as a potential vaccine candidate[10]. Due to highly divergent nature of FetA VR, 412 FetA VR peptides have been identified. These peptide sequences could be resolved into nine distinct families (F1, F2, F3, F4, F5, F6, F7, F8 and F9). The sequence changes were suggested to be a likely consequence of selection pressure imposed by the host immune responses[12]. Diversity of FetA allows it to be used as a marker of molecular characterization along with PorA VRs and multi locus sequence typing (MLST).

The precise characterization of *N. meningitidis* from cases of invasive meningococcal meningitis is essential for the management and control of meningococcal disease[13,14]. In case of meningococci, the European Monitoring Group for Meningococci (EMGM) recommends the use of genogrouping, MLST and clonal complex[15], *por*A VRs and *fet*A VR[16] as methods of molecular characterization.

Antigen sequence typing of *por*A and *fet*A genes is now widely accepted and used for highly discriminating and precise typing of *N. meningitidis*[17]. This study was undertaken to characterize outer membrane protein FetA antigen of *N. meningitidis* isolates obtained from patients with invasive meningococcal meningitis from in Delhi and adjoining areas.

Material & Methods

A total of 15 isolates of *N. meningitidis* serogroup A obtained from the cases of invasive meningococcal meningitis in New Delhi, India, during 2005-2011, were included in this study. Eight of these were from occasional cases of meningococcal meningitis reported in 2008-2011. All these were isolated from routine diagnostic laboratories of Microbiology of the three hospitals of Delhi including All India Institute of Medical Sciences (AIIMS), Vardhman Mahavir Medical College (VMMC) and Safdarjung Hospital and National Center for Disease Control (NCDC). CSF (1-2 ml) was extracted from suspected cases of acute bacterial meningitis. Of these eight isolates, one was collected from AIIMS, four were collected from VMMC and Safdarjung Hospital and three were from NCDC. The remaining seven were taken from those stored at AIIMS from the outbreaks of meningococcal meningitis in Delhi and its adjoining areas during 2005-2006.

All isolates were stored at -70 °C in brain heart infusion broth with 10 per cent glycerol and recovered by plating on chocolate agar. For each isolate, the growth obtained from the surface of a single petri dish after overnight incubation in an atmosphere of 5 per cent CO_2 was used to prepare an opaque cell suspension in 500 µl deionized water and meningococcal DNA was extracted by using nucleic acid extraction kit (QIAGEN, Germany). For serogroup predictions, PCR was applied using primers designed for genes that

are specific for each serogroup[18]. This was done by targeting orf-2 region of *myn* B gene for serogroup A.

All the PCRs were performed at the laboratory of Microbiology department in AIIMS, New Delhi. Primers mentioned in the Multi locus sequence typing website for FetA VR[19] were used. The forward and reverse primers used were S1 5'-CGGCGCAAGCGTATTCGG-3' and S8 5'- CGCGCCCAATTCGTAACCGTG-3' (Fermentas, life Sciences, USA), respectively. Amplified product was of size 1200 bp. PCR products were prepared for sequencing using PCR product purification kit (Axyprep PCR cleanup kit, Axygen Biosciences USA). Sequencing reactions were performed using ABI PRISM Big Dye Terminator cycle sequencing ready reaction kit (Ver 3.1, Applied Biosystems, USA). Sequencing of DNA was carried out in sixteen capillaries ABI Prism 3130XL genetic analyzer (Applied Biosystems, USA). Consensus sequences were submitted to the PubMLST website[19] and the *fetA* allele was obtained. DNA sequence of *fet*A gene was submitted to the genebank (Genebank Accession No. JN182195-JN182196). General information of *N. meningitidis* isolate (AIIMS/08) was also submitted to the MLST database and id number 18485 obtained.

The study protocol was approved by the ethics committee of the AIIMS and patients' consent was taken for the extraction of CSF.

Results & Discussions

Meningococcal meningitis is endemic in Delhi for the last 50 yr. The aim of the study was to document diversity in outer membrane protein *fet*A gene of *N. meningitidis* serogroup A isolated from invasive meningococcal meningitis cases in New Delhi, India during 2005-2011. All 15 isolates of *N. meningitidis* were serogroup A and carried the same *fet*A VR allele, F3-1 (Genebank accession No. JN182195-96). The nomenclature of all isolates as proposed by the EMGM was A: F3-1.

The findings add to our knowledge about the stability found in outer membrane protein of *N. meningitidis* serogroup A in Delhi, India, as no new allele was found. All of these were associated with the same type of *fetA* allele *i.e.* 3-1.

Reported studies showed that *fet*A 3-1 allele was found in both invasive and carrier state though mainly it is isolated from cases of invasive disease. Extensive MLST database[19] search on reported studies showed that of the 49 isolates, 35 (71.4%) are from invasive disease including meningitis, six (12.2%) from carriers and the rest unspecified. *f*3-1 allele was most commonly associated with MLST clonal complex ST5 complex / subgroup III and porA type P1.20, 9 of serogroup A *N. meningitides*.

Earlier reported studies and search through global MLST database[19] of serogroup A with sequence type ST5/clonal complex III in "meningitis belt" of Africa showed the allele type of *fet*A 3-1. However, it was noted that FetA characterization of *N. meningitidis* of serogroup A with sequence type ST5/clonal complex III was not done for all the entries in the database. Those few entries of Africa where FetA 3-1, were from Morocco in 1994, Burkinofaso in 2007, Slovenia in 2006 and Mali in 1997, 2009, 2010. Sequence type and genosubtyping were the same for all.

MLST database[19] reveals that serogroup A sequence type 4789 of ST5/clonal complex III has earlier been reported from Bangladesh (2002), France (2006), Israel (2006), USA (2009), Italy (2009) and UK (2011). All the genosubtyping enteries for ST 4789 were found to be the same type P1.20, 9. The analysis of database reveals that USA, UK and Italy reported *fet*A allele as 3-1 similar to our findings. *fet*A allele 3-1 was found to be associated with other serogroups and sequence types in other geographical regions. Serogroup Y from Spain was also found to be associated with sequence type 4789 but further antigen sequence typing was not done[19].

Extensive search of the database has led to the conclusion that serogroup A *N. meningitidis* appears to be a stable organism in relation with clonal complex, *por*A typing and *fet*A allele. The outer membrane protein (FetA) has potential to elicit immune response which has bactericidal activity. But variation in this region can result in the ineffectiveness of this as a vaccine component. According to a few studies available, stability is found in the genetic structure coding for *fet*A associated with serogroup A. Hence more studies on *Fet*A typing are required from different parts of world in their respective serogroup, sequence type and *por*A type to know the degree of variation. Also the sequence diversity and length of the FetA VR make it suitable for use as a molecular marker.

In conclusion, serogroup A of *N. meningitidis* seems to be stable serogroup in its association with

similar type of *fet*A allele. The limitation of this study was the small number of isolates characterized from a single outbreak and a few sporadic cases. Hence the degree of variation or stability of FetA antigen is likely to be underestimated.

References

1. Rosenstein NE, Fischer M, Tappero JW. Meningococcal vaccines. *Infect Dis Clin North Am* 2001; *15* : 155-69.
2. Peltola H, Nors T, Salminen S, Kayhty H, Sivonen A, Jousimies-Somer H, *et al*. Were the meningococcal vaccination campaigns in 1974-76 of benefit?. *Duodecim* 1983; *99* : 332-41.
3. Sachdeva A, Kukreja S, Jain V, Dutta AK. Meningococcal disease--outbreak in Delhi. *Indian Pediatr* 2005; *42* : 547-56.
4. Patel PT. Cerebro-spinal fever in Bombay. A study of 170 consecutive cases during the years 1921-24. *Lancet* 1926; *11* : 539-41.
5. Sinclair D, Preziosi MP, Jacob John T, Greenwood B. The epidemiology of meningococcal disease in India. *Trop Med Int Health* 2010; *15* : 1421-35.
6. Beucher M, Sparling PF. Cloning, sequencing, and characterization of the gene encoding FrpB, a major iron-regulated, outer membrane protein of *Neisseria gonorrhoeae*. *J Bacteriol* 1995; *177* : 2041-9.
7. Pettersson A, Maas A, van Wassenaar D, van der Ley P, Tommassen J. Molecular characterization of FrpB, the 70-kilodalton iron-regulated outer membrane protein of *Neisseria meningitidis*. *Infect Immun* 1995; *63* : 4181-4.
8. Van der Ley P, van der Biezen J, Sutmuller R, Hoogerhout P, Poolman JT. Sequence variability of FrpB, a major iron regulated outer-membrane protein in the pathogenic neisseriae. *Microbiology* 1996; *142* : 3269-74.
9. Black JR, Dyer DW, Thompson MK, Sparling PF. Human immune response to iron-repressible outer membrane proteins of *Neisseria meningitidis*. *Infect Immun* 1986; *54* : 710-3.
10. Marsh JW, O'Leary MM, Shutt KA, H. Harrison LH. Deletion of *FetA* gene sequences in serogroup B and C *Neisseria meningitidis* isolates. *J Clin Microbiol* 2007; *45* : 1333-5.
11. Petterson A. The Nobel lectures in immunology. The Nobel Prize for Physiology or Medicine, 1919, awarded to Jules Bordet 'for his discoveries relating to immunity'. *Scand J Immunol* 1990; *32* : 425-8.
12. Thompson EA, Feavers IM, Maiden MC. Antigenic diversity of meningococcal enterobactin receptor FetA, a vaccine component. *Microbiology* 2003; *149* : 1849-58.
13. Stuart JM, Monk PN, Lewis DA, Constantine C, Kaczmarski EB, Cartwright KA. Management of clusters of meningococcal disease. PHIS Meningococcus Working Group and Public Health Medicine Environmental Group. *Commun Dis Rep CDR Rev* 1997; *7* : R3-5.
14. Caugant DA. Population genetics and molecular epidemiology of *Neisseria meningitidis*. *APMIS* 1998; *106* : 505-25.
15. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, *et al*. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA* 1998; *95* : 3140-5.
16. Jolley KA, Brehony C, Maiden MC. Molecular typing of meningococci: recommendations for target choice and nomenclature. *FEMS Microbiol Rev* 2007; *31* : 89-96.
17. Fox AJ, Taha MK, Vogel U. Standardized nonculture techniques recommended for European reference laboratories. *FEMS Microbiol Rev* 2007; *31* : 84-8.
18. Orvelid P, Backman A, Olcen P. PCR identification of the group A *Neisseria meningitidis* gene in cerebrospinal fluid. *Scand J Infect Dis* 1999; *31* : 481-3.
19. PubMLST website. Available from: *http://pubmlst.org/neisseria/fetA/*, accessed on February 13, 2015.

Reprint requests: Dr Bimal Kumar Das, Department of Microbiology, All India Institute of Medical Sciences
Ansari Nagar, New Delhi 110 029, India
e-mail:tezpur.bimal@gmail.com