©Biotechnology Society

www.bti.org.in
ISSN 0974-1453
Research Article

EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF A MENINGOCOCCAL POLYSACCHARIDE VACCINE (GROUP A, C, Y AND W-135) IN ADULT HUMAN SUBJECTS

Ashish Prakash[1*], M.P. Singh[2], B Balaraju[3], Anuradha Makkar[4]

[1*]Yashodha hospital and research centre, Nehrunagar, Ghaziabad.,[2] Sir T General Hospital Bhavnagar, Gujarat,[3]Department of Medicine, Osmania Medical College, Hyderabad,[4]Department of Microbiology, Army College of Medical Sciences, Delhi cantt.

***Corresponding author: ashprakash@yahoo.com**

ABSTRACT

Meningococcal disease (septicemia and/or meningitis) caused by *Neisseria meningitides (N. meningitidis)* leads to morbidity and mortality in the children and adults worldwide. In India, both bivalent (A, C) and quadrivalent (A, C, Y, W-135) polysaccharide vaccines are available. The present study focuses on the evaluation of safety and immunogenicity of the indigenously manufactured tetravalent vaccine (A, C, Y, and W-135) in Indian subjects. A multicentre, open, controlled and non-comparative phase III clinical trial was conducted in India. A single dose of QuadriMeningo™vaccine was administered in subjects aged 18-68 years. The subjects were evaluated post vaccination for safety and immunogenicity of the vaccine. The safety and immunogenicity results obtained 2-4 weeks post vaccination indicated an acceptable safety profile and a good immune response. Overall, the vaccine was well tolerated in all the subjects. The subjects (100%) showed ≥4 fold rise in bactericidal antibody titre for all the serogroups.

Keywords: Meningococcal Disease, Polysaccharide Vaccine, Quadrivalent, *Neisseria meningitides*.

INTRODUCTION

Meningococcal disease is a severe bacterial infection caused by *Neisseria meningitidis* (*N. meningitidis*), a diplococcal, Gram negative bacteria that colonize the nasopharyngeal mucosa (Crum and Sullivan,2016). The clinical manifestations of the meningococcal disease includes fever, headache, vomiting, nausea, stiffness of neck and rashes (Rosenstein *et al.,* 2001). The incidence rate is higher in infants<1 year of age, with second peak observed in adolescents, the population with the highest carrier (Lalwani *et al.,* 2015).There has been 10-15% case-fatality rates from invasive meningococcal disease and even as high as 50% to 60% among patients with meningococcaemia having blood stream infection and shock (Ferguson *et al.*, 2002 , Zalmanovici Trestioreanu *et al.,* 2013). The World Health Organization reported 21,649

Center	Male (n)	Female (n)	Age (Mean ± SD)	Past immunized No. of patients without antibodies
I (N=40) (18-68 Years)	40	0	33.6 ±9.74	2
II (N=47) (22-57) Years	32	15	33.6 ±10.27	6
III (N=45) (19-52) Years	40	5	32.1 ±6.56	5

Table 1. Basic Demographic Characteristics

*****N=Total number of subjects, n= number of subjects, SD= Standard deviation

meningitis cases in 2015 with 1577 deaths - an overall case fatality ratio of 7.3% (Ferguson *et al*., 2002; (Ferguson *et al.,* 2002 , Trestioreanu *et al.,* 2013)) . Since 2005, in India, there were a number of outbreaks, all attributable to serogroup A. According to WHO, 111 cases and 15 deaths were reported within 3 months of 2005 in India (Paul *et al.,* 2010). As per MedIndia report on prevalence and deaths in India due to meningococcal infections it was reported a total of 16, 217 cases and 300 deaths between 2014 and 2015, of which Bihar was listed at the top rank with 8871 cases registered in 2015 (Ngilneii, 2017).

The majority of invasive meningococcal disease (90%) can be attributed to one of the six immunologically distinct serogroups, *i.e*., A, B, C, W-135, X and Y (MacNeil and Cohn 2011, Lalwani *et al.,* 2015, Crum and Sullivan 2016). The disease outbreaks are found in various proportions all over the world; with A as a predominant serogroup in the Sub-Saharan Africa in addition to W, C and X which are responsible for a smaller number of cases. The introduction of group A polysaccharide vaccine in Africa has almost entirely eradicated Men A epidemic disease. The vaccine's effectiveness has been established by various African field trials. The trials reported that the vaccine induces a solid antibody response in persons between 1 and 29 years of age (Sow *et al.,* 2011)

In the US and Canada, serogroups B, C and Y are responsible for majority of the meningococcal infections, and B and C are the predominating serogroups in Europe (Crum and Sullivan 2016). In N. America, serogroups other than serogroup C are more prevalent, therefore a combination polysaccharide-protein vaccine, including C, Y and W135 serogroups, are more used in this region (Pollard and Scheifele, 2001).

Of 55 cases diagnosed as confirmed/probable the mortality rate was 14·6%. Meningitis was reported in 60% of cases and meningococcaemia in 40% (Nair *et al.,* 2009). To prevent such occasional outbreaks (since 2009) in India, the use of quadrivalent polysaccharide vaccines has been recommended routinely in individuals at a higher risk(John *et al.,* 2013).The quadrivalent vaccines consist of combinations of the purified capsular polysaccharide from serogroups A, C, Y and W-135. The present study focuses on the evaluation of the reactivity and immunogenicity of QuadriMeningo™ (Bio-Med (P) Ltd., Ghaziabad, India), atetravalent meningococcal polysaccharide vaccine (A, C, Y and W 135) in Indian subjects.

MATERIALS AND METHODS

Study Design

This was a phase III, multicentre, open, controlled and non-comparative study which was conducted at three different centers in India- Medical College Bhavnagar (Center I), Osmania Medical College, Hyderabad (Center II) and Santosh Medical College, Ghaziabad (Center III) (Table 1, Fig.1). The study was conducted

as per the protocols approved by Drugs Controller General (India). The study was approved by the respective ethical review committees. All the centers cleared the protocol and had ethics clearance.

A written informed consent form was obtained from each subject prior to the study. Three consecutive batches of Quadrimeningo™ fulfilled the requirement of Indian Pharmacopoeia. They were well tested and approved from Central Drugs Laboratory (CDL), Kasauli (H.P). Batch No. N010103, N020103 and N030103were used for clinical trial at Santosh Medical College, Medical College Bhavnagar and Osmania Medical College, respectively.

Subjects of either gender (18-68 years) were included in the study. Those suffering from fever, diarrhea, infections and those with history of any disease, previous administration of meningococcal vaccine, drug intake (except vitamins and immunosuppressant in past two weeks of vaccination date), hypersensitive to any component of vaccine were excluded from the study.

Study population

A total of 132 subjects were included in the study where center I, center II and center III consisted of 40 (18-68 years; mean age: 33.6±9.74 years), 47 (22-57 years; mean age: 33.6±10.27 years) and 45 subjects (19-52 years; mean age: 32.1±6.56 years), respectively. Table 1: Basic Demographic Characteristics and Figure 1 (Subject Disposition)

Vaccines

A single dose of lyophilized QuadriMeningo™vaccine when reconstituted in 0.5 ml normal saline contains 50 µg of purified polysaccharide of each group (A, C, Y, and W-135). The vaccine was administrated in the subjects by intramuscular route.

Immunogenicity

Blood sample (2-3 ml) for immunogenicity analyses from each subject was aseptically collected prior to vaccination (Day 0) and post vaccination (after 2-4 weeks). The samples were processed by centrifugation and stored at -20°C in pre-coded sterile vials. Immunogenicity of the vaccines was evaluated by carrying out bactericidal assay on paired sera from each subject. The antibody titers were expressed as the reciprocal of the highest dilution that shows 50% or greater killing of the test organism. Bactericidal assay was performed as per W.H.O. T.R.S. 594, Annex 2, 1974 page no.72-73.This is one of the standard methods for testing immune response to Meningococcal Polysaccharide Vaccine and it is particularly important as immunity to meningococcal disease is strongly correlated with the presence of serum bactericidal antibodies. We used Rabbit serum which was three-four weeks old as complement. Each lot of complement was tested against the strain (s) of Neisseria meningitis (Group A, C, Y, W135) as the test antigen to determine whether the complement alone has antibodies, which can kill the organism. The positive response was indicated by a ≥ 4 fold increase in the antibody titer.

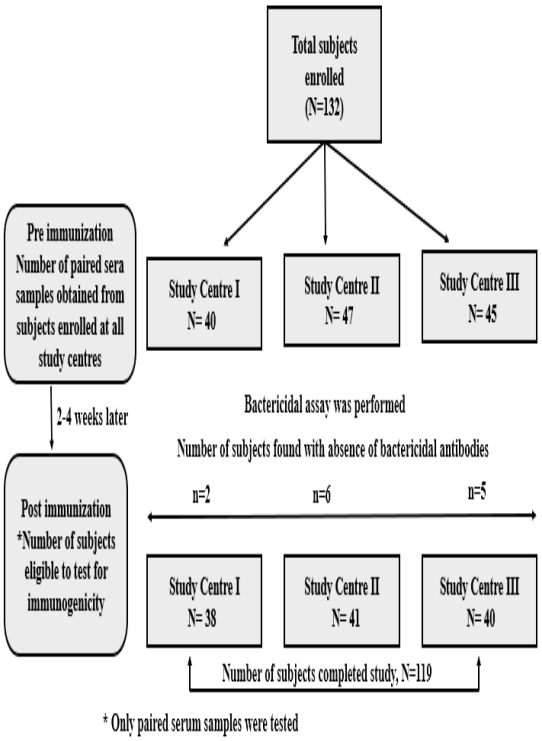

Figure 1: Subject Disposition Safety

All the subjects were observed for local injection site adverse reactions (Ars); pain, erythema, inflammation and other ARs at 30 min, between 4-6 hours and between 24-48 hours post vaccination. The severity of ARs were measured on a scale of excellent, good, fair and bad; if they exhibited no inflammation and normal temperature, inflammation < 2 cm in diameter and temperature <38.3°C, inflammation >2 cm in diameter and temperature <38.3°C and inflammation > 2 cm and temperature >38.3 °C, respectively.

Table 2: Antibody Titre of Serogroups.
SD= Standard deviation, *: p value≤0.001 is significant

Centre	Antibody Titre (Mean ± SD)				*p* Value
	A	C	W-135	Y	
I	9.9±1.43	8.0±2.28	9.3±2.09	9.03±2.99	0.0036
II	10.4±1.80	8.1±1.75	9.5±1.61	9.3±2.09	0.0010*
III	10.1±2.19	8.6±1.71	9.4±2.28	8.7±2.09	0.0059

Statistical Analysis

The continuous data are presented as mean ± standard deviation (SD) and categorical in terms of percentage (%). One way ANOVA was used to compare the mean fold rise in titre between the groups. A*p value* ≤ 0.001was considered to be statistically significant.

RESULTS

Overall, vaccine had a good safety profile and was well tolerated in all the subjects. A total of 3 volunteers at Centre II and 7 of Centre III reported pain at the site of injection, which lasted for less than 48 hours. None of the subjects in the overall population developed erythema, inflammation, fever or any other AR. A significant rise in the antibody titre was observed for all the serogroups post vaccination (Table 2). Rise in Bactericidal Antibody Titre of Serogroups A, C, W-135 and Y)**.** The rise in antibody titres for serogroups A, C, Y and W-135 for Center I was [9.9±1.43, 8.0±2.28, 9.3±2.09 and 9.03±2.99; *p*=0.0036], respectively. For Center II and Center III the rise in antibody titre for serogroup A, C, W-135 and Y was [10.4±1.80, 8.1±1.75, 9.5±1.61 and, 9.3±2.09; *p*<0.001] and [10.1±2.19, 8.6±1.71, 9.4±2.28, and 8.7±2.09; *p*=0.0059], respectively. The rise in antibody titre for all the serogroups was almost similar in all the centers. Prior to vaccination, all the subjects showed less than 1:2 bactericidal antibody titre. It was observed that post vaccination, all the subjects (100%) showed ≥4 fold rise in bactericidal antibody titre for all the serogroups. A single subject from Centre I showed <4 fold rise in bactericidal antibody titre for serogroup Y. Only those subjects whose paired serum samples were available were tested for immunogenicity (Centre I-38, Centre II-41 and Centre III-40).

DISCUSSION

Meningococcal disease is an important cause of mortality worldwide (Shao *et al.,* 2009). The disease can be controlled by implementation of appropriate vaccination strategies at an early stage (Assaf and Dbaibo, 2016). The antimicrobial chemoprophylaxis serves as the conventional therapy for the meningococcal disease (Chung 2005). The antibiotics were effective (up to 2-4 weeks post treatment) for eradication of *N. meningitides*. However, in addition to the mild AEs associated with them; development of resistance against these antibiotics is a major cause of concern (Trestioreanu *et al.,* 2013).

Although antimicrobial chemoprophylaxis provides an immediate and effective measure for the prevention of the disease; immune prophylaxis is considered as a more appropriate method for the reduction of future risks of spread of the disease (Chung, 2005). Unlike

antibiotics, vaccines are effective for a longer duration and provide immunity thereby, decreasing the overall number of *N. meningitis* cases (Lipsitch *et al.,* 2016).

Currently, both polysaccharide and conjugated polysaccharide meningococcal vaccines are available for the prophylaxis of meningococcal disease. Polysaccharide vaccines are available in various forms, *viz.* bivalent (groups A and C), trivalent (groups A, C and W-135) or quadrivalent (groups A, C, Y and W-135) (Crum and Sullivan, 2016). Conjugated vaccines are the new generation of vaccines that are generated by chemical technologies and recombinant DNA technologies for covalently linking bacterial polysaccharides to proteins (Tagliabue and Rappuoli, 2018). Reverse vaccinology is another new approach using genomic sequencing to design vaccines that has resulted in a first important protein vaccine against meningococcus B that is now in use worldwide. (Serruto *et al.,* 2012)

Specifically, the effectiveness of polysaccharide vaccines against diseases such as meningococcal meningitis is limited because they do not stimulate a T cell response, which is required to activate long term immunological memory. Also, polysaccharide vaccines induce poor responses in infants. As the polysaccharide vaccine against meningitis A and C does not induce the production of immunological memory cells and is relatively ineffective in children aged under 2, it does not protect these vulnerable groups. However with conjugate vaccines, it involves attaching the required polysaccharide component of the bacteria against which the immune response is to be directed to a protein (Berger, 1998). The conjugated antigen induces a more powerful, T-cell-based immune response in infants, which is developed by the time they are 2 months of age. This response occurs at all ages (Goldblatt, 2000).

Vaccines containing purified polysaccharide antigens of serogroups A, C, Y, W-135 are available in various combinations for past30 years (Shao *et al.,* 2009). To predict the safety and acceptability of the tetravalent (A, C, Y, W-135) polysaccharide vaccines; Hankins *et al.* conducted a clinical trial and reported that the combined effect of this vaccine was well tolerated and clinically acceptable (Hankins *et al.,* 1982).The present study focused on the evaluation of the safety and immunogenicity of tetravalent meningococcal polysaccharide vaccine. A ≥4 fold (post vaccination) rise in serum bactericidal antibodies in Centre I (p=0.0022), Centre II (p<0.001) and Centre III (p=0.0059) was observed for all the serogroups. None of the subject had any serious AEs post vaccination; except mild pain at the site of injection.

A clinical trial conducted by Yadav *et al*., (2014) exhibited the safety and immunogenicity of the quadrivalent meningococcal diphtheria toxoid conjugate vaccine, on administrating a single dose of the vaccine in 300 subjects, the geometric mean titres (GMT) showed ≥8 fold rise for all the serogroups in most of the participants. Mild and short lived AEs were reported for few subjects. The findings of our study are consistent with the previous studies demonstrating the safety and immunogenicity of the tetravalent polysaccharide vaccine. Shao *et al.* (2009) observed that the polysaccharide vaccine was safe and immunogenic in adults and children aged 2-30 years and during the study, 39% of the subjects experienced mild pain at site of injection and fever. According to Chung, severe reactions towards the vaccine were rarely reported; only mild and transient AEs lasting for ≤ 2 days were observed post vaccination (Chung, 2005).

Similarly, Gabarro *et al.* (2007 conducted a case study during an epidemic in Burkina Faso and observed that the vaccine was 83.6% effective against serogroups A and W-135. They concluded that the trivalent (A, C and W-135) meningococcal polysaccharide vaccine was highly effective against the epidemic. Since

then, the vaccine is used in Africa to control the meningococcal outbreak (Soriano-Gabarró *et al.,* 2007). The conjugation of carrier proteins to the polysaccharide vaccines (conjugate vaccines) induces the T-cell dependent response (Dbaibo *et al.,* 2012).

Then on-conjugated tetravalent polysaccharide vaccines are effective in preventing the meningococcal disease however; they do not show a long lasting immunological memory (only lasts for 3-5 years). The immune response is more robust and longer lasting especially in children less than 2 years old. The conjugated vaccines are generally much more expensive than the non-conjugated types. Campbell *et al.,* (2002) reported that on administrating a single dose of quadrivalent (A, C, Y and W-135) diphtheria toxoid conjugate vaccine in 89 healthy individuals (18-55 years old), acceptable tolerance and high immunogenicity was exhibited against *N.meningitidis*, only two subjects reported fever and AEs such as local pain, chills, anorexia and malaise.

Though the polysaccharide meningococcal vaccines have some of its limitations, they are more affordable and can be used to control the disease outbreak in the absence of conjugate vaccines. Polysaccharide vaccines can be used as a routine vaccination for the individuals at higher risk of the disease(such as those in armed forces, training camps and travelers to epidemic areas) as well as for individuals suffering from inherited immunological deficiencies (Shao *et al.*, 2009).

CONCLUSION

Polysaccharide vaccines have strong potential to be used in outbreaks. The QuadriMeningo™ polysaccharide vaccine is safe in subjects 2-3 weeks post vaccination. The antibody titres of the sera from 100% of the subjects showed a fourfold or greater rise in antibody titre of each sero group (Group A, C, Y and W-135) after immunization. Overall vaccine showed good safety profile.

FUNDING SOURCES: The test vaccine was provided by M/s Biomed Private Limited

CONFLICT OF INTEREST: None

ACKNOWLEDGEMENTS: The author acknowledges Knowledge Isotopes Pvt. Ltd. (http://www.knowledgeisotopes.com) for the medical writing assistance.

REFERENCES

Assaf-Casals A, Dbaibo G (2016). Meningococcal quadrivalent tetanus toxoid conjugate vaccine (MenACWY-TT, Nimenrix™): A review of its immunogenicity,safety,co-administration, and antibody persistence. Hum Vaccin Immunother. 12: 1825-1837.

Berger A (1998). Science commentary: Why conjugate vaccines protect longer. BMJ. 316: 1571.

Campbell JD, Edelman R, King Jr JC, Papa T, Ryall R, Rennels MB (2002): Safety, reactogenicity and immunogenicity of a tetravalent meningococcal polysaccharide-diphtheria toxoid conjugate vaccine given to healthy adults. J Infect Dis. 186: 1848-1851.

Chung P (2005). Prevention of meningococcal disease. http://www.clevelandclinicmeded.com/medicalpubs/pharmacy/julyaug2005/default.htm. Accessed on: 22/11/2017

Crum-Cianflone N, Sullivan E (2016). Meningococcal vaccinations. Infect. Dis Ther. 5: 89-112.

Dbaibo G, Macalalad N, Reyes MR, Dimaano E, Bianco V, Baine Y,

Miller JM (2012). The immunogenicity and safety of an investigational meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine (ACWY-TT) compared with a lincensed meningococcal tetravalent polysaccharide vaccine: a randomized, controlled non-inferiority study. Hum Vaccin Immunother. 8: 873-880.

Ferguson LE, Hormann MD, Parks DK, Yetman RJ (2002). Neisseria meningitides: presentation, treatment and prevention. J Pediatr Health Care. 16: 119-124.

Goldblatt D (2000). Conjugate vaccines. Clin Exp Immunol. 119: 1-3.

Hankins WA, Gwaltney Jr JM, Hendley JO, Farquhar JD, Samuelson JS (1982). Clinical and serological evaluation of a meningococcal polysaccharide vaccine groups A,C, Y and W135. Proc Soc Exp Biol Med. 169: 54- 57.

John TJ, Gupta S, Chitkara AJ, Dutta AK, Borrow R (2013). An over view of meningococcal disease in India: knowledge gaps and potential solutions. Vaccine. 31: 2731-2737.

Lalwani S, Agarkhedkar S, Gogtay N, Palkar S, Agarkhedkar S, Thatte U, Vakil H, Jonnalagedda R, Pedotti P, Hoyle M, Bhusal C (2015). Safety and immunogenicity of an investigational meningococcal ACWY conjugate vaccine (MenACWY-CRM) in healthy Indian subjects aged 2 to 75 years. Int J Infect Dis. 38: 36-42.

Lipsitch M, Siber GR (2016). How can vaccines contribute to solving the antimicrobial resistance problem? MBio. 7: e00428-16.

MacNeil J,Cohn A (2011). Meningococcal disease. CDC Manual for the surveillance of vaccine-preventable diseases(5th ed) Center for Disease Control and Prevention, Atlanta GA.

Nair D, Dawar R, Deb M, Capoor MR, Singal S, Upadhayay DJ, Aggarwal P, Das B, Samantaray JC (2009). Outbreak of meningococcal disease in and around New Delhi, India, 2005–2006: a report from a tertiary care hospital. Epidemiol Infect. 137: 570-576.

Ngilneii C (2017). Medindia Report on Meningococcal Meningitis - Prevalence and Deaths in India. Available at URL: https://www.medindia.net/health_statistics/diseases/meningococcal-meningitis-india.asp

Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L (2010). Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. Antimicrob Agents Chemother. 54: 4851-4863

Pollard AJ, Scheifele D (2001). Meningococcal disease and vaccination in North America. J Paediatr Child Health. 37: 20-27.

Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM (2001). Meningococcal disease. N Eng J Med. 344: 1378-1388.

Serruto D, Bottomley MJ, Ram S, Giuliani MM, Rappuoli R (2012). The new multicomponent vaccine against meningococcal serogroup B, 4CMenB: immunological,

functional and structural characterization of the antigens. Vaccine. 30: B87-B97.

Shao PL, Chang LY, Hsieh SM, Chang SC, Pan SC, Lu CY, Hsieh YC, Lee CY, Dobbelaere K, Boutriau D, Tang H (2009).Safety and immunogenicity of a tetravalent polysaccharide vaccine against meningococcal disease. J Formos Med Assoc. 108: 539-547.

Soriano-Gabarró M, Toé L, Tiendrebeogo SR, Nelson CB, Dabal M, Djingarey MH, Plikaytis B, Rosenstein N (2007). Effectiveness of a trivalent serogroup A/C/W135 meningococcal polysaccharide vaccine in Burkina Faso, 2003. Vaccine. 25: A92-A96.

Sow SO, Okoko BJ, Diallo A, Viviani S, Borrow R, Carlone G, Tapia M, Akinsola AK, Arduin P, Findlow H, Elie C (2011). Immunogenicity and safety of a meningococcal A conjugate vaccine in Africans. N Eng J Med. 364: 2293-2304.

Tagliabue A, Rappuoli R (2018).Changing priorities in vaccinology: antibiotic resistance moving to the top. Front Immunol 9: 1068. Published online 2018 May 30. doi: 10.3389/fimmu.2018.01068

Yadav S, Manglani MV, Narayan DA, Sharma S, Ravish HS, Arora R, Castells VB, Arya S, Oster P (2014).Safety and immunogenicity of a quadrivalent meningococcal conjugate vaccine (MenACYW-DT): A multicenter, open-label, non-randomized, phase III clinical trial. Indian Pediatr. 51: 451-456.

Trestioreanu AZ, Fraser A, Gafter-Gvili A, Paul M, Leibovici L (2013). Antibiotics for preventing meningococcal infections. The Cochrane Library.