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Transplacental Transfer and Age-Related Levels of Serum IgG Antibodies to the Capsular Polysaccharides of *Streptococcus pneumoniae* Types 14 and 19 in Korea

Little is known about the prevalence of naturally acquired IgG antibodies to the capsular polysaccharides of *Streptococcus pneumoniae* (pneumococcal IgG) in Korea. In the present study, we investigated transplacental transfer and age-related levels of pneumococcal IgG to provide background seroepidemiologic data for *S. pneumoniae* in Korea. One hundred thirty eight sera were assayed by ELISA for IgG to pneumococcal polysaccharide capsular serotypes 14 and 19, the predominant serotypes for under 15 yr of age in Korea. The subjects were divided into 7 subgroups according to age. The cord/maternal geometric mean titer of pneumococcal were 4.47±5.88/5.21±5.88 for serotype 14, and 4.68±5.55/6.55±6.92 for serotype 19 (mean±standard deviation, μg/mL). After birth, the geometric mean titers of pneumococcal IgG for serotypes 14 and 19 expressed in μg/mL were 1.18±2.12 and 1.41±2.17 in the 0-6 months group, 0.27±0.19 and 0.69±0.93 in 7-12 months, 0.21±0.22 and 0.64±1.32 in 1-2 yr, 0.69±0.78 and 2.65±2.46 in 3-6 yr, 2.52±2.72 and 8.29±4.24 in 7-10 yr, respectively. In conclusion, reduced transplacental transfer and very low serum concentrations of pneumococcal IgG may contribute to the susceptibility of neonates, infants, and young children to *S. pneumoniae* infection.

Key Words : *IgG; Streptococcus pneumoniae; Polysaccharides; Enzyme-Linked Immunosorbent Assay; Child*

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INTRODUCTION

Even with the advent of antibiotics, *Streptococcus pneumoniae* infection persists as an important cause of morbidity and mortality of infants and children in all societies. With the introduction and wide spread usage of efficacious vaccines against the *Haemophilus influenzae* type b, another major cause of invasive infections in children, the relative importance of *S. pneumoniae* as a cause of invasive infections is obviously increasing. The annual incidence of invasive infections caused by *S. pneumoniae* ranges between 45.3-104 per 100,000 in children younger than 5 yr of age (1, 2).

The need for a vaccine to prevent pneumococcal disease in children has been emphasized by the fact that pneumonia, often caused by pneumococci, is a common cause of infant mortality in developing countries and by the emergence of pneumococcal strains resistant to antimicrobials in developed countries (3). Currently available pneumococcal vaccine consists of purified capsular polysaccharides of 23 *S. pneumoniae* serotypes. However, purified capsular polysaccharide vaccine is a poor immunogen in children, especially in regard to pneumococcal serotypes 6, 14, 19 and 23 commonly responsible for severe infections in children (4-6). This low efficacy is due to polysaccharide antigens' inability to stimulate T helper cells (7). To enhance immunogenicity and to improve vaccines for pediatric use, capsular polysaccharides have been conjugated to protein antigens. The first polysaccharide-protein conjugate vaccine, *H. influenzae* type b vaccine, has already been shown to be immunogenic and protective among infants (8, 9). Most recently pneumococcal conjugate vaccines have been undergoing clinical trials (3, 10-12).

Since it is likely that a pneumococcal conjugate vaccine will be available in the near future in Korea, seroepidemiologic data of *S. pneumoniae* will be needed to aid in planning the use of such a vaccine. In the present study we applied the ELISA method to investigate transplacental transfer and age-related levels of serum IgG antibodies to the capsular polysaccharides of *S. pneumoniae* types 14 and 19 (pneumococcal IgG) to provide background seroepidemiologic data for *S. pneumoniae* and to be served for the design of new vaccination program of pneumococcal conjugate vaccine in Korea.

MATERIALS AND METHODS

Serum specimens

Sera were obtained from nineteen mothers at full term delivery and from the umbilical vein of their healthy neonates. And one hundred infants and children aged 0 month to 10 yr were selected at random from the nursery and well baby clinic of Korea University Medical Center and an elementary school in Seoul, which is the capital city of Korea. The subjects were divided into 7 subgroups according to age, that is, mother (adult), cord, 0-6 months, 7-12 months, 1-2 yr, 3-6 yr, 7-10 yr of age (Table 1). We obtained 3 mL of blood from subjects and the blood samples were immediately centrifuged and stored at -70℃.

Reagents

Types 14 and 19 pneumococcal capsular polysaccharides (PPS) were purchased from the American Type Culture Collection (Rockville, MD, U.S.A.). Cell wall polysaccharide (CWPS) was obtained from American Cyanamid Company Lederle-Praxis Biological Division (West Henrietta, NY, U.S.A.). Alkaline phosphatase-conjugated antisera (affinity purified goat anti-human IgG) were purchased from Jackson Immunoresearch Labs, Inc. (West Grove, PA, U.S.A.). Pneumococcal standard was a mixture of 90 serum samples tested against the reference serum 89SF received from US Food and Drug Administration (Bethesda, MD, U.S.A.). After a minimum of 20 assays, an IgG value was assigned for each serotype to be tested.

ELISA

Microtiter plates (Polysorp; Nunc, Roskilde, Denmark) were coated with the PPS (ATCC, Rockville, MD, U.S.A.) in pyrogen-free PBS (14, 1 μg/mL; 19, 10 μg/mL) by incubating for 12 hr at 37℃. Plates were stored at 4℃.

Sera and pneumococcal standard were diluted 1:50 in PBS/0.05% Tween 20 containing 500 μg/mL CWPS to neutralize anti-CWPS antibodies. After 30 min of incubation with the CWPS at room temperature, three sequential dilutions were made in PBS/0.05% Tween 20 on the dilution plate.

After four washings of the microtiter plates with PBS/0.1% Tween 20, we transferred 50 mL of diluted pneumococcal standards and sera from dilution plate to the corresponding wells of each of the microtiter plates, which were incubated for 2 hr at room temperature. Alkaline phosphatase-conjugated antihuman IgG was diluted in PBS/0.05% Tween 20. Plates were washed four times as above, then conjugate was added to each well and plates were incubated for 2 hr at room temperature. *P*-nitrophenyl phosphate substrate (Sigma, St. Louis, MO, U.S.A.) was diluted in diethanolamine buffer and was added to each well and plates were incubated for 40 min at room temperature. After substrate incubation 3 N NaOH was added to each well and incubated for 5 min at room temperature.

Plates were read at 405 nm on an EIA reader (EL 311 SX microwell reader). Kinetical calculator program was used to calculate results. Pneumococcal IgG concentrations were calculated by comparing the titers of the pneumococcal standards. Results were given as microgram per milliliter calculated on the basis of the assigned IgG value of the 89 SF reference serum. We rejected the run if the correlation coefficient was less than 0.995. Coefficient of variation between assays was less than 25%.

Statistical methods

Statistical comparisons were made with Student t test or paired t test and correlation test. Results were given as geometric mean titers (GMTs), and proportions of children with $\geq$1 μg/mL antibody.

Table 1. Number of subjects

Groups	Male	Female	Total
Cord	9	10	19
0-6 month	12	8	20
7-12 month	18	2	20
1-2 yr	11	9	20
3-6 yr	14	6	20
7-10 yr	11	9	20
Mother (Adult)		19	19
Total	75	63	138

RESULTS

Transplacental transfer of pneumococcal IgG

The cord sera had lower levels of type 14 and 19 pneumococcal IgG concentrations than paired maternal sera at 4.47±5.88 vs. 5.21±5.88 and 4.68±5.55 vs. 6.55±6.92 μg/mL, respectively (Table 2, Fig. 1). But the difference was significant only for type 19 (p<0.001). The mean cord:maternal ratios of serum concentrations

Table 2. Geometric mean titers of pneumococcal IgG in cord and maternal blood

Serotype	Cord	Mother	Cord/Mother ratio
14	4.47±5.88*	5.21±5.83	0.78±0.42
19	4.68±5.55†	6.55±6.92†	0.64±0.22

*Antibody levels are in μg/mL; †$p<0.01$

of pneumococcal IgG were 0.78±0.42 for type 14 and 0.64±0.22 for type 19. There was significant correlation between pneumococcal IgG concentration of maternal serum and cord serum for both type 14 (r=0.85, $p<0.001$) and 19 (r=0.98, $p<0.001$) (Fig. 2).

Age-related levels of pneumococcal IgG

Geometric mean titers (GMTs) of pneumococcal IgG according to age for each of the pneumococcal serotype 14 and 19 are shown in Table 3 including those of cord and maternal serum (Table 3). From birth the GMTs of pneumococcal IgG decreased with age and reached a nadir between ages 1 and 2 yr. Thereafter they increased with ages.

According to age, the proportion of children with ≥1 μg/mL pneumococcal IgG showed a tendency similar to GMTs of pneumococcal IgG (Table 3). At a nadir between ages 1 and 2 yr there were no children with ≥1 μg/mL pneumococcal IgG to serotype 14 and only 10% of children with ≥1 μg/mL pneumococcal IgG to serotype 19.

There was significant correlation between age and concentration of pneumococcal IgG after birth for both type 14 (r=0.35, $p<0.001$) and type 19 (r=0.67, $p<0.001$) (Fig. 3).

DISCUSSION

Neonatal antibody concentrations reflect maternal antibody production and placental transport during gestation (13, 14). Maternal IgG transfer to the fetus across the placenta begins in 16th week of gestation and is

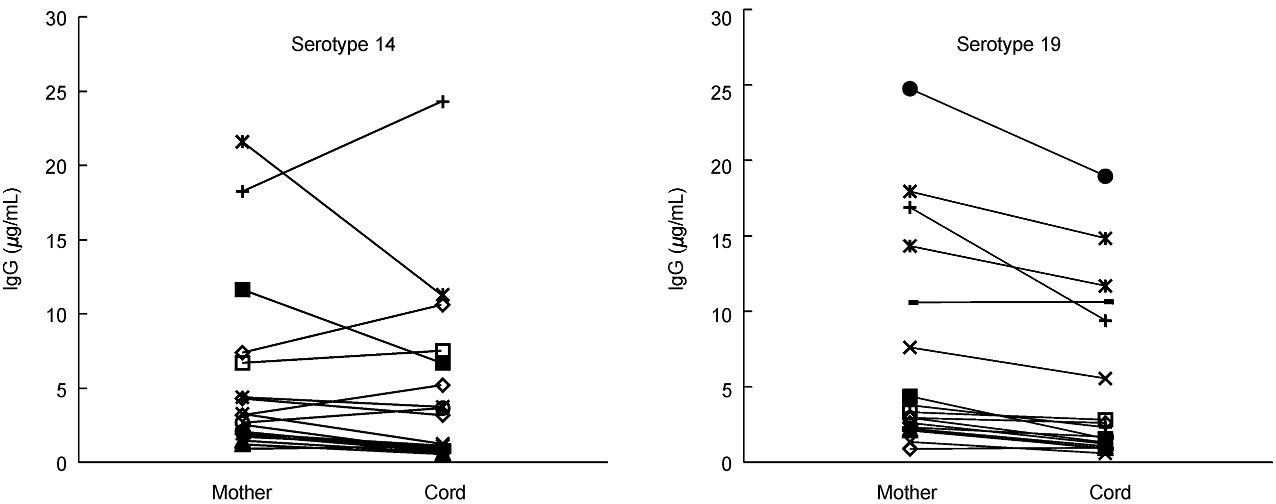

Fig. 1. Transplacental transfer of pneumococcal IgG to serotype 14 and serotype 19.

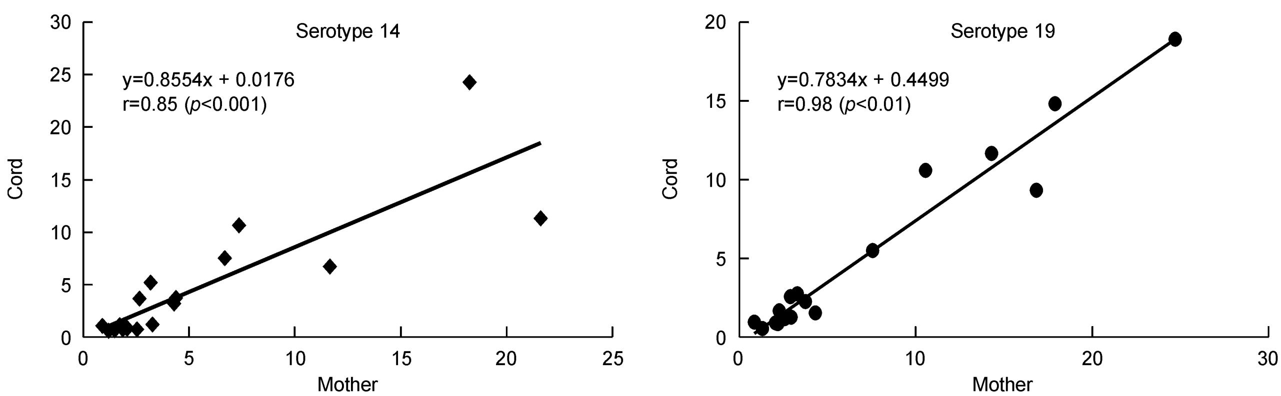

Fig. 2. Correlation between maternal and cord blood of pneumococcal IgG to serotype 14 and serotype 19.

Table 3. Geometric mean titers of pneumococcal IgG in groups

Groups	Serotype 14		Serotype 19	
	GMT*	% with ≥1.0 μg/mL	GMT	% with ≥1.0 μg/mL
Cord	4.47±5.88	68.4	4.68±5.55	73.7
0-6 month	1.18±2.12	20	1.41±2.17	35
7-12 month	0.27±0.19	0	0.69±0.93	20
1-2 yr	0.21±0.22	0	0.64±1.32	10
3-6 yr	0.69±0.78	10	2.65±2.46	65
7-10 yr	2.52±2.72	65	8.29±4.24	100
Mother	5.21±5.83	94.7	6.55±6.92	94.7

*GMT: geometric mean titers in μg/mL

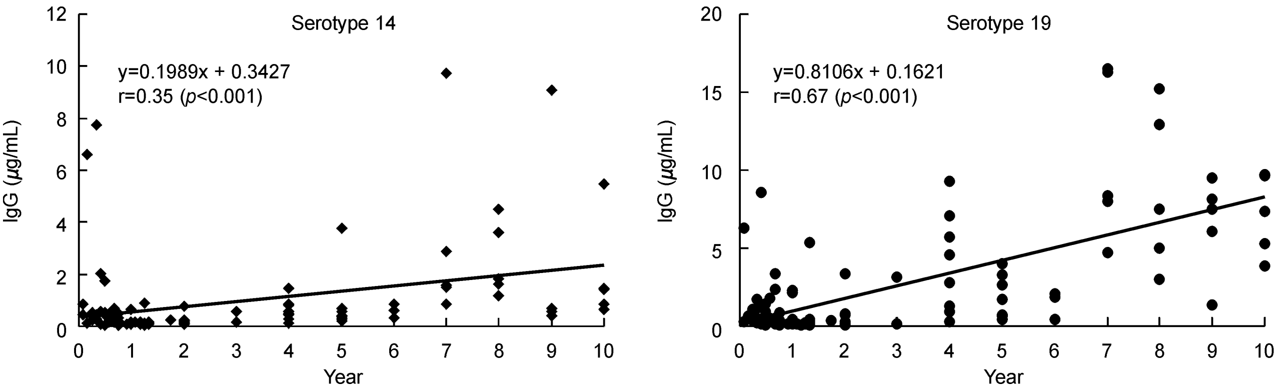

Fig. 3. Correlation between age and pneumococcal IgG to serotype 14 and serotype 19.

mainly completed during the last 6 weeks of gestation (15). As for specific IgG transfer, it is controversial whether the neonatal: maternal serum ratio of specific antibodies is higher, similar, or lower as compared with that of total antibodies (16-20). Sato et al. (16) showed that higher concentrations of neutralizing antibodies to measles, mumps, and rubella viruses were present in cord sera than in paired maternal sera. Our previous studies of measles and rubella antibodies have shown similar results (19-20). However, in the present study the cord serum had lower concentrations of specific antibodies to type 14 and 19 pneumococcal capsular polysaccharide than paired maternal serum. Our finding of reduced transfer of specific antibodies to pneumococcal capsular polysaccharides is consistent with data from others. Chudwin et al. (21) reported that the maternal-fetal transfer of total IgG was higher than the transfer of the 7F pneumococcal polysaccharide specific IgG. Antibody against pneumococcal capsular polysaccharide may be largely of the IgG_2 subclass. Our finding of reduced maternal-fetal transfer of pneumococcal capsular polysaccharide antibodies support the possibility of reduced transport of specific IgG_2 antibodies across the placenta (22, 23).

Mechanism of action for production of natural specific immunity is probably through antigenic stimulation by homologous or cross-reacting antigens. Nasopharyngeal carriage of pneumococci in childhood has been shown to be associated with acquisition of serum antibody (24) and cross-reactivity has been reported between pneumococcal capsular polysaccharide and surface antigens of *Klebsiella* species, *Escherichia coli*, and some strains of α-hemolytic and nonhemolytic streptococci (25, 26). Again the importance of age as a factor in immunity to pneumococci should be considered. Gwaltney et al. (24) noted that production of type-specific antibodies in response to nasopharyngeal colonization appeared to be strongly correlated with age. However, during the first 2 yr of life antibody production was unrelated to nasopharyngeal pneumococcal carriage. Therefore, anti-polysaccharide antibody titers reach a nadir between ages 6 and 18 months because of the disappearance of maternal antibody and the slow maturation of the infant's anti-polysaccharide antibody production (27). In the present study, there was a tendency for the GMTs of pneumococcal IgG to decrease with age after birth with a nadir reached between ages 1 and 2 yr.

In previous studies, it was demonstrated that pneumococcal antibody level steadily increased with age (6) and reached a plateau around the 10 yr of age (28). The reason for this phenomenon was also attributed to nasal carriage of pneumococci. Paton et al. (28) demonstrated that nasal carriage of a particular pneumococcal serotype correlates well with a significantly increased serum antibody level to that type. Loda et al. (29) reported that the pneumococcal carriage rate in white children in North Carolina remained steady at approximately 50% up to the age of 6 yr but decreased to 12% at 8 to 10 yr of age. Thus there is an approximate correlation between the age at which natural acquisition of type-specific antibody ceases and the age at which antigenic stimulation through carriage of pneumococci diminishes. Our data in this study supports the results published from previous studies. The GMTs of pneumococcal IgG to serotype 14 and 19 have shown the tendency to increase with age until the age group 7-10 yr in which the GMTs were not significantly differed from those of adults both for serotype 14 and 19.

The protective level of ELISA antibody levels is not known and it may, indeed, differ according to the pneumococcal serotypes and clinical entities. Serum concentrations of 1.0 μg/mL have been reported to be protective against pneumococcal otitis media in an animal model (30). Although the level of $\geq$1.0 μg/mL in this study and several others has not been demonstrated to be associated with protection, using 1.0 μg/mL as an arbitrary cutoff seems reasonable and conservative until newer data are available.

Pneumococcal conjugate vaccines have recently been produced based on the technology and experience from *H. influenzae* type b conjugate vaccines. A conjugate vaccine can probably not be a 23-valent because of logistical difficulties in the manufacturing process and the attendant high cost. Therefore, the most important types must be chosen for conjugation. The goal is to have a 7- to 10-valent vaccine containing the most frequent pediatric pneumococcal types, to cover the majority of invasive and noninvasive infections (31). Which leaves us with the difficult question of how to design a single pneumococcal conjugate vaccine suitable for pediatric use conferring protection against the 90 pneumococcal serotypes? The distribution of serotypes that cause invasive disease in children and otitis media differ according to the populations (32-36). Moreover, the differences in the serotypes of invasive disease isolates in developed and developing countries suggests that it may be necessary to formulate different vaccines for use in different populations. Unfortunately, epidemiological data for most parts of the world are insufficient. Epidemiological data on the occurrence and risk factors of these diseases are needed to aid in planning for use of conjugate pneumococcal vaccine. In Korea, Park et al. (37) reported that serotypes 6A, 6B, 14, 19F, and 23F comprised 67% of all isolates from pediatric patients and 82% of these serotypes were associated with significant resistance to current antibiotics therapy.

In conclusion, we have shown that reduced transplacental transfer and very low serum concentrations of IgG antibodies to the casular polysaccharide of *S. pneumoniae* types 14 and 19. To protect infants and young children from pneumococcal infection, the development and evaluation of pneumococcal conjugate vaccine in Korea is mandatory. Keeping in mind of possible future development of conjugate vaccines, further studies must explore nationwide epidemiological data on invasive pneumococcal infections in neonates, infants, and children in Korea.

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REFERENCES

1. Dagan R, Engelhard D, Piccard E. *Epidemiology of invasive childhood pneumococcal infections in Israel. The Israeli Pediatric Bacteremia and Meningitis Group. JAMA 1992; 268: 3328-32.*
2. Eskola J, Takala AK, Kela E, Pekkanen E, Kalliokoski R, Leinonen M. *Epidemiology of invasive pneumococcal infections in children in Finland. JAMA 1992; 268: 3323-7.*
3. Kähty H, Åman H, Rönberg PR, Tillikainen R, Eskola J. *Pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine is immunogenic in infants and children. J Infect Dis 1995; 172: 1273-8.*
4. Robbins JB, Austrian R, Lee CJ, Rastogi SC, Schiffman G, Henrichsen J, Mäkelä PH, Broome CV, Facklam RR, Tiesjema RH, Parke JC Jr. *Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. J Infect Dis 1983; 148: 1136-59.*
5. Leinonen M, Sakkinen A, Kalliokoski R, Luotonen J, Timonen M, Mäkelä PH. *Antibody response to 14-valent pneumococcal capsular polysaccharide vaccine in preschool age children. Pediatr Infect Dis J 1986; 5: 39-44.*
6. Douglas RM, Paton JC, Duncan SJ, Hansman DJ. *Antibody response to pneumococcal vaccination in children younger than five years of age. J Infect Dis 1983; 148: 131-7.*
7. Stein KE. *Thymus-independent and thymus-dependent responses to polysaccharide antigens. J Infect Dis 1992; 165 (Suppl 1): S49-52.*

8. Breinman RF, Spika JS, Navarro VJ, Darden PM, Darby CP. *Pneumococcal bacteremia in Charleston County, South Carolina: a decade later. Arch Intern Med 1990; 150: 1401-5.*
9. Musfun MA, Oley G, Hughey D. *Pneumococcal disease in a medium-sized community in the United States. JAMA 1982; 248: 1486-9.*
10. Istre GR, Tarpay M, Anderson M, Pryor A, Welch D. *Invasive disease due to Streptococcus pneumoniae in an area with a high rate of relative penicillin resistance. J Infect Dis 1987; 156: 732-5.*
11. Burman LA, Norrby R, Trollfors B. *Invasive pneumococcal infections: incidence, predisposing factors, and prognosis. Rev Infect Dis 1985; 7: 133-42.*
12. King JC Jr, Vink PE, Farley JJ, Smilie M, Parks M, Lichtenstein R. *Safety and immunogenecity of three doses of a five-valent pneumococcal conjugate vaccine in children younger than two years with and without human immunodeficiency virus infection. Pediatrics 1997; 99: 575-80.*
13. Salazar JC, Daly KA, Giebink GS, Lindgren BR, Liebeler CL, Meland M, Le CT. *Low cord blood pneumococcal immunoglobulin G (IgG) antibodies predict early onset acute otitis media in infancy. Am J Epidemiol 1997; 145: 1048-56.*
14. Oxelius VA, Svenningsen NW. *IgG subclass concentrations in preterm neonates. Acta Paediatr Scand 1984; 73: 626-30.*
15. Stiehm ER. *Fetal defence mechanisms. Am J Dis Child 1975; 129: 438-43.*
16. Sato H, Albrecht P, Reynolds DW, Stango S, Ennis FA. *Transfer of measles, mumps, and rubella antibodies from mother to infant. Its effect on measles, mumps, and rubella immunization. Am J Dis Child 1979; 133: 1240-3.*
17. Einhorn MS, Granoff DM, Nahm MH, Quinn A, Shackleford PG. *Concentrations of antibodies in paired maternal and infant sera:relationship to IgG subclass. J Pediatr 1987; 111: 783-88.*
18. Baker CJ, Webb BJ, Kasper DL, Yow MD, Beachler CW. *The natural history of group B streptococcal colonization in the pregnant woman and her offspring. Am J Obstet Gynecol 1980; 137: 39-42.*
19. Cheon HW, Shin YK, Choung JT, Tockgo YC. *Rubella-specific IgG in children and maternal sera. Korea Univ Med J 1998; 35: 13-26.*
20. Park HY, Shin YK, Choung JT, Tockgo YC. *Measles specific IgG antibody level of term pregnant women and neonatal umbilical cords. J Korean Pediatr Soc 1996; 39: 1224-30.*
21. Chudwin DS, Wara DW, Schiffman G, Artrip SG, Ammann AJ. *Maternal-fetal transfer of pneumococcal capsular polysaccharide antibodies. Am J Dis Child 1985; 139: 378-80.*
22. Wang AC, Faulk WP, Stuckey MA, Fudenberg HH. *Chemical differences of adult, fetal and hypogammaglobulinemic IgG immunoglobulins. Immunochemistry 1970; 7: 703-8.*
23. Hay FC, Hull MG, Torrigiani G. *The transfer of human IgG subclasses from mother to foetus. Clin Exp Immunol 1971; 9: 355-8.*
24. Gwaltney JM Jr, Sande MA, Austrian R, Hendley JO. *Spread of Streptococcus pneumoniae in families. II. Relation of transfer of S. pneumoniae to incidence of colds and serum antibody. J Infect Dis 1975; 132: 62-8.*
25. Robbins JB. *Vaccines for the prevention of encapsulated bacterial diseases: current status, problems and prospects for the future. Immunochemistry 1978; 15: 839-54.*
26. Yurchak AM, Austrian R. *Serologic and genetic relationships between pneumococci and other respiratory streptococci. Trans Assoc Am Physicians 1966; 79: 368-75.*
27. Gray BM, Converse GM III, Huhta N, Jonhston RB Jr, Pichichero ME, Schiffman G, Dillon HC Jr. *Epidemiologic studies of Streptococcus pneumoniae in infants: antibody response to nasopharyngeal carriage of types 3, 19, and 23. J Infect Dis 1981; 144: 312-8.*
28. Paton JC, Toogood IR, Cockington RA, Hansman D. *Antibody response to pneumococcal vaccine in children aged 5 to 15 years. Am J Dis Child 1986; 140: 135-8.*
29. Loda FA, Collier AM, Glezen WP, Strangert K, Clyde WA Jr, Denny FW. *Occurrence of Diplococcus pneumoniae in the upper respiratory tract of children. J Pediatr 1975; 87: 1087-93.*
30. Giebink GS, Koskela M, Vella PP, Harris M, Le CT. *Pneumococcal capsular polysaccharide-meningococcal outer membrane protein complex conjugate vaccines: immunogenicity and efficacy in experimental pneumococcal otitis media. J Infect Dis 1993; 167: 347-55.*
31. Dagan R, Muallem M, Melamed R, Leroy O, Yagupsky P. *Reduction of pneumococcal nasopharyngeal carriage in early infancy after immunization with tetravalent pneumococcal vaccines conjugated to either tetanus toxoid or diphtheria toxoid. Pediatr Infect Dis J 1997; 16: 1060-4.*
32. Shapiro ED, Austrian R. *Serotypes responsible for invasive Streptococcus pneumoniae infections among children in Connecticut. J Infect Dis 1994; 169: 212-4.*
33. Parkinson AJ, Davidson M, Fitzgerald MA, Bulkow LR, Parks DJ. *Serotype distribution and antimicrobial resistance patterns of invasive isolates of Streptococcus pneumoniae: Alaska 1986-1990. J Infect Dis 1994; 170: 461-4.*
34. Verhaegen J, Glupczynski Y, Verbist L, Blogie M, Verbiest N, Vandeven J, Yourassowsky E. *Capsular types and antibiotic susceptibility of pneumococci isolated from patients in Belgium with serious infections, 1980-1993. Clin Infect Dis 1995; 20: 1339-45.*
35. Orange M, Gray BM. *Pneumococcal serotypes causing disease in children in Alabama. Pediatr Infect Dis J 1993; 12: 244-6.*
36. Block SL, Harrison CJ, Hedrick JA, Tyler RD, Smith RA, Keegan E, Chartrand SA. *Penicillin-resistant Streptococcus pneumoniae in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. Pediatr Infect Dis J 1995; 14: 751-9.*
37. Park JY, Kim MR, Jang SH, Lee HJ, Choi KW, Kim EJ, Kim JH. *Serotypes and antimicrobial susceptibilities of Streptococcus pneumoniae isolated from clinical specimen. Korean J Infect Dis 1994; 26: 9-19.*