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Research Article / Araştırma Makalesi

The Prevalence and Serogroup Analysis of Nasopharyngeal Meningococcal Carriage in Turkish Citizens Living in Belgium, Germany, and the Netherlands During Their Visits to Turkey

Belçika, Almanya ve Hollanda'da Yaşayan Türk Vatandaşlarının Türkiye'ye Seyahatleri Sırasında Nazofaringeal Meningokokkal Taşıyıcılık Prevalansı ve Serogrup Dağılımı

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Abstract: Invasive meningococcal disease (IMD) is among the leading causes of morbidity and mortality worldwide. Epidemiological studies in Turkey have shown that serogroups B and W are the most prevalent serogroups associated with IMD cases and meningococcal carriage. Many Turkish citizens who live in Europe visit their homeland of Turkey every year. However, no data is available on meningococcal carriage in this population. In addition, the effects of the presence of these citizens on meningococcal epidemiology in Turkey are unknown. This study aimed to examine the presence of *Neisseria meningitidis* carriage and serogroup distribution in Turkish citizens who live in Germany, Belgium, and the Netherlands and have visited Turkey. The study sample comprised 361 volunteers aged 2–85 who lived in Germany, Belgium, and the Netherlands and arrived at the Eskisehir airport between June 1 and August 22, 2016. A polymerase chain reaction assay for *N. meningitidis* was performed on the nasopharyngeal samples of these participants. Of the 361 volunteers, the nasopharyngeal meningococcal carriage rate was 0.6% (two subjects from Belgium). Serogroup X was isolated in a 46-year-old man, and non-groupable *N. meningitidis* was isolated in an 18-year-old girl. Previous studies showed that serogroup W is the one of the predominant strain in Turkey, was associated with having participated in the Hajj and the Umrah, have been controlled with routine immunization before travel. In contrast, the meningococcal carriage rate was low among Turkish citizens arriving each year from European countries; therefore, these visitors had no effect on meningococcal seroepidemiology in Turkey.
Keywords: *Neisseria meningitidis*, meningococci, meningococcal disease, carriage, Turkey

Özet: İnvaziv meningokok enfeksiyonları, tüm dünyada önemli morbidite ve mortalite nedenleri arasında yer almaktadır. Türkiye'de yapılan epidemiyolojik çalışmalarda, invaziv meningokok enfeksiyonu ve taşıyıcılık için en sık görülen serogrupların B ve W olduğu gösterilmiştir. Avrupa'da yaşayan birçok Türk vatandaşı her yıl Türkiye'yi ziyarete gelmektedir. Ancak bu kişilerde meningokok taşıyıcılığı ile ilgili çalışma bulunmamaktadır. Buna ek olarak, bu taşıyıcılığın, Türkiye'de meningokok epidemiyolojisi üzerine etkisi ile ilgili de veri bulunmamaktadır. Bu çalışmada, Almanya, Belçika ve Hollanda'da yaşayan ve Türkiye'ye ziyarete gelenlerde, *Neisseria meningitidis* taşıyıcılığı ve serogrup dağılımının değerlendirilmesi planlandı. Çalışmaya 1 Haziran- 22 Ağustos 2016 tarihleri arasında Almanya, Belçika ve Hollanda'dan Eskişehir Havalimanı'na gelen 2-85 yaş arasında 361 gönüllü dahil edildi. Nazofaringeal örneklerde *N. meningitidis* polimeraz zincir reaksiyonu ile değerlendirildi. Çalışmaya dahil edilen 361 gönüllüde, meningokok taşıyıcılığı %0.6 (Belçika'dan gelen iki olguda) olarak saptandı. 46 yaşında bir erkekte serogrup X, 18 yaşında bir kadında da gruplandırılamayan *N. meningitidis* saptandı. Daha önceki çalışmalarda serogrup W, Türkiye'de en sık saptanan etkenlerden biri olup, Hac ve Umre ile ilişkili olduğu düşünülmüştür ve seyahat öncesi rutin aşılama ile kontrol altına alınmıştır. Bunun aksine Avrupa ülkelerinden her yıl Türkiye'ye çok sayıda Türk vatandaşı gelmesine rağmen, bu çalışmada bu ziyaretçilerin Türkiye'de meningokok seroepidemiyolojisine etkisi saptanmamıştır.
Anahtar Kelimeler: Neisseria meningitidis, meningokok, meningokok hastalığı, taşıyıcılık, Türkiye

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1. Introduction

Invasive meningococcal disease (IMD) results from infection with *Neisseria meningitidis* and is associated with high fatality rates and, in 10–20% of cases, long-term sequelae, including neurologic complications, loss of limbs, and hearing loss (1–2). Each year, 1.2 million new meningococcal infection cases occur globally, 135,000 of which result in death (3). Twelve serogroups of *N. meningitidis* have been identified on the basis of their polysaccharide structures. However, the majority of IMD cases are attributed to serogroups A, B, C, W, X, and Y. Serogroup distribution is unpredictable and depends on geographical location (1–5). Adequate surveillance of meningococcal infections is the key element in maintaining accurate epidemiological data and developing potential vaccine prevention strategies (1).

IMD is transmitted by respiratory droplets. IMD infection results from exposure to the respiratory secretions of infected patients or asymptomatic nasopharyngeal carriers. Therefore, close respiratory contact, particularly with household members, increases the risk of contracting the disease. Nationwide meningococcal carriage rates make up one of the main indicators used to determine the epidemiology of IMD. Meningococcal carriage rates are typically highest in adolescents and young adults (1, 2, 6, 7). Other factors associated with nasopharyngeal carriage include prior bacterial or viral upper respiratory infections, smoking, low socioeconomic status, and visits to crowded places (2, 6, 7). Participation in the Hajj or the Umrah in Saudi Arabia is also associated with an increased risk of meningococcal carriage, particularly carriage of serogroup W (8).

The incidence of IMD has decreased over the years. However, *N. meningitidis* has been the primary cause of acute bacterial meningitis in children since the onset of the widespread use of conjugated pneumococcal and *Haemophilus influenzae* type b vaccines (9–12). In Turkey, *N. meningitidis* is the leading cause of bacterial meningitis; however, the serogroup distributions of IMD are dynamic and unpredictable and differ between neighboring countries (4, 5). Serogroups W and B are the major causes of IMD in Turkey, but serogroups A, Y, and X have also been observed in this country (9–11,13). Since 2005, there have been no cases of meningococcal serogroup C in Turkey, even in the absence of the routine use of the meningococcal conjugated C vaccine (5, 11). Serogroup W has been important serogroup of IMD in Turkey since 2005, and a major route for the transfer of serogroup W to Turkey is attendance to the Hajj (4, 5, 8, 11).

Determination of carriage rates and serogroup distributions is particularly important for the development of immunization programs and other strategies to combat diseases and epidemic conditions. Many Turkish citizens have been living and working in Europe, mainly Germany, the Netherlands and Belgium, for many years. These citizens visit Turkey every year, especially during the summer. No data is available on meningococcal carriage by Turkish citizens living abroad; therefore, the effects of these groups on meningococcal carriage in Turkey are unknown. This study aimed to evaluate, regardless of age and gender, the carriage rates and serogroup distributions of *N. meningitidis* in Turkish citizens living in Germany, Belgium, and the Netherlands who visited Turkey in the summer.

2. Materials and Methods

The present study involved the evaluation of the nasopharyngeal carriage rate of Turkish citizens living in Germany, Belgium, and the Netherlands who had visited Turkey. According to the official records, 13281 Turkish citizens from Belgium, 900 from the Netherlands, and 78 from Germany arrived at Eskisehir airport in 2015. Ethical approval for this study was obtained from the Local Ethics Committee of Eskisehir Osmangazi University (December, 2015/6). To conduct this study at the airport, necessary permissions were received from the city's legal office. This study received financial support from the Scientific Research Project of Eskisehir Osmangazi University. Informed consent Informed consent was obtained from each participant and/or a parent or legal guardian for every individual participant included to this study.

The present study enrolled Turkish citizens of all ages from Germany, Belgium, and the Netherlands who were visiting Turkey during the summer period. Data concerning age, gender, country of residence, and previous years of participation in the Hajj or the Umrah were recorded for each participant. Nasopharyngeal samples were obtained from incoming passengers either upon arrival at the airport or within three days of entry into the country. These samples were then placed in a transport medium and sent to the laboratory of the Department of Pediatric Infectious Diseases at Hacettepe University. This laboratory performed DNA isolation on each specimen, assessed the presence of *N. meningitidis*, and performed serogroup analyses on all the positive samples.

Laboratory analysis: The reagents and cartridges of Fujifilm QuickGene DNA Tissue Kit S (Fujifilm Wako Pure Chemical Corp., Tokyo, Japan) and the QuickGene Mini80™ Nucleic Acid isolation tool (Autogen/FujiFilm, Holliston, MA, USA) were used to isolate nucleic acids from the nasopharyngeal swab samples. A polymerase chain reaction (PCR) analysis was performed to identify the bacterial agent. CtrA was the target gene for *N. meningitidis*. Definition of *N. meningitidis* and serogroup analyses were performed according to the protocol used in our previous nasopharyngeal meningococcal carriage study (14).

Statistical analysis: SPSS for Windows, Version 16.0 software (SPSS, Inc., Chicago, IL) was used to analyze the statistical frequencies of factors such as meningococcal infections, vaccination, Hajj pilgrimage, and gender. A p value < 0.05 was considered significant.

3. Results

The present study enrolled 361 volunteers (197 males, 164 females) aged between 2 and 85 years. The volunteers arrived in Eskisehir between June 1 and August 22, 2016. Of the 361 participants, 251 were from Belgium (133 males and 118 females), 93 were from Germany (58 males and 35 females), and 17 were from the Netherlands (6 males and 11 females). The mean age was 46.9 ± 19.1 years.

The participants were divided into five groups on the basis of age:

- Group I: 21 participants (15 boys and 6 girls) aged 0–14 years
- Group II: 38 participants (21 males and 17 females) aged 15–24 years
- Group III: 98 participants (41 males and 47 females) aged 15–44 years
- Group IV: 140 participants (75 males and 65 females) aged 45–64 years
- Group V: 67 participants (45 males and 22 females) aged >65 years.

Forty-four (12.2%) participants (all above 45 years of age) had previously visited Saudi Arabia for the Hajj and/or the Umrah. A total of 113 participants (31.3%) had previously received plain polysaccharide or conjugated meningococcal vaccines. The immunization rates by age group were as follows: Group I 90.4% (19/21); Group II 71.0% (27/38); Group III 24.2% (23/95); Group IV 12.1% (17/140); and Group 1: 40.2% (27/67). Of the participants with histories of participation in the Hajj or the Umrah, 44 (n = 17, 4.7% in Group IV; n = 27, 7.4% in Group V) had been immunized with the quadrivalent meningococcal polysaccharide vaccine. The remaining 69 participants also had histories of immunization with the monovalent meningococcal C conjugate vaccine and/or the quadrivalent meningococcal conjugate vaccine.

The overall meningococcal carriage rate found in this study was 0.6% (2/361). These two cases, which were from Groups II and IV, corresponded to carriage rates of 2.6% in Group II and 0.7% in Group IV. Both of these two participants were from Belgium. During the serogroup analysis, a non-groupable serogroup was isolated in an 18-year-old female participant who had previously been immunized with a meningococcal C vaccine during her childhood. Additionally, serogroup X was isolated in a 46-year-old male (with no previous history of participation in the Hajj or the Umrah) who had not been previously immunized.

4. Discussion

Previous studies have found participation in the Hajj and the Umrah to be associated with the prevalence and serogroup distribution of *N. meningitidis* in Turkey, particularly for serogroup W (4, 5, 8). Each year, especially in the summer, the majority of Turkish citizens living in Europe (mainly Germany and Belgium) visit their homeland of Turkey. According to the records of the Turkish Ministry of Tourism, more than five million people from Germany, one million people from the Netherlands, and six hundred thousand people from Belgium visited Turkey as tourists in 2015 (15). Meningococcal carriage has not been previously studied in these groups. The determination of meningococcal carriage rates and distributions of serogroups within this population, as well as their effects on meningococcal carriage in Turkey, is of great importance to vaccination programs.

The present study evaluated meningococcal carriage in 361 participants who visited Turkey in the summer of 2016 from Germany, Belgium, and the Netherlands. A meningococcal carriage rate of 0.6% (two subjects from Belgium) was found in this sample. In 2014; 2,760 confirmed cases of IMD were reported in the European region according to the European Centre for the Disease Prevention and Control. Majority of the patients with IMD are infants and children below five years old, and the majority of IMD cases have been caused by serogroup B, followed by serogroup C). The incidence of IMD has been found to be per 100,000 individulas, 0.8 cases in Belgium, 0.4 cases in Germany, and 0.5 cases in the Netherlands (16).

Nasopharyngeal meningococcal carriage is important because of its significant role in the development of IMD. The meningococcal carriage rate is 5–10% in general population and varies depending on age (6–7). Studies in Europe and North America have indicated meningococcal carriage rates of 3% in children aged four years and below and rates of 24–37% in young adults (17). Meningococcal carriage rates in other countries have been reported as follows: 9.9% in the Czech Republic, 10.6% in Greece, 6.2% in

Nigeria, 9.6% in Norway, 2.7% in Morocco, and 1.7% in Sudan (14). In a 2015 study of 1,518 adolescents and young adults aged 10–24 years in Turkey, Tekin et al. (14) found a meningococcal carriage rate of 6%, with serogroup W being the most prevalent. A 2016 study in Istanbul (18) found an N. *meningitidis* carriage rate of 0.6% among 1,000 subjects aged 0–79 years. The present study found a meningococcal carriage rate of 0.6% among Turkish citizens living in Belgium, Germany, and the Netherlands.

Carriage rates, such as the incidence of invasive disease, tend to vary with age. In Europe and North America, meningococcal carriage rates increase rapidly during adolescence and young adulthood but are very low during the first years of life (6, 7, 17). A 2011 review of 27 European countries found that the carriage rate of *N. meningitidis* increased during childhood and during the age range of 15–24 years, even though it varied by country (19). The meningococcal carriage rate found in the present study was lower than those found in other countries. Similar to other invasive diseases, meningococcal carriage rates and related serogroups vary geographically.

An ongoing study conducted by Ceyhan and colleagues over a period of 12 years (since 2005) in Turkey showed that *N. meningitidis* was the most commonly isolated agent in pediatric bacterial meningitis, with serogroups B and W being the most prevalent (8–11). No infections related to serogroup C, which is very common in many European countries, were detected in any of the participants between 2005 and 2017 (8–11). Further, Tekin et al. (14) did not detect serogroup C in a nasopharyngeal carriage study of Turkish 10–24-year-olds. In the present study, serogrup C was not detected. A non-groupable serogroup was isolated in an 18-year-old participant while serogroup X was isolated in a 46-year-old participant. Serogroup X has been sporadically reported in the European region; however, serogroup X is an emerging cause of IMD in sub-Saharan Africa, where it has been responsible for many outbreaks (20).

One limitation of the present study was that the volunteer sample was obtained from a population of differing age groups, mainly from middle-aged and elderly adults. The number of children and adolescents, in whom meningococcal carriage is most common, was limited. No analyses of genetic strain characteristics or lineages were performed on the isolates.

The present study revealed that Turkish citizens arriving in Turkey from European countries had a low meningococcal carriage rate. Serogroup C was not detected in this group, possibly due to the high rate and long history of serogroup C-related monovalent conjugate vaccines in their countries of residence. In Turkey, the majority of pediatricians and infectious disease specialists reported believing that meningococcal vaccines should be a part of the National Immunization Program, especially for healthy children as well as groups at risk of developing IMD (21). In summary, the development of vaccine strategies for meningococcal infections, ongoing surveillance studies, and strategies to monitor travelers and other high-risk groups are important in preventing the spread of IMD both in Turkey and worldwide.

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