Prevalence of *sea, seb, sec, sed, and tsst-1* genes of *Staphylococcus aureus* in nasal carriage and their association with multiple sclerosis

Zahra Pakbaz[1], Mohammad Ali Sahraian[2], Samira Sabzi[3], Mahmood Mahmoodi[4], Mohammad Reza Pourmand[5,]*

Abstract

Background **Microbial superantigens might initiate or exacerbate autoimmune responses against particular tissues, organs or systems. This study aimed to examine the prevalence of *sea, seb, sec, sed,* and *tsst-1* genes of *Staphylococcus aureus* in nasal carriage and their association with multiple sclerosis (MS).**

Methods **Nasal swabs were collected from 150 MS patients and 150 healthy individuals (control group) to isolate *S. aureus* and investigate their superantigen genes (*sea, seb, sec, sed* and *tsst-1*) using PCR.**

Results **A total of 300 participants were enrolled in the study, matched for age and gender (150 patients in the MS group and 150 in the control group). The prevalence of *S. aureus* colonization in MS patients and control groups was 42% and 23.3%, respectively. There was a statistically significant association between *S. aureus* colonization and MS disease ($p<0.001$; odds ratio 2.4; 95% confidence interval 1.4-3.9). No significant association was observed between the presence of *S. aureus* harboring *sea, seb, sec, sed* and *tsst-1* genes with MS disease.**

Conclusion **The rate of *S. aureus* nasal carriage is higher in patients with MS. Our study's results suggest that further investigation into whether there is a connection between MS and nasal exposure to staphylococcal superantigens is warranted.**

Keywords **Multiple sclerosis, *Staphylococcus aureus*, superantigen, nasal carriage.**

Introduction

Multiple sclerosis (MS) is an inflammatory autoimmune disease characterized by demyelination of the central nervous system (CNS).[1] The demyelination process is associated with infiltrating T cells specific for major myelin proteins into the CNS. The disease has a peak onset between 20 and 40 years old and affects women approximately twice as often as men.[2-4] The relapsing-remitting MS (RR-MS) form of disease is the most frequent one, involving about 85% to 90% of all the cases. This form of the disease is characterized by defined attacks, followed by partial or complete recovery.[5,6]

The causes of MS remain unknown, but evidence indicates that it is a complex and multifactorial neurodegenerative disease. A combination of genetic predisposition and environmental factors, potentially including microbial agents, appears to be responsible.[7-9] The possible ways whereby pathogens can initiate or perpetuate autoimmunity, are the activation and clonal expansion of autoreactive T lymphocytes. The autoimmune molecular mimicry of bacterial

Received: 21 February 2017; revised: 15 August 2017 and 12 October 2017; accepted: 22 October 2017.

[1]PhD, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Poursina St., Tehran, Iran; [2]MD, MS Research Center, Neuroscience Institute, Neurology Department, Tehran University of Medical Science, Sina Hospital, Hasan Abad Sq., Tehran, Iran; [3]MSc, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Poursina St., Tehran, Iran; [4]PhD, Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Poursina St., Tehran, Iran; [5]PhD, Department of Pathobiology, School of Public Health, and Biotechnology Research Center, Tehran University of Medical Sciences, Poursina St., Tehran, Iran.

*Corresponding author: Mohammad Reza Pourmand, PhD, Department of Pathobiology, School of Public Health, and Biotechnology Research Center, Tehran University of Medical Sciences, PourSina St., Tehran, Iran. mpourmand@tums.ac.ir

Published December 2017

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

or viral antigens is one of the important mechanisms that represent an explanation for the activation of autoreactive T cells in MS.[10,11] However, the superantigens (SAgs), because of their strong T cell mitogenic activity, can initiate or exacerbate autoimmune responses against antigens of the central nervous system. SAgs bind to a wide variety of major histocompatibility complex class II (MHC-II) molecules on antigen-presenting cells and T cell receptors (TCR) on T cells. This binding leads to massive polyclonal activation of as much as 20% of the helper T cell repertoire. However, the conventional peptide antigens bind only 1 in 10^5-10^6 naive T cells.[12,13]

It has been suggested that *S. aureus* carriage is associated with various autoimmune diseases including Wegener's granulomatosis, lupus erythematosus and rheumatoid arthritis through SAgs such as staphylococcal enterotoxins (SEs) or toxic shock syndrome toxin-1 (tsst-1).[14-16] About 20–30% of the normal human population are asymptomatic carriers of *S. aureus* in the anterior nares.[17] More than 20 SAgs have been identified in *S. aureus* strains. Reports show that at least 80% of the clinical strains of *S. aureus* harbor a minimum of one SAg.[18] The SAg can be efficiently absorbed from nasal mucosa, either directly or facilitated by other exotoxins, such as cytolysins.[19] Recurrent or chronic systemic exposure to an extremely small amount of SAg in *S. aureus* carriers can also cause absorption of the SAg. The absorption of the SAg can lead to activation of the autoreactive T and B-lymphocytes in genetically predisposed individuals. The activation of such autoreactive cells under appropriate conditions can precipitate an autoimmune disease.[18-20] This study aims to identify the prevalence of *staphylococcal enterotoxin A* (*sea*), *seb, sec, sed,* and *tsst-1* genes of S. *aureus* in nasal carriage and their association with multiple sclerosis.

Methods

Study population

This study was carried out between March 2015 and March 2016 in the MS Research Center, Tehran University of Medical Sciences (TUMS), Iran. Ethical approval for this study was obtained from TUMS ethics committee (Approval number: IR.TUMS.REC.1394.2194). A total of 150 MS patients who had the diagnosis of RR-MS and 150 healthy individuals as a control group, were included in the study. The control group was roughly matched with the patients in age and sex. None of the patients and healthy individuals had evidence of infection or active respiratory disease before sampling.

Sample processing and bacteriological methods

Samples were collected from anterior nares using sterile cotton swab. The nasal swabs were inoculated onto blood agar and incubated at 37°C overnight and they were examined for growth. *S. aureus* was identified by standard methods, colony morphology, Gram staining, catalase test, fermentation of mannitol, DNase enzyme production and slide and tube coagulase test.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed for each *S. aureus* isolate by the disc diffusion method against cefoxitin, gentamicin, erythromycin, clindamycin, ciprofloxacin, co-trimoxazole, chloramphenicol, rifampin, doxycycline, quinupristin dalfopristin, linezolid and mupirocin. Vancomycin susceptibility testing was also performed by the microdilution method using the Clinical and Laboratory Standards Institute (CLSI) guidelines (2015).

PCR assay for *sea, seb, sec, sed* and *tsst-1* genes

The DNA was extracted from culture isolates of *S. aureus* by Bioneer kit (Daejeon, Korea). The primers specific to *sea, seb, sec, sed* and *tsst-1* genes were designed and multiple alignments were carried out. The sequence of primers is listed in Table 1.

According to the kit instruction (PCR Master Mix Kit, SinaClon, Tehran, Iran), each PCR procedure was performed with 5 µL DNA, 12.5 µL 2x master mix, 20 pmol each of the forward and reverse primers) in 25 µL of final volume. The PCR products were analyzed by 1% agarose

gel electrophoresis with KBC staining (0.5 µg/mL) (Kowsar, Tehran, Iran).

Table 1. Base sequences and sizes of PCR products for the *sea, seb, sec, sed* and *tsst-1* genes.

Gene	Oligonucleotide sequence (5′_ 3′)	Size of PCR product (bp)	Reference
sea	F: GGGAACAGCTTTA GGCAATC R: ATTTGAATACTGTC CTTGAGC	564	This study
seb	F: CCAGATCCTAAAC CAGATGAG R: TGCAGGCATCATG TCATACC	599	This study
sec	F:AGATGAAGTAGT TGATGTGTATGG R: CACACTTTTAGAAT CAACCG	451	21
sed	F: GTGGTGAAATAGA TAGGACTGC R: GAAGGTGCTCTGT GGATAATG	381	This study
tsst-1	F: TGCTAGACTGGTA TAGTAGTGG R: GTTCCTTCGCTAG TATGTTGG	212	This study

Statistical analysis

Statistical analysis was performed using SPSS version 22 (IBM Corp, Armonk, NY, USA). Associations between categorical variables were examined by Fisher exact test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. P-values of <0.05 were considered statistically significant.

Results

A total of 300 nasal swabs were collected (150 from the MS patients and another 150 from the control group). The patient group consisted of 99 (66%) females and the remaining 51 (34%) males. Their age ranged from 20 to 58 years with a mean age and standard deviation (SD) of 32±9 years. The control group was comprised of 97 (64.7%) females and 53 (35.3%) males. Their age ranged from 19 to 59 years with a mean age of 29±7 years. The prevalence of *S. aureus* colonization among the MS patients group and the control group accounted for 63 (42%) and 35 (23.3%) individuals, respectively. There was a statistically significant association between *S. aureus* colonization and MS disease ($p<0.001$; OR 2.4; 95%CI 1.4-3.9). However, no statistically significant associations were found between the colonization with *S. aureus* and clinical traits (including age, sex, disease duration). Demographic traits of *S. aureus* nasal carriers in MS patients group are shown in Table 2.

No significant association was observed between colonization with antibiotics-resistant *S. aureus* and MS disease. The prevalence of methicillin resistant *S. aureus* (MRSA) in the patient group was 22.2% (n=14) while in the control group it was 16.7% (n=5). The antibiotic resistance pattern is shown in Table 3.

Among the MS patients who tested positive for *S. aureus*, the least prevalent SAg gene identified was *sed* (accounting for 6.3% of the patients) while the most prevalent one was *seb* (accounting for 28.6% of the patients) – Table 4. In the control group, the least prevalent SAg gene was also *sed* (accounting for 5.7%) while the most prevalent SAg gene was *sea* (accounting for 28.6% of the colonized individuals in the control group). The prevalence of *sea* in the MS patients group and *seb* in the healthy group was 25.4% and 17.1%, respectively. The findings indicated that there is no significant association between the presence of *S. aureus* harboring *sea*, *seb*, *sec*, *sed* and *tsst-1* genes with MS disease.

Table 2. Demographic traits of *S. aureus* nasal carriers in the MS patients group

Demographic traits		Nasal carriage of *S. aureus*, no. (%) Positive N=63	Negative N=87	Total N=150	OR	95%CI	p-value
Age (years)	20-30	26 (37.1)	44 (62.9)	70	–	–	0.714
	31-40	21 (42.0)	29 (58.0)	50			
	41-50	12 (54.5)	10 (45.5)	22			
	51-60	2 (40.0)	3 (60.0)	5			
	61-70	2 (66.7)	1 (33.3)	3			
Sex	Male	27 (53.0)	24 (47.0)	51	0.825	0.417-1.633	0.604
	Female	36 (36.4)	63 (63.6)	99			
Disease duration (years since diagnosis)	1-5	29 (42.0)	40 (58.0)	69	–	–	0.860
	6-10	22 (46.8)	25 (53.2)	47			
	11-15	7 (35.0)	13 (65.0)	20			
	16-20	3 (42.8)	4 (57.2)	7			
	>20	2 (28.6)	5 (71.4)	7			
Relapse at the time of sampling	Yes	28 (41.2)	40 (58.8)	68	0.917	0.479-1.759	0.869
	No	35 (42.7)	47 57.3)	82			

MS – multiple sclerosis; **OR** – odds ratio; **95%CI** – 95% confidence interval.

Table 3. Antibiotic susceptibility pattern of *S. aureus isolates*

Antibiotic	Resistant, no. (%) MS patients (n=63)	Healthy individuals (n=35)	Total resistant (n=98)	OR	95% CI	p-value
Cefoxitin	14 (22.2)	5 (16.7)	19 (19.3)	1.714	0.581-5.242	0.426
Gentamicin	8 (12.7)	4 (11.4)	12 (12.2)	1.127	0.314-4.048	1
Erythromycin	26 (41.3)	9 (25.7)	25 (25.5)	2.030	0.818-5.038	0.186
Clindamycin	17 (26.9)	7 (20.0)	24 (17.3)	1.478	0.545-4.009	0.475
Ciprofloxacin	11 (17.5)	5 (16.7)	16 (16.3)	1.269	0.402-4.003	0.781
Co-trimoxazole	15 (23.8)	4(11.4)	19 (19.3)	2.422	0.735-7.975	0.185
Chloramphenicol	7 (11.1)	4 (11.4)	11 (11.2)	0.969	0.263-3.571	1
Rifampin	3 (4.8)	0 (0.00)	3 (3.1)	0.952	0.901-1.006	0.551
Doxycycline	23 (36.5)	8 (22.8)	31 (31.6)	1.941	0.757-4.973	0.182
Quinupristin-dalfopristin	0 (0.0)	0 (0.0)	0 (0.0)	–	–	–
Linezolid	0 (0.0)	0 (0.0)	0 (0.0)	–	–	–
Mupirocin	3 (4.8)	1 (2.8)	4 (4.1)	1.700	0.170-16.989	1
Vancomycin	0 (0.0)	0 (0.0)	0 (0.0)	–	–	–

MS – multiple sclerosis; **OR** – odds ratio; **95%CI** – 95% confidence interval.

Table 4. Rate of *sea, seb, sec, sed* and *tsst-1* genes among MS patients and control groups

Gene	MS patients N=63	Healthy individuals n=35	Total (n=98)	OR	95% CI	p-value
sea	16 (25.4)	10 (28.6)	26 (26.5)	0.851	0.337-2.151	0.813
seb	18 (28.6)	6 (17.1)	26 (26.5)	1.600	0.593-4.316	0.469
sec	6 (9.5)	5 (14.2)	11 ((11.2)	0.632	0.178-2.241	0.515
sed	4 (6.3)	2 (5.7)	6 (6.1)	1.119	0.194-6.438	1
tsst-1	13 (20.6)	8 (22.8)	21 (21.4)	0.878	0.324-2.379	0.802
At least one of five superantigens	40 (63.5)	21 (60.0)	61 (62.2)	0.863	0.369-2.016	0.824
Multiple superantigens (2 or more superantigens)	14 (22.2)	7 (20.0)	21 (21.4)	0.875	0.316-2.485	1

MS – multiple sclerosis; **OR** – odds ratio; **95%CI** – 95% confidence interval.

Discussion

Patients with MS are more likely to have impaired T lymphocyte function. Hence, they may be considered as immune-compromised. The prevalence of *S. aureus* nasal carriage among immunocompromised patients is high compared to the healthy population.[10] In the present study, the nasal carriage rate of *S. aureus* in MS patients was 42% whilst in the control group it was 23.3%. Similarly, a study in Turkey reported prevalences of 46.6% and 20% among MS patients and control groups, respectively.[22] The relatively high nasal carriage rate of *S. aureus* may imply that MS patients are more vulnerable to *S. aureus* colonization due to reduced immunity. In contrast, a study in Canada reported a lower nasal carriage rate of *S. aureus* among MS patients (27%) and relatively higher in non-MS group (30%). They showed that MS patients were not more susceptible to *S. aureus* colonization, but in their study, the control groups were not matched with patients in terms of age and sex.[23]

In the current study sex classification revealed that female and male patients were not significantly different in the prevalence of *S. aureus* colonization. Additionally, the age of patients and disease duration had no significant association with *S. aureus* colonization in MS patients. No differences were observed for the rate of *S. aureus* in relapsing and non-relapsing patients.

S. aureus strains show considerable diversity in antibiotic resistance globally. Our study compared the frequency of antibiotic resistance of *S. aureus* in MS patients and healthy individuals. The *S. aureus* isolates from the healthy individuals and MS patients indicated almost similar resistance rates to antibiotics. All the isolates were susceptible to quinupristin-dalfopristin, vancomycin and linezolid. The highest rate of resistance was observed for doxycycline and erythromycin. MRSA is one of the major drug-resistant pathogens in the world and treating MRSA infections is problematic because MRSA strains carry resistance genes to other antimicrobial agents.[24,25] A report from a systematic review showed that the rates of MRSA in Iran ranged from 20.4% to 90%.[26] The overall resistance rates of *S. aureus* isolates to methicillin were 19.3% (22.2% in MS patients and 16.7% in the healthy population). Based on the molecular detection of MRSA, Melek et al. reported MRSA in 18.1% of MS patients.[22] Differences in the rate of MRSA carriage could be related to the use of different techniques for identifying methicillin resistance.

Among the MS patients and the control group the most prevalent superantigens were *seb* and *sea*, separately. However, there was no statistically significant association between *seb* gene and MS disease. Another study reported that *sea* was more prevalent in MS patients versus control group (30% in non-MS individuals, 21.2% in MS stable

and 27.2% in MS exacerbation groups). They revealed a significant association between *S. aureus* harboring *sea* gene and MS exacerbation.[23] In our study, we were unable to evaluate exacerbation in patients with MS, therefore we cannot draw any conclusions regarding the risk of exacerbation.

Conclusions

The risk of *S. aureus* nasal carriage in our study was higher in MS disease. *S. aureus* superantigens have been implicated in some immunological diseases such as lupus erythematosus, Wegener's granulomatosis syndrome and rheumatoid arthritis.[14-16] However, the findings of previous studies are not enough to support the role of superantigens in the induction or exacerbation of MS disease. Further investigation is needed to understand whether SAg positive *S. aureus* decolonization among MS patients could reduce MS disease severity.

Authors' contributions statement: ZP prepared the manuscript and performed experimental procedures. MAS performed physical examinations of MS patients. SS performed manuscript revision. MM participated in the acquisition of data and statistical analysis. MRP was involved in study concept and design, and in the development of the study. All authors read and approved the final manuscript.

Conflicts of interest: All authors – none to disclose.

Funding: This research was supported by Tehran University of Medical Sciences, Tehran, Iran (grant no: 30902).

References

1. Waxman SG. Multiple sclerosis as a neuronal disease. Arch Neurol 2000;57:22-4. [Crossref] [PubMed]
2. Ozenci V, Kouwenhoven M, Link H. Cytokines in multiple sclerosis: methodological aspects and pathogenic implications. Mult Scler. 2002;8:396-404. [Crossref] [PubMed]
3. Liguori M, Marrosu MG, Pugliatti M, et al. Age at onset in multiple sclerosis. Neurol Sci 2002;21:825-9. [Crossref] [PubMed]
4. Debouverie M. Gender as a prognostic factor and its impact on the incidence of multiple sclerosis in Lorraine, France. J Neurol Sci 2009;286:14-7. [Crossref] [PubMed]
5. Goldenberg MM. Multiple sclerosis review. P T 2012;37:175-84. [PubMed] [FullText]
6. Lublin FD, Reingold SC. Guidelines for clinical trials of new therapeutic agents in multiple sclerosis: relations between study investigators, advisors, and sponsors. Neurology 1997;48:572-4. [Crossref]
7. Disanto G, Morahan JM, Ramagopalan SV. Multiple sclerosis: risk factors and their interactions. CNS Neurol Disord Drug Targets 2012;11:545-55. [Crossref] [PubMed]
8. Balto JM, Ensari I, Hubbard EA, Khan N, Barnes JL, Motl RW. Individual and co-occurring SNAP risk factors: smoking, nutrition, alcohol consumption, and physical activity in people with multiple sclerosis. Int J MS Care 2016;18:298-304. [Crossref] [PubMed] [FullText]
9. Sahraian MA, Naghshineh H, Shati M, Jahromi SR, Rezaei N. Persian adaptation of a questionnaire of environmental risk factors in multiple sclerosis (EnvIMS-Q). Mult Scler Relat Disord 2016;10:82-5. [Crossref] [PubMed]
10. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. J Clin Invest 2001;108:1097-104. [Crossref] [PubMed] [FullText]
11. Libbey JE, McCoy LL, Fujinami RS. Molecular mimicry in multiple sclerosis. Int Rev Neurobiol 2007;79:127-47. [Crossref] [PubMed]
12. Torres BA, Kominsky S, Perrin GQ, Hobeika AC, Johnson HM. Superantigens: the good, the bad, and the ugly. Exp Biol Med (Maywood) 2001;226:164-76. [Crossref] [PubMed]
13. Thomas D, Dauwalder O, Brun V, et al. *Staphylococcus aureus* superantigens elicit redundant and extensive human Vbeta patterns. Infect Immun 2009;77:2043-50. [Crossref] [PubMed] [FullText]
14. Stegeman CA, Tervaert JW, Sluiter WJ, et al. Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. Ann Intern Med. 1994;120:12-7. [Crossref] [PubMed]
15. Chowdhary VR, Tilahun AY, Clark CR, Grande JP, Rajagopalan G. Chronic exposure to staphylococcal superantigen elicits a systemic inflammatory disease mimicking lupus. J Immunol 2012;189:2054-62. [Crossref] [PubMed] [FullText]
16. Jackson MS, Bagg J, Gupta MN, Sturrock RD. Oral carriage of staphylococci in patients with rheumatoid arthritis. Rheumatology (Oxford) 1999;38:572-5. [Crossref] [PubMed]
17. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 2005;5:751-62. [Crossref] [PubMed]
18. Xu SX, McCormick JK. Staphylococcal superantigens in colonization and disease. Front Cell Infect Microbiol 2012;2:52. [Crossref] [PubMed] [FullText]
19. Spaulding AR, Salgado-Pabón W, Kohler PL, Horswill AR, Leung DY, Schlievert PM. Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev 2013;26:422-47. [Crossref] [PubMed] [FullText]
20. Sfriso PA, Ghirardello C, Botsios M, et al. Infections and autoimmunity: the multifaceted relationship. J Leukoc Biol 2010;87:385-95. [Crossref] [PubMed]

21. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000;38:1032-5. [PubMed] [FullText]
22. Melek IM, Duran N, Duran GG, Duman T, Okuyucu E. The frequency of slime, adhesin and methicillin resistance genes among staphylococci isolated from nasal samples of multiple sclerosis patients. Afr J Microbiol Res 2011;5:5453-60.
23. Mulvey MR, Doupe M, Prout M., et al. *Staphylococcus aureus* harbouring Enterotoxin A as a possible risk factor for multiple sclerosis exacerbations. Mult Scler 2011;17:397-403. [Crossref] [PubMed]
24. Ohadian Moghadam S, Pourmand MR, Mahmoudi M, Sadighian H. Molecular characterization of methicillin-resistant *Staphylococcus aureus*: characterization of major clones and emergence of epidemic clones of sequence type (ST) 36 and ST 121 in Tehran, Iran. FEMS Microbiol Lett 2015;362:fnv043. [Crossref] [PubMed]
25. Dağı HT, Fındık D, Demirel G, Arslan U. Detection of methicillin resistance and various virulence factors in *Staphylococcus aureus* strains isolated from nasal carriers. Balkan Med J 2015;32:171-5. [Crossref] [PubMed] [FullText]
26. Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, Naderinasab M. Epidemiology of mecA-methicillin resistant *Staphylococcus aureus* (MRSA) in Iran: a systematic review and meta-analysis. Iran J Basic Med Sci 2012;15:1010-9. [PubMed] [FullText]

Please cite this article as:
Pakbaz Z, Sahraian MA, Sabzi S, Mahmoodi M, Pourmand MR. Prevalence of *sea, seb, sec, sed, and tsst-1* genes of *Staphylococcus aureus* in nasal carriage and their association with multiple sclerosis. GERMS 2017;7(4):171-177. doi: 10.18683/germs.2017.1123.