Compare the polymerase chain reaction (PCR) versus anterior nair swab culture for detecting nasal carriage of staphylococcus aureus

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Abstract: Staphylococcus aureus has long been recognized as an important pathogen in human disease. Diabetes introduced as one of the risk factors in increasing the probability of staphylococcus aureus colonization in nasal anterior cavities. This study aimed at comparing the polymerase chain reaction (PCR) versus anterior nair swab culture for detecting nasal carriage of staphylococcus aureus. In this descriptive-analytical study, samples were taken from nasal mucus of 126 diabetic patients using cotton swab and polymerase chain reaction (PCR). A questionnaire consisting of information such as patients' personal particulars including age, gender, duration of the diseased, how the diabetes is controlled, HbA1C (based on the recent test of the patients which should not belong for more than three months ago) was filled for all patients. Out of all 126 understudy diabetic patients, 26 cases (20.6%) were known as carriers of staphylococcus aureus in nasal mucus. Out of all 126 understudy diabetic patients, 32 cases (25.4%) were known as carriers of staphylococcus aureus with PCR. Mean duration of detected diabetes was 7.96±6.61 and 7.76±7.51 years among subjects with positive culture of staphylococcus aureus and nuc, respectively. According to the statistical analysis, there was not any significant relationship in this regard (P=0.42). Mean HbA1C was 7.76% and 8.52% in groups with positive culture of staphylococcus aureus and nuc^{+}, respectively (P=0.045). In diabetic patients, frequency of staphylococcus aureus colonization of nasal mucus in patients with poor control of blood sugar is not significantly different than polymerase chain reaction (PCR).
[Mojtaba Varshochi , Alka Hasani. **Compare the polymerase chain reaction (PCR) versus anterior nair swab culture for detecting nasal carriage of staphylococcus aureus.** *J Am Sci* 2013;9(10s):75-79]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 14

Keywords: Staphylococcus aureus; Nasal cavity; Diabetes mellitus; polymerase chain reaction (PCR); Anterior nair swab culture

1. Introduction

Staphylococcus aureus is a bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. Although S. aureus is not always pathogenic, it is a common cause of skin infections, respiratory, and food poisoning.(Tarran et al. 2013; Zhong et al. 2013) Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic S. aureus is a worldwide problem in clinical medicine.(Zimmerman et al. 2012; Haley et al. 2012) In contrast to the substantial body of epidemiologic information that exists regarding the risks of *S. aureus* carriage, relatively little is known of the basic host surface components or mechanisms that mediate attachment of staphylococci to the nasal mucosa.(Shnit-Orland et al. 2012; Obidike et al. 2011) Investigators have demonstrated binding to desquamated nasal epithelial cells, to ferret airway mucu, bovine submaxillary mucin, as well as immunoglobulin A (IgA) salivary mucin complexes and respiratory tract glycolipids. Due to an increasing number of infections caused by methicillin-resistant S. aureus (MRSA) strains, therapy has become problematic. Therefore, prevention of staphylococcal infections has become more important.(Folescu et al. 2012; Santos et al. 2012) Carriage of S. aureus appears to play a key role in the epidemiology and pathogenesis of infection. The ecological niches of S. aureus are the anterior nares. In healthy subjects, over time, three patterns of carriage can be distinguished: about 20% of people are persistent carriers, 60% are intermittent carriers, and approximately 20% almost never carry S. aureus.(Kumari et al. 2011; Bragadeeswaran et al. 2011) The molecular basis of the carrier state remains to be elucidated. In patients who repeatedly puncture the skin and patients with human immunodeficiency virus (HIV) infection, increased carriage rates are found. Carriage has been identified as an important risk factor for infection in patients undergoing surgery, those on hemodialysis or CAPD, those with HIV infection and AIDS, those with intravascular devices, and those colonized with MRSA.(Bernstein et al. 2011; Ito et al. 2011) Elimination of carriage has been found to reduce the infection rates in surgical patients and those on hemodialysis and CAPD. Elimination of carriage appears to be an attractive preventive strategy in patients at risk. Further studies are needed to optimize this strategy and to define the groups at

risk.(Bernstein et al. 2010; Su 2011) The aim of this study was to compare the polymerase chain reaction (PCR) versus anterior nair swab culture for detecting nasal carriage of staphylococcus aureus.

2. Material and Methods

In this descriptive-analytical study conducted at infectious and glands clinics of Iman Reza and Sina hospitals from May 2012 to May 2013, 126 insulin-dependent and insulin-independent diabetic patients were evaluated. This study was approved by ethic committee of Tabriz university of medical sciences. Written consent was obtained from all the patients. A questionnaire consisting of information such as patients' personal particulars including age, gender, duration of the diseased, how the diabetes is controlled (oral or insulin or both), diabetic ulcer, HbA1C (based on the recent test of the patients which should not belong for more than three months ago) was filled for all patients. The control will be regarded as poorly one if HbA1C is higher than 7%. Then, samples were taken from nasal anterior mucus of all patients. Positive HIV, uremic or dialysis patients, IVDU (injective addicts), anemia (Hb<14 for men, Hb<11 in women during their menstruation age, Hb<12 in women after menopause, and Hb<10 in pregnant women), being a health care personnel (HCWs), recent hospitalization (during last 4 weeks), history of recently receiving antibiotics (during the last 4 weeks), not using medical ways to treat diabetes were regarded as the exclusion criteria. Nasal swab (using cotton swab) was used as the sampling method and anterior naires was the sampling point. The sampling was done once and they were transferred to the laboratory in the pipes including Nutrient broth Nacl 6.5%+. Blood agar and mannitol salt agar with modified pH were the cultivation media used to isolate staphylococcus aureus. Sterilization was conducted using autoclave in a temperature of 121c^{o} and pressure of 15 Ibs for 15 minutes. All plates were stored at a temperature of 4-8 c^{o} and for 2 weeks, at maximum. In PCR technique, Synthetic oligonucleotide primers of 21 and 24 bases, respectively, were used in the polymerase chain reaction (PCR) to amplify a sequence of the nuc gene, which encodes the thermostable nuclease of Staphylococcus aureus. A DNA fragment of approximately 270 bp was amplified from lysed S. aureus cells or isolated DNA. The PCR product was detected by agarose gel electrophoresis or Southern blot analysis by using a 33-mer internal nuc gene hybridization probe. With S. aureus cells the lower detection limit was less than 10 CFU, and with the isolated target the lower detection limit was 0.69 pg of DNA. The primers recognized 90 of 90 reference or clinical S. aureus strains. Amplification was not recorded when 80 strains representing 16 other staphylococcal species were tested or when 20 strains representing 9 different nonstaphylococcal species were tested. Some of the non-S. aureus staphylococci produced thermostable nucleases but were PCR negative. The PCR product was generated when in vitro-cultured S. aureus was used to prepare simulated clinical specimens of blood, urine, cerebrospinal fluid, or synovial fluid. No PCR product was generated when the sterile body fluids were tested. A positive PCR result was recorded when a limited number of clinical samples from wounds verified to be infected with S. aureus were tested, while the PCR product was not detected in materials from infections caused by other bacteria. Generation of PCR products was not affected by exposure of S. aureus to bactericidal agents, including cloxacillin and gentamicin, prior to testing, but was affected by exposure to UV radiation. The PCR for amplification of the nuc gene has potential for the rapid diagnosis of S. aureus infections by direct testing of clinical specimens, including specimens from patients with ongoing antimicrobial therapy.

Statistical analysis

The data was statistically analyzed using descriptive statistical methods (frequency, percentage, and mean), Chi-square test, or Fischer's exact test and mean difference test for independent groups. Also, SPSS version 15 was used as the statistical software. In this study, P<0.05 were regarded statistically significant

3. Results

In this study, 126 diabetic patients were studied. Mean age of patients with positive cultivation in terms of staphylococcus aureus of nasal mucus was 53.12±12 years and that of the negative staphylococcus aureus was 54.56±12.35 years. There was not any significant relationship between positive staphylococcus aureus and patients' age (P=0.29). The understudy subjects were consisted of 82 women (65.1%) and 44 men (34.9%). Out of all 126 understudy diabetic patients, 26 cases (20.6%) were known as carriers of staphylococcus aureus in nasal mucus. Out of 26 patients with isolated staphylococcus aureus, 18 subjects (69.2%) were women and 8 cases (30.8%) were men. Statistical analysis does not referred to any relationship between gender and staphylococcus aureus colonization (P=0.45). Out of all 126 understudy diabetic patients, 32 cases (25.4%) were known as carriers of staphylococcus aureus with PCR. Mean age of patients with nuc^{+} in terms of staphylococcus aureus of nasal mucus was 54.22±11 years and that of the nuc^{-} was 55.54±11.45 years. There was not any significant relationship between nuc and patients' age (P=0.24). Out of 32 patients with nuc^{+}, 20 subjects (62.5%) were women and 12 cases (27.5%) were men.

Statistical analysis does not referred to any significant relationship between gender and nuc (P=0.42). Out of the understudy subjects, 80 (63.5%), 36 (28.6%), and 10 (7.9%) patients controlled their blood sugare relying on oral, injective (insulin), and both methods (insulin + oral medication), respectively. Out of 80 oral cases, 19 cases (23.75%) experienced staphylococcus aureus isolation and 22 cases (27.5%) were nuc^+. There was no significant difference in two methods (P=0.64). Additionally, out of 36 cases who controlled their blood sugare using insulin, 4 cases (11.1%) underwent staphylococcus aureus isolation while 6 cases (16.7%) were nuc^+. The statistical analysis did not refer to any significant relationship (P=0.32). Mean duration of detected diabetes was 7.96±6.61 and 7.76±7.51 years among subjects with positive culture of staphylococcus aureus and nuc, respectively. According to the statistical analysis, there was not any significant relationship in this regard (P=0.42). Mean HbA1C was 6.43 ± 0.66 and 9.38 ± 1.79 in groups with positive culture of staphylococcus aureus and nuc^+, respectively. Evidently, it was higher in the group with positive culture of staphylococcus aureus. The statistical test (independent T-test) demonstrates that the difference is significant (P=0.045)(Table 1).

Table 1. Comparing the two methods for diagnosing the staphylococcus aureus

		Anterior nair swab culture	PCR
Age (year)		53.12±12	54.22±11
Gender	Male	8 (30.8%)	12 (37.5%)
	Female	18 (69.2%)	20 (62.5%)
Diabetes	Type 1	0 (0%)	6 (18.7%)
	Type 2	26 (100%)	26 (81.3%)
History of diabetes (year)		7.96±6.61	7.76±7.51
Method of blood sugar control	Oral	20 (76.9%)	24 (75%)
	Injection	5 (19.2%)	6 (18.7)
	Both	1 (3.9%)	2 (6.3%)
HbA1c		6.43 ± 0.66	9.38 ± 1.79

4. Discussions

Diabetic subjects probably have a higher risk of the following infections: asymptomatic bacteriuria, lower extremity infections, reactivation tuberculosis, infections in surgical wounds after sternotomy and total hip replacement, and group B streptococcal. Support for these associations comes from controlled observational studies in all cases, except for lower extremity infections, where the magnitude of the association between foot and ankle infection and diabetes from hospital-based data appears too great to be explained by detection, selection, or other potential biases.(Robb and Bendig 2010; Canton and del 2010) Local and systemic immunologic defects probably account for higher infection rates in diabetic patients. Autonomic and sensory neuropathy probably account for higher bacteriuria and lower extremity infection rates, while systemic immunologic effects of diabetes may be responsible for the increased propensity to surgical wound infection and tuberculosis reactivation.(Zahm et al. 2010; Kesavan et al. 2010) The nasal passageway is close to other niches like the lungs, mouth, and throat. The nasal passage is influenced by the bacteria colonies of Staphylococcus aureus. Successful colonization depends not only on the ability of S. aureus to survive host factors but also on coexistence with other bacteria. An airborne bacterium also causes allergies and irritation.(Kasai et al. 2010; Jervis-Bardy et al. 2009) Tamang et al. demonstrated that 1 causes of Bacterial infections causing nasal discharge are related to diabetes, or a family history of diabetes (from a list of 3 total causes). These diseases and conditions may be more likely causes of bacterial infections causing nasal discharge if the patient has diabetes, is at risk of diabetes, or has a family history of diabetes.(Tamang et al. 2009) Several risk factors have been introduced for staphylococcus aureus colonization.(Kandan and Thappa 2009; Khalil 2009) In their study, Kieffer et al. reported the rate as 3.8% in adults referring to the emergency ward. In diabetic patients, chronic renal disease and recent injections were more observed. (Kieffer et al. 2008) Miyake et al. introduced maleness, youngness, having contact with health system personnel, injection, and acne as risk factors of colonization.(Miyake et al. 2007) Diabetes is one of the risk factors introduced for staphylococcus aureus colonization in nasal anterior mucus. Tuazon et al., suggested that staphylococcus aureus carriers in insulin-dependent diabetics were three times more than normal population. (Tuazon et al. 1975) Rogers et al. studied type II diabetic patients and demonstrated that carrier rate in patients receiving insulin, oral medications, and non-diabetic subjects was 35.3%, 13.8%, and 10.7% respectively. They introduced consumption of insulin, hospitalization

during the last 6 months, past history of diabetes for more than 6 years, FBS>111, and consumption of antibiotics within the last 6 months as colonization risk factors in diabetics. There was not any significant relationship between colonization in diabetics and recent consumption of antibiotics, age, race, or diabetes duration. It had a reverse relationship with glucose control (based on FBS and HbA1C.(Rogers et al. 2009) In contrary, some studies have not confirmed diabetes as an effective factor in staphylococcus aureus colonization.(Below et al. 2009; Azawi et al. 2008) In this study, authors compared the polymerase chain reaction (PCR) versus anterior nair swab culture for detecting nasal carriage of staphylococcus aureus. Except for mean HbA1C, the other risk factors had not any significant difference (P>0.05). In their study Lima et al. demonstrated that duration of detected diabetes was statistically more in polymerase chain reaction (PCR) than anterior nair swab culture for detecting nasal carriage of staphylococcus aureus (P<0.05).(Lima et al. 2008) Belle et al. demonstrated that HbA1C was statistically more in polymerase chain reaction (PCR) than anterior nair swab culture for detecting nasal carriage of staphylococcus aureus (P<0.05).(Belle et al. 2008) Malaty et al. demonstrated that. type of medication and age were no statistically different in polymerase chain reaction (PCR) and anterior nair swab culture for detecting nasal carriage of staphylococcus aureus (P>0.05). (Malaty and Antonelli 2008).

Conclusion

In diabetic patients, frequency of staphylococcus aureus colonization of nasal mucus in patients with poor control of blood sugar is not significantly different than polymerase chain reaction (PCR).

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9/22/2013