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Discrimination of influenza virus-infected nasal fluids by Vis-NIR spectroscopy

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ABSTRACT

Background: Influenza patients show a severe condition of the respiratory tract with high temperature. Efficient treatment of influenza requires early use of oseltamivir, and thus rapid diagnosis is needed. Recently, rapid diagnostic methods such as immunochromatography have been developed; however, immunochromatography is not an optimal technique because it is relatively expensive and has low sensitivity.
Methods: Visible and near-infrared (Vis-NIR) spectroscopy in the region 600–1100 nm, combined with chemometrics analysis such as principal component analysis (PCA) or soft modeling of class analogy (SIMCA), was used to develop a potential diagnostic method for influenza based on nasal aspirates from infected patients.
Results: The Vis-NIR spectra of nasal aspirates from 33 non-influenza patients and 34 influenza patients were subjected to PCA and SIMCA to develop multivariate models to discriminate between influenza and non-influenza patients. These models were further assessed by the prediction of 126 masked measurements [30 from non-influenza patients, 30 from influenza patients and 66 from patients infected with respiratory syncytial virus (RSV)]. The PCA model showed some discrimination of the masked samples. The SIMCA model correctly predicted 29 of 30 (96.7%) non-influenza patients, and 30 of 30 (100%) influenza patients from the Vis-NIR spectra of masked nasal aspirate samples. Nasal aspirates of RSV-infected patients were predicted as 50% non-influenza and 50% influenza by the SIMCA model, suggesting that discrimination between patients infected with influenza virus and those infected with RSV was difficult.
Conclusions: Although the study sample was small and there was difficulty in discriminating between influenza virus and RSV infection, these results suggest that Vis-NIR spectroscopy of nasal aspirates, combined with chemometrics analysis, might be a potential tool for diagnosis of influenza.

1. Introduction

Influenza viruses are divided into three types (A, B, and C) based on the antigenicity of nucleoprotein (NP) and matrix protein (M1) [1]. The influenza A virus genome encodes two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), for which antigenicity defines 16 distinct HA and 9 distinct NA subtypes [2,3]. Among the various types and subtypes of influenza virus, A/H1N1, A/H3N2 and B have been mainly associated with human infection [1]. Influenza virus infection causes a severe upper respiratory tract infection in infants, young children, and older individuals, with an estimated 3–5 million cases of severe illness and 250,000–500,000 deaths per year worldwide [1].

Currently, influenza virus infection cannot be reliably diagnosed on clinical features alone [4], because it is difficult to differentiate it from other respiratory diseases, such as infection with adenovirus, parainfluenza virus, respiratory syncytial virus (RSV) [5], hemolytic streptococcus [6], and metapneumovirus [7]. In particular, when RSV and influenza epidemics coincide and hospitalization rates are at their highest, the discrimination between RSV infection and influenza virus infection becomes important and difficult [8]. Furthermore, it is important to discriminate viral infection from bacterial infection in order to decrease the misuse of antibiotics, to minimize the emergence of antibiotic-resistant strains of bacteria, and to reduce the length of hospital stay [9].

Several methods for detecting influenza virus have been developed, such as reverse transcription polymerase chain reaction (RT–PCR), enzyme-linked immunoassay (EIA), immunofluorescence assay, rapid antigen test (immunochromatography) and serological tests [10]. In particular, immunochromatography is useful as a rapid diagnostic assay of influenza virus infection and is broadly used for practical diagnosis in the clinic. In addition, although immunochromatography provides a relatively accurate diagnosis with a specificity for influenza of 98.2%, it has low sensitivity (62.3%) [11]. It's positive and negative likelihood ratios are 34.5 and 0.38, respectively, suggesting that a positive test result is unlikely to be a false positive, but a negative result has a reasonable likelihood of being a false negative and should be confirmed

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by other laboratory diagnostic tests. More importantly, this method is not optimal in terms of cost-effectiveness. To overcome these problems, an alternative diagnostic method using an instrument that facilitates cost-effective diagnosis is needed.

Visible and near-infrared (Vis-NIR) spectroscopy is a spectroscopic method that uses visible light and NIR radiation. Moreover, Vis-NIR spectroscopy requires no sample preparation and no reagents [12], resulting in lower costs and less waste. As a result, Vis-NIR spectroscopy is widely used as an analytical technique in the agricultural, pharmaceutical, chemical and petrochemical industries [12,13]. Vis-NIR spectroscopy has also been used in a broad range of clinical applications [12,14].

2. Materials and methods

2.1. Nasal aspirate samples and body temperature measurement

This research project was approved by the Ethics Committee of Osaka University, and written informed consent was obtained from the parents of all influenza, non-influenza and RSV-infected patients before samples were collected.

Clinical nasal aspirates were collected from pediatric patients attending the Baba pediatric clinic (Tables 1, 2). Before the collection, core body temperature was measured by a C202 axillary electric thermometer (Termo Corp., Tokyo, Japan). The nasal aspirate was collected as follows. Saline was introduced into the nasal cavity, then fluid was collected using a Belvital nasal aspirator (Melisana, Nogent-sur-Marne, France). To remove cell debris, the nasal fluid was filtered using a stainless steel mesh [200 grids per inch (25.4 mm)]. The nasal aspirates were subjected to immunochromatography for influenza viruses using a kit (Esprine kit; Fujirebio Inc., Tokyo, Japan). The nasal aspirates from 34 patients with influenza, diagnosed on the basis of immunochromatography, and those from 35 patients not infected with influenza were used as test samples to develop a calibration model for PCA [15] and SIMCA [16] (Table 1). A further 126 measurements comprising 10 samples from non-influenza patients each with n = 3 spectra; 10 samples from influenza patients each with n = 3 spectra; and 22 samples from RSV-infected patients each with n = 3 spectra were masked and used for prediction (Table 2). RSV infection was analyzed by an immunochromatography kit (Bionx Now kit; Eiken Chemical Co. Ltd., Tokyo, Japan).

2.2. Instrument and data collection

Spectral data from nasal aspirates diluted 10-fold with phosphate buffered saline (PBS) was collected as absorbance values [$\log(1/T)$], where T = transmittance in the wavelength range from 600 to 1100 nm. For each sample, three consecutive Vis-NIR spectra were acquired at 2-nm resolution with an FQA-NIRGUN spectrophotometer (Japan Fantec Research Institute, Shizuoka, Japan) at 37 °C (Fig. 1).

2.3. Data processing

Pirouette software (ver. 3.11; Infometrics, Woodinville, WA) was employed for data processing. To minimize differences in spectra caused by baseline shifts and noise, prior to calibration spectral data were mean-centered and transformed by standard normal variates (SNVs) [17] and smoothing based on the Savitsky–Golay algorithm [18]. To identify the predominant absorbance peaks in the spectra, the PCA [15] and SIMCA [16] algorithms were further applied to develop, respectively, PCA and SIMCA models of influenza diagnosis. To visualize the SIMCA approach, we used Coomans plot [19], which plots "class" distances against one other. Coomans plot can assess the classification performance of the SIMCA model by predicting class membership in terms of distance from the model. The critical distance from the model corresponded to the 0.05 level and was defined as the 95% tolerance interval. The mathematical formulas used are available in the Pirouette manual. The difference in core body temperature was evaluated by Kruskal–Wallis test followed by Dunn's analysis. A value of $P<0.05$ was considered to be statistically significant.

3. Results and discussion

Rapid diagnosis of influenza is important because the efficacy of oseltamivir is maximized by early commencement of treatment. Clinical benefits are seen only when oseltamivir is given within 48 h of the onset of symptoms [20].

Recently, we have demonstrated the possibility of diagnosing various diseases by Vis-NIR spectroscopy using various types of samples such as blood [21–25], cell culture medium [26], and tissues [27–29]. In this study, the potential spectroscopic diagnosis of influenza using nasal aspirates was investigated. Nasal aspirates from influenza and non-influenza patients, as well as those from RSV-infected patients, were subjected to Vis-NIR spectroscopy coupled with multivariate analysis such as PCA and SIMCA, in order to explore a novel method for the diagnosis of influenza.

Discrimination between the nasal aspirates from influenza patients and those from non-influenza patients using test samples was seen in PCA scores using first principal component (PC1) and second principal component (PC2) analysis (Fig. 1A). In addition, the SIMCA model enabled distinct separation of the Vis-NIR spectra of 100 of 105 (95.24%) non-influenza samples and 95 of 102 (93.13%) influenza samples (Table 3). SIMCA analysis using Coomans plot demonstrated that the nasal aspirate classes of "influenza patients" and "non-influenza patients"

Table 1
Test samples used for developing a calibration model[a].

Sample name	Male/female	Core body temperature [°C]	Sample name	Male/female	Core body temperature [°C]
N 6	F	36.3	I 7	M	36.4
N 7	F	36.3	I 8	M	36.0
N 8	F	36.3	I 9	M	36.0
N 9	M	36.4	I 10	F	36.3
N 10	F	36.3	I 11	F	36.0
N 11	M	36.6	I 12	F	36.7
N 12	F	36.0	I 13	M	36.3
N 14	F	36.3	I 15	M	36.4
N 15	F	36.3	I 16	M	36.1
N 16	M	36.1	I 19	F	36.1
N 17	F	36.4	I 20	F	36.4
N 18	M	36.1	I 21	M	36.4
N 19	F	36.3	I 22	F	36.4
N 20	M	36.2	I 23	M	36.0
N 21	F	36.5	I 24	F	36.3
N 22	M	36.4	I 25	M	36.1
N 23	F	36.4	I 26	F	36.0
N 24	M	36.4	I 27	F	36.0
N 25	F	36.3	I 28	F	36.3
N 26	M	36.3	I 29	F	36.3
N 27	F	36.3	I 31	M	36.4
N 28	F	36.1	I 32	M	36.1
N 29	M	36.1	I 33	M	36.0
N 31	F	36.6	I 34	M	36.0
N 32	M	36.6	I 35	F	36.0
N 33	F	36.4	I 37	M	36.2
N 34	F	36.4	I 39	M	36.3
N 35	M	36.4	I 40	M	36.4
N 36	M	36.3	I 41	M	36.4
N 37	M	36.2	I 42	M	36.4
N 38	M	36.4	I 43	F	36.3
N 40	F	36.4	I 44	F	36.4
N 41	M	36.1	I 45	F	36.1
N 42	M	36.3	I 47	M	36.6
N 43	F	36.2			

[a] N6-N43 are non-influenza samples, while I7-I47 are influenza samples. Core body temperatures of non-influenza, influenza, and RSV-infected patients at the time when nasal aspirates were collected from patients are shown.