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Visible and near-infrared spectra collected from the thumbs of patients with chronic fatigue syndrome for diagnosis

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ABSTRACT

Background: Currently, diagnosis of chronic fatigue syndrome (CFS) is based on clinical symptoms and therefore relies on the experience and skill of the doctors. Here, we have examined the possible diagnosis of CFS based on spectral information and chemometrics analysis, such as principal component analysis (PCA) and soft modeling of class analogy (SIMCA).
Methods: Visible and near-infrared (Vis-NIR) spectroscopy was used to examine possible changes in the region of 600–1100 nm in thumbs and assessed.
Results: The Vis-NIR spectra of thumbs from 57 CFS patients and 74 healthy volunteers were subjected to PCA and SIMCA to develop multivariate models to discriminate between CFS patients and healthy individuals. The model was further assessed by the prediction of 120 determinations (60 in the healthy group and 60 in the CFS patient group). The PCA model predicted a discrimination of the masked samples; specifically the SIMCA model correctly predicted 51 of 60 (83.3%) healthy volunteers and 42 of 60 (70%) CFS patients.
Conclusions: Despite the relatively small number of subjects involved in this trial, who were exclusively Japanese, our results imply that Vis-NIR spectroscopy of the thumb combined with chemometrics analysis may provide a valuable tool for diagnosing CFS.

1. Introduction

Chronic fatigue syndrome (CFS) is a persistent weakened condition associated with a variety of somatic and psychological symptoms [1]. The socio-economic impact of CFS is substantial given the chronic nature and seriousness of the illness [2]. The prominent features of CFS are self-reported impairments in concentration and short-term memory, sleep disturbances, musculoskeletal pain, extreme fatigue and cognitive dysfunction [1]. Cytokines, neuropeptides or neurotransmitters are thought to be responsible for the abnormal immune response [3] and disrupted hypothalamo–pituitary–adrenal (HPA) axis [3], which is characteristic of CFS patients. Nonetheless, the precise pathophysiology of this condition is currently unknown. In addition, there is no clear consensus with regard to changes in blood composition or genetic factors, such as polymorphisms, which might predispose certain individuals to CFS [4–7]. Therefore, due to the absence of reliable biochemical markers, the diagnosis of CFS is currently based on clinical symptoms alone [8]. As this diagnostic procedure relies on the experience and skill of the person making the judgement, CFS can be accurately diagnosed by only a limited number of skilled medical practitioners. A further complication arises because some clinicians, researchers and patients use alternative designations for CFS such as myalgic encephalomyelitis (ME), fibromyalgia (FM) [9] and chronic fatigue and immune dysfunction syndrome (CFIFS) [10,11]. This confusion is due, at least in part, to the current CFS diagnostic procedure. The most commonly used criteria for CFS are defined by the Centers for Disease Control and Prevention (CDC) [1], which are often criticized for being too inclusive and putting insufficient emphasis on neurocognitive dysfunction, abnormal fatigability and post-exertional malaise [12]. These diagnostic criteria have also been found to be flawed by the same researchers [13,14]. Hence, an additional diagnostic method that enables objective judgment to be made is urgently needed.

Visible and near-infrared (Vis-NIR) spectroscopy is a spectroscopic method using visible light and NIR radiation. Moreover, Vis-NIR spectroscopy requires no sample preparation and no reagents and enables non-invasive and non-destructive analysis [15]. Therefore, Vis-NIR spectroscopy has become a widely used analytical method in the agricultural, pharmaceutical, chemical and petrochemical industries [15,16]. Vis-NIR spectroscopy has also been used for a broad range of clinical applications [15,17–19] including CFS [20–22].

We previously reported that CFS can be objectively diagnosed by principal component analysis (PCA) and soft modeling of class

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analogy (SIMCA) of Vis-NIR spectra obtained from sera [20]. Furthermore, Vis-NIR spectral changes in the thumb of patients with CFS were also reported [22]. In this study, we have extended our analysis to the possibility of using Vis-NIR spectroscopy of thumbs coupled with multivariate analysis for a novel and non-invasive procedure of CFS diagnosis.

2. Materials and methods

2.1. Samples

At the Medical Hospital of Osaka City University, we examined the thumbs of 77 CFS patients (35.8 ± 7.8 years; male/female: 29/48) (Table 1), who were diagnosed on the basis of clinical criteria proposed by the Centers for Disease Control and Prevention (CDC) [1]. The thumbs of 94 healthy volunteers (38.5 ± 10.7 years; male/female: 52/42) were also analyzed. Spectra (i.e., test samples) obtained from the thumbs of 57 CFS patients and 74 healthy volunteers were used to develop a calibration model of PCA [23] and SIMCA [24]. In addition, another 120 determinations (40 samples ×3 spectra) including 60 obtained from 20 healthy volunteers (35.4 ± 8.6 years; male/female: 15/5) (×3 spectra) and 60 determinations (20 CFS patient samples (35.9 ± 9.4 years; male/female: 7/13) (×3 spectra) were masked and used for prediction (Table 1). This research project was approved by the Ethics Committee of Osaka City University and written informed consent was obtained from all CFS patients and healthy volunteers.

2.2. Instrumentation and data collection

Three consecutive Vis-NIR spectra were measured at 2 nm resolution using a FQA-NIRGUN instrument (Japan Fantec Research Institute, Shizuoka, Japan) at 25 °C (Fig. 1). During measurement by Vis-NIR, it was important to regulate the pressure to the thumbs because there was a possibility that this may affect the Vis-NIR spectrum. Therefore, we normalized the pressure to the thumb using a rubber band. In addition, to reduce the effect of keeping the thumbs for an extended period of time in the instrument, the collection of Vis-NIR spectra were performed within 1 min. The spectral data were collected as absorbance values [log(1/T)], where T = transmittance was in the wavelength range from 600 to 1100 nm.

2.3. Data processing

Pirouette software (ver. 3.11; Infometrics, Woodinville, WA) was employed for data processing. Prior to calibration, spectral data were mean-centred and standard normal variate (SNV) transformations [25] were performed with smoothing based on the Savitzky–Golay algorithm [26]. This procedure minimized differences between spectra caused by shifts in baseline and background noise. To identify

Table 1
Samples used in this study.

	N	Age	Male/Female
All samples			
Healthy	94	38.5 ± 10.7	52/42
CFS	77	35.8 ± 7.8	29/48
Test samples			
Healthy	74	39.2 ± 11.0	37/37
CFS	57	35.9 ± 7.5	22/35
Masked samples			
Healthy	20	35.4 ± 8.6	15/5
CFS	20	35.9 ± 9.4	7/13

Fig. 1. Spectrophotometer used for collection of Vis-NIR spectra. Vis-NIR spectra collected from the thumb by FQA-NIRGUN spectrophotometer were used for the potential diagnosis of CFS.

the predominant absorbance peaks in the spectra, PCA and SIMCA methods were further applied to develop novel models for CFS diagnosis. We used Cooman's plot [27] as a method of visualizing the SIMCA approach, which plots class distances against each other. Cooman's plot was applied to 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 used corresponded to the 0.05 level and defined a 95% tolerance interval. The mathematical formulas used for this procedure are available in the Pirouette manual.

3. Results and discussion

The main problems in CFS studies can be attributed to the objectivity of diagnosis, because the diagnosis of CFS is based solely on clinical symptoms [8]. To test the feasibility of spectroscopic diagnosis for CFS by Vis-NIR spectroscopy, the thumbs of CFS patients and healthy volunteers were subjected to Vis-NIR spectroscopy coupled with multivariate analysis, including PCA and SIMCA. If successful, the technique would provide a novel means of objectively diagnosing CFS in a non-invasive manner.

Although incomplete, some discrimination was observed between CFS patients and healthy volunteers as seen from the PCA scores using first principal component (PC1) and fourth principal component (PC4) (Fig. 2A). The SIMCA model facilitated reasonably accurate separation of Vis-NIR spectra i.e., 179 of 222 (80.6%) healthy volunteers and 132 of 171 (77.2%) CFS patients were correctly identified (Table 2). SIMCA analysis using the Cooman's plot demonstrated that classes of healthy volunteers and CFS patients displayed some separation in multivariate space, providing validation for the class separation (Fig. 3A). In addition, further trials were also carried out using the Vis-NIR spectroscopic data in order to test the PCA and SIMCA models. Discrimination of the masked Vis-NIR spectral data derived from CFS patients and healthy volunteers were assessed using the PCA and SIMCA models (Figs. 2B, 3B). PCA showed some discrimination of the masked samples between healthy volunteers and CFS patients. SIMCA correctly predicted 51 of 60 (85.0%) healthy volunteers and 42 of 60 (70.0%) CFS patients from their respective Vis-NIR spectra (Table 3).

The spectral information modeled by PCA and SIMCA can be inferred from the corresponding loadings or discriminating power, respectively. In terms of PC1, the loadings positively peaked around

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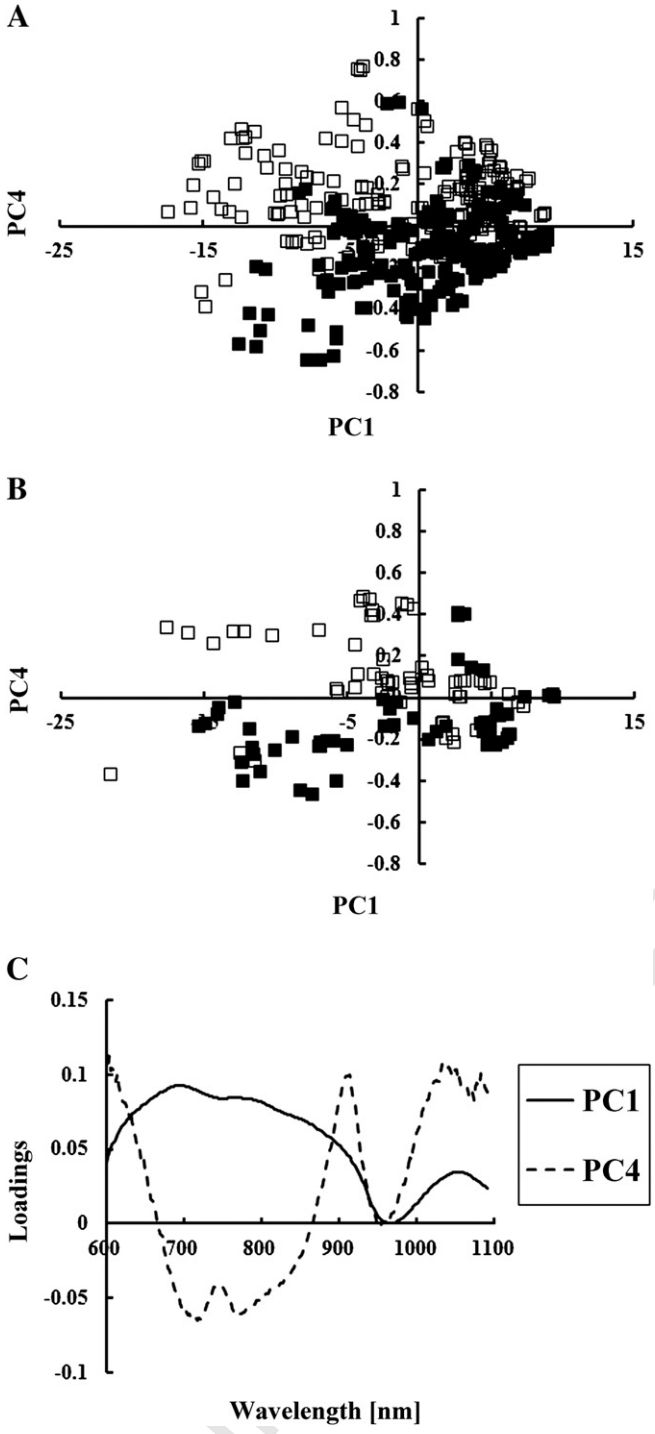

Fig. 2. Principal component analysis (PCA) (first and fourth principal components) of visible and near-infrared (Vis-NIR) calibration and diagnostic prediction of chronic fatigue syndrome (CFS). Thumbs of healthy volunteers and CFS patients were subjected to Vis-NIR spectroscopy. Spectral data were then pre-processed and subjected to PCA calibration modeling to develop a multivariate model to diagnose CFS, which was referenced on the basis of Centers for Disease Control and Prevention (CDC) criteria. The PCA score plot of the first principal component (PC1) *versus* the fourth principal component (PC4) for Vis-NIR spectra of test samples (A) and masked samples (B) using the PCA model showed clear discrimination between healthy volunteers (open squares) and CFS patients (closed squares). (C) PC1 (solid line) and PC4 (dot line) loadings of the PCA.

1050 nm with a broad peak between 600 and 900 nm (Fig. 2C). PC4 loadings positively peaked around 900 and 1030 nm, with minima around 710 nm and 770 nm (Fig. 2C). PCA loadings were generally consistent with the discriminating power of the SIMCA model

Table 2
Prediction of CFS patients and healthy volunteers in test samples of the SIMCA model.

	Predicted healthy	Predicted CFS	No match
Actual healthy	179	43	0
Actual CFS	38	132	1

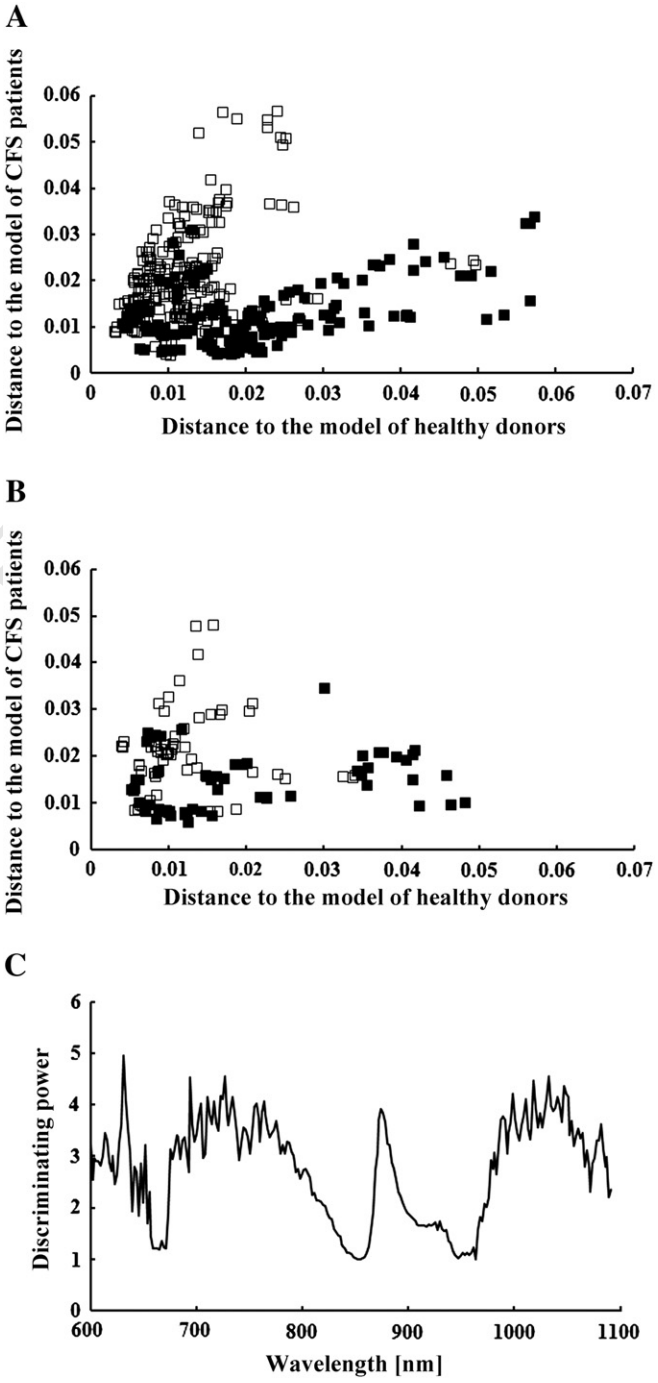

Fig. 3. Soft modeling of class analogy (SIMCA) of Vis-NIR calibration and prediction of CFS diagnosis. Vis-NIR spectral data of thumbs from healthy volunteers and CFS patients were pre-processed and subjected to SIMCA calibration modeling to develop a multivariate model to diagnose CFS. Cooman's plot of SIMCA demonstrating that the healthy volunteer class (open squares) and CFS patient class (closed squares) of test samples (A) and masked samples (B) did not share multivariate space. (C) Discriminating power from the SIMCA calibration model.

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Table 3
Prediction of CFS patients and healthy volunteers in masked samples of the SIMCA model.

	Predicted healthy	Predicted CFS	No match
Actual healthy	51	9	0
Actual CFS	17	42	1

(Fig. 3C). The most prominent discriminating power, which represents independent variables (wavelengths) important in discriminating two classes (CFS from healthy) were the sharp peak at around 900 nm and broad peaks at around 700–800 and 1000–1050 nm (Fig. 3C).

Oxyhemoglobin has greater absorbance at 850 nm than at 760 nm, whereas deoxyhemoglobin absorbs more at 760 nm than at 850 nm. In addition, the ratio of absorbance at 605 nm to 620 nm correlates with the oxidation of heme $a + a_3$ in cytochrome c oxidase [28]. Similarly, the ratio of absorbance at 830 nm to 780 nm correlates with the oxidation of copper in cytochrome *c* oxidase [29]. Intriguingly, the absorbance of these wavelengths in thumbs is different between CFS and healthy volunteers [22]. This differentiation in spectral characteristics may be due to a change of oxyhemoglobin/deoxyhemoglobin and oxidation of heme $a + a_3$ in cytochrome *c* oxidase in CFS patients [22]. Indeed, this change appears to influence the discriminating power in SIMCA derived from Vis-NIR spectra of thumbs (Fig. 3). The peak around 900 nm was also close to a water band at around 950 nm [16]. The peak near 800 nm was previously assigned to an amine [16]. However, further studies will be necessary to accurately perform band assignment for the important peaks of PCA loadings and discriminating power. Further information obtained from detailed analysis of NIR spectra may make significant contributions not only to the diagnosis of CFS but also to a better understanding of the pathogenesis of this condition.

These findings indicate that Vis-NIR spectra of the thumb combined with chemometrics analysis achieves separation of CFS patients from healthy volunteers. Although the current study involved only a small number of individuals, all of whom were Japanese, our approach deserves further evaluation as a potential strategy for objective diagnosis of CFS. Further studies will be necessary to confirm whether this method can be used for different population groups (i.e., ethnicity, age, etc.). Improved band assignment for the important peaks of PCA loadings and SIMCA discriminating power will be required in order to develop a more reliable diagnostic method. Detailed analyses of these Vis-NIR spectra may also help identify reliable biochemical markers for CFS as well as increase our understanding of the pathophysiology of this disease, which will inevitably lead to effective treatments. In addition, we previously showed that sera and fingernails of CFS patients could be distinguished from those of healthy controls [20,30]. These observations suggest that CFS patients share some factors and abnormalities in sera and fingernails, although we do not currently know whether such changes are related to the present observation of thumbs. Further study will be necessary to conclude whether there is a definite correlation between these observations. Noteworthy is the fact that non-invasive approaches to diagnosis of CFS have been shown to be particularly valuable. In addition, it will be interesting to ascertain whether other illnesses such as psychiatric disorders, whose diagnosis is based on clinical symptoms similar to CFS, could be diagnosed by this technique.

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