Potential use of visible and near-infrared spectroscopy for the analysis and diagnosis of chronic fatigue syndrome (Review)

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Received August 11, 2015; Accepted June 30, 2016

DOI: 10.3892/mmr.2016.5476

Abstract. At present, chronic fatigue syndrome (CFS) is diagnosed on the basis of clinical symptoms. Although various psychological, endocrinological and immunological abnormalities of patients with CFS have been reported, no clear consensus exists regarding the symptoms for this disorder. Thus, an objective diagnostic method for CFS is urgently required. The present study investigated the diagnosis and analysis of CFS using visible and near-infrared (Vis-NIR) spectroscopy. Previous studies have demonstrated the potential of Vis-NIR spectroscopy for diagnosing CFS by analyzing either serum samples as an invasive approach or thumbs as a non-invasive approach. Analysis of the Vis-NIR spectra of blood and thumbs suggested that factors absorbing in this spectral region are altered in patients with CFS compared with healthy individuals. These findings are likely to facilitate the search for biomarkers associated with CFS and to increase our understanding of the pathophysiology of the disorder. The current review aimed to outline the latest studies and discuss the future perspectives for CFS made possible by Vis-NIR spectroscopy.

Contents

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Key words: chronic fatigue syndrome, diagnosis, infrared spectroscopy, thumb, visible and near-infrared spectroscopy

1. Current status of chronic fatigue syndrome

Chronic fatigue syndrome (CFS) is a disabling condition in which the patient is affected by long-term fatigue. Symptoms, which can lasts for ≥6 months, include tiredness, pain, breathing problems, depression leading to digestive disturbances, low-grade fever, difficulty in concentrating, and weakness of the immune system and muscles (1). One characteristic of this disease is that the symptoms are not relieved by increased rest (1). Another characteristic of CFS is the absence of intervention or medication that is universally effective in treating the symptoms (2). Furthermore, CFS causes not only personal problems, but also economic problems. Several previous studies have demonstrated that the incidence of CFS is 0.4% in the USA and other countries (3) and 0.26% in Japan (4). CFS is present in 522/100,000 females and 291/100,000 males in the USA, indicating that CFS incidence in females is higher compared with in males (2). In addition, economic losses due to CFS are estimated to be as high as $9.1 billion per annum in the USA (5) and ¥408 billion per annum in Japan (4). Therefore, CFS has an impact on society in general, as well as on the individual patient.

While patients affected by CFS sometimes suffer from recognized symptoms, they often experience other social problems, which makes the disorder difficult to recognize. Research performed by the Centers for Disease Control and Prevention (Atlanta, GA, USA) estimates that <20% of patients with CFS in the USA have been successfully diagnosed (3,6). Clearly, the establishment of a reliable diagnostic test for CFS will improve our understanding of this disorder. However, several obstacles are preventing the accomplishment of this goal. One such obstacle is the high degree of heterogeneity in the symptoms of patients with CFS (7). Thus, at present, CFS is diagnosed on the basis of the presenting symptoms, coupled with the exclusion of other medical disorders (1). The other predominant barriers to diagnosing patients with CFS are an absence of biophysical and biochemical signs that identify the disease, and a lack of diagnostic laboratory tests (7). Consequently, typical screening results of blood samples for CFS are often unrevealing or negative. As a result, CFS diagnosis requires experience and techniques that can be performed only by skilled doctors on the basis of exclusion of all other possible causes of chronic fatigue as listed.

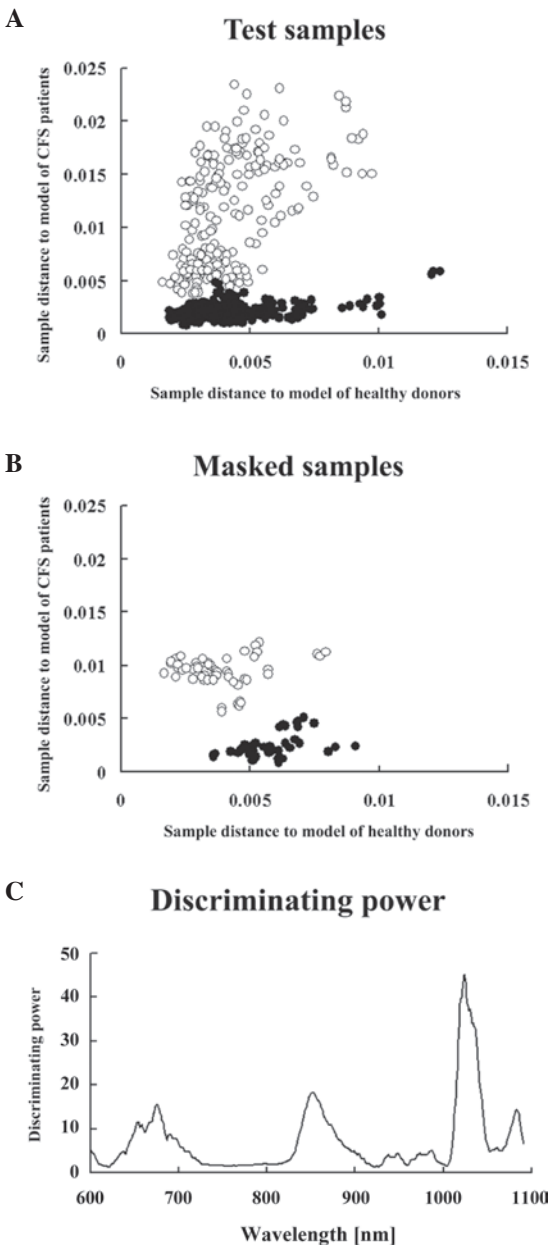

Figure 1. Vis-NIR spectroscopic analysis of sera for the diagnosis of CFS. A blood sample was obtained from the antecubital vein of CFS patients and healthy donors. Sera from 77 patients with CFS and 71 healthy donors were used as test samples to develop a calibration model for diagnosis. A further 99 samples were masked and used for prediction. The Vis-NIR spectral data of the test samples were pre-processed and subjected to SIMCA (8) calibration modeling to develop a multivariate model to diagnose CFS. Pirouette software (version 3.11; Infometrics, Woodinville, WA, USA) was employed for spectral data processing. The Coomans plot (9), which plots class distances against each other, demonstrated that the healthy donor class (open circles) and CFS patient class (closed circles) of (A) test samples and (B) masked samples did not share multivariate space. (C) The discriminating power of the SIMCA calibration model indicates important wavelengths for differentiating Vis-NIR spectra between CFS patients and healthy donors. Modified from Fig. 2 in Sakudo *et al* (10) with permission from Elsevier. Vis-NIR, visible and near-infrared spectroscopy; CFS, chronic fatigue syndrome; SIMCA, soft independent modeling of class analogy.

2. CFS abnormalities and research

Despite various data on the psychological, endocrinological and immunological abnormalities observed among CFS patients, no clear consensus exists on the symptoms for this disorder (12). Several indications of CFS have been reported, including altered levels of cytokines (13), immunoglobulins (13), autoantibodies (14), RNase L (15), 2'-5'oligo-adenylate synthetase (15), melatonin (16), dehydroepiandrostenedione (16), growth hormones (17), acylcarnitine (18), folic acid (19), vitamins (20), amino acids (20), carnitine Coenzyme Q10 (20), fatty acids (20) and minerals (20,21). Altered cell populations and activity of the immune system have also been reported (13,22). In addition, alterations in T-cell phenotype and proliferative response, along with the specific signature of the NK cell phenotype, have been reported in certain individuals with CFS (23). Other homeostatic changes involving the opioid system (24) and arginine vasopressin system (24) may be associated with CFS. Furthermore, adrenocorticotropic hormone and the cortisol response appear to be aberrant among patients with CFS (16).

The severe levels of fatigue and disability associated with CFS may be associated with peripheral inflammation and immune activation of blood cells, as is the case with neuroinflammatory and autoimmune illnesses (25). The mental and physical fatigue associated with CFS appears to be the consequence of interactions between multiple systemic and central pathways that take place via immune-inflammatory and neuroinflammatory networks (26). Such interactions would be supported by the activation of cytokines and immune cells, which has been reported in numerous previous studies (27,28).

This background to CFS prompted us to investigate fundamental abnormalities in patients with CFS and to develop an objective diagnostic method for this disease. As a result, our research group has recently explored an approach based on visible and near-infrared (Vis-NIR) spectroscopy.

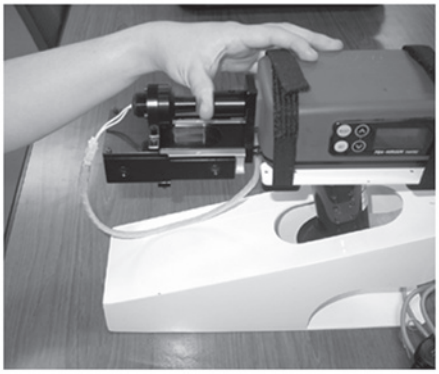

Figure 2. Spectrophotometer used for collecting Vis-NIR spectra from thumbs. Transmittance Vis-NIR spectra in the wavelength range 600-1,100 nm were non-invasively measured using an FQA-NIRGUN instrument (Japan Fantec Research Institute, Shizuoka, Japan). Reproduced from Fig. 1 in Sakudo *et al* (11) with permission from Elsevier. Vis-NIR, visible and near-infrared spectroscopy.

3. Vis-NIR spectroscopy for CFS research

The analysis of Vis-NIR spectra from the sera of patients with CFS identified certain characteristics that could be distinguished from the corresponding spectra of healthy

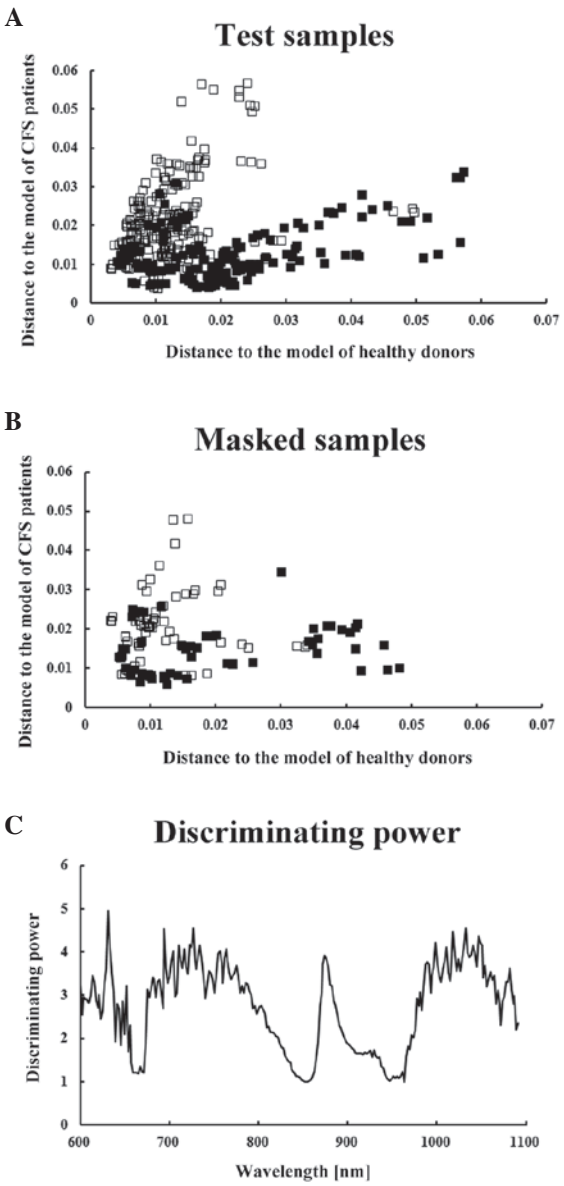

Figure 3. SIMCA of Vis-NIR calibration and prediction of CFS using thumb spectra. Vis-NIR spectral data obtained from the thumbs of healthy volunteers and CFS patients were pre-processed and subjected to SIMCA calibration modeling to develop a multivariate model to diagnose CFS. The Coomans plot of SIMCA demonstrated that the healthy volunteer class (open squares) and CFS patient class (closed squares) of (A) test samples and (B) masked samples did not share multivariate space. (C) Discriminating power of the SIMCA calibration model. Modified from Fig. 3 in Sakudo *et al* (11) with permission from Elsevier. Vis-NIR, visible and near-infrared spectroscopy; CFS, chronic fatigue syndrome.

donors (12,29). A chemometrics analysis, termed soft independent modeling of class analogy (SIMCA), was applied to develop a multivariate model to discriminate between the Vis-NIR spectra of patients with CFS and those of healthy individuals (Fig. 1). As a result, the SIMCA models correctly predicted 54/54 (100%) healthy subjects and 42/45 (93.3%) patients with CFS based on the analysis of Vis-NIR spectra from masked serum samples (10).

Subsequently, to develop a non-invasive approach, Vis-NIR spectroscopic analysis of thumbs was performed (Fig. 2). The SIMCA models were applied to the Vis-NIR spectra of thumbs from patients with CFS and healthy individuals

Table I. Spectroscopic comparison of the ratio of oxyhemoglobin to deoxyhemoglobin in the thumbs of patients with CFS and healthy controls.

	Absorbance at 850-760 nm	
Characteristic	Male	Female
Healthy	0.6137±0.0073	0.5005±0.0082
CFS	0.6517±0.0108	0.5352±0.0080
D'Agostino-pearson test (parametric data or not)	Yes	Yes
Unpaired t test (P-value)	(0.0027)[a]	(0.0027)[a]
Mann-Whitney test (P-value)	-	-

The mean Vis-NIR spectra of patients with CFS and healthy controls showed sharp peaks at 694, 970 and 1,060 nm, and broad peaks in the regions of 740-760 and 830-850 nm. The broad peak at 740-760 nm is associated with deoxyhemoglobin (30), whereas that at 830-850 nm is associated with oxyhemoglobin (31) and the oxidation status of cytochrome *c* oxidase (31,32). Therefore, the ratio of absorbance at 850 to that at 760 nm in smoothing- and standard normal variate-corrected Vis-NIR spectra was compared in males and females (69 healthy males, 42 CFS males, 53 healthy females and 61 CFS females). An increased ratio of oxyhemoglobin to deoxyhemoglobin in CFS thumbs was observed in the two groups. Absorbance is shown as the average ± standard error of the mean. [a]P<0.01. Reproduced from Table III in Sakudo *et al* (33) with permission from Elsevier. CFS, chronic fatigue syndrome; Vis-NIR, visible and near-infrared spectroscopy.

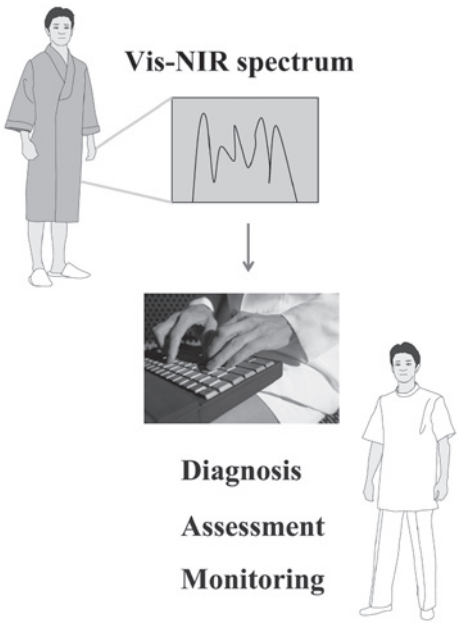

Figure 4. Future perspectives of Vis-NIR spectroscopy for CFS. Currently, CFS can be diagnosed on the basis of clinical symptoms only by skilled doctors. Thus, clinician experience and specialist techniques are required for the diagnosis of this condition. Vis-NIR spectroscopy may facilitate an objective and rapid diagnosis for CFS. In addition to diagnosis, Vis-NIR spectroscopy may also contribute to disease monitoring and assessment. To enable diagnosis, assessment and monitoring of CFS by Vis-NIR spectroscopy, the construction of a robust model by chemometrics analysis of Vis-NIR spectra will be important. Vis-NIR, visible and near-infrared spectroscopy; CFS, chronic fatigue syndrome.

(Fig. 3). The model successfully predicted 51/60 (83.3%) healthy subjects and 42/60 (70%) patients with CFS (11).

The discriminating power of the calibration models from these previous studies suggested the presence of common factors among the sera and thumbs of patients with CFS (Figs. 1C and 3C). One of these factors may be associated with blood flow and energy metabolism, since the Vis-NIR spectra of thumbs suggested that patients with CFS have a significantly higher oxyhemoglobin content (Table I), and significantly increased oxidation of heme $a+a_3$ and copper in cytochrome *c* oxidase (33).

IR spectroscopy is a form of vibrational spectroscopy. As such, the principal of IR spectroscopy is similar to that of Vis-NIR spectroscopy (34), although there are certain differences. The absorption observed in IR is due to fewer overtones as compared with Vis-NIR, resulting in sharper bands with higher intensity. Thus, IR spectroscopy is a powerful tool for CFS research. A previous study using IR spectroscopy found that fingernails of patients with CFS showed a decrease in α-helix content and increased β-sheet content compared with those of healthy individuals, suggesting reduced levels of normal protein elements of the nail plate (35).

4. Conclusion

As described above, previous studies have demonstrated the potential of Vis-NIR spectroscopy for the diagnosis of CFS using serum samples and thumbs. Furthermore, analysis of the Vis-NIR and IR spectra of sera, thumbs and fingernails suggests that factors absorbing in this spectral region are altered in patients with CFS. Combining chemometric analysis of spectra obtained from CFS samples with analysis of a spectra database may enable us to identify potential candidates for CFS biomarkers. Currently, although vast IR spectra databases, including the KnowItAll Informatics System (Bio-Rad Laboratories, Inc., Philadelphia, PA, USA), are commercially available, no similarly large NIR spectra databases exist. Thus, construction of an NIR spectra database would be necessary for a chemometrics-based study. Further analysis using such a spectra database would facilitate the search for biomarkers for CFS and aid our understanding of the pathophysiology of this disorder.

Although the current review has focused on previous studies using Vis-NIR spectroscopy to examine CFS, this approach can be applied to other diseases, including cancer (36,37), diabetes (38,39), Alzheimer's disease (40) and epilepsy (41,42). Therefore, the application of Vis-NIR spectroscopy to the diagnosis and analysis of disease in general is likely to increase. Its applications include not only diagnosis, but also assessment and monitoring of diseases (Fig. 4). In addition, Vis-NIR spectroscopy coupled with quantitative chemometrics analyses, including partial least squares and principal component regression, can be used to predict levels of biochemical constituents, including triglycerides, cholesterols, urea and lactate in body fluids, including blood (43,44) and hematocrit in the body (45). Therefore, this method may contribute to advances in clinical laboratory testing as well as maintaining human health in the form of a home testing kit.

Acknowledgements

The present study was supported by Grant-in-Aids for Scientific Research from the Japan Society for the Promotion of Science.

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