Visible and near-infrared interactance spectroscopy is a non-invasive technique which can be used to evaluate the hemoglobin concentration in endometriotic cyst fluid

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Abstract. Hemoglobin concentrations in endometriotic cyst fluids have been found to be associated with distinct clinical manifestations, such as pelvic pain and infertility, as well as with malignant transformation. However, the measurement of the hemoglobin concentration in cyst fluid is an invasive procedure. The present study aimed to evaluate the usefulness of visible and near-infrared interactance spectroscopy as a non-invasive technique for estimating the hemoglobin concentration in endometriotic cystic fluid. Optical fibers were directly placed onto sliced raw pork (up to 10-mm-thick as an anatomical barrier on the cyst's surface) that covers a cuvette containing hemoglobin solution or endometriotic cyst fluid. Partial least square regression based on the second derivative using visible and near-infrared interactance spectroscopy (wavelength region, 500-1,200 nm) was used to estimate the hemoglobin concentration. The samples were categorized into the evaluation sets (i.e., calibration set) to create calibration curves and test sets (i.e., validation set) to validate equations. The cyst fluid at 5 mm of pork thickness achieved a high correlation between actual and predicted hemoglobin concentrations (calibration (R^2=0.977) and validation (R^2=0.874) data). However, the correlation slightly decreased at 10-mm pork thickness (i.e., calibration (R^2=0.979) and validation (R^2=0.580) data). Interactance spectroscopy may thus be a non-invasive tool which can be used to estimate the hemoglobin concentration in endometriotic cyst fluid when the anatomical barrier is 5 mm. This technology is a reliable modality for predicting the severity of dysmenorrhea and infertility, as well as malignant transformation, in a number of patients with endometriotic cysts. Such quantitative optical spectroscopic imaging technologies may enable the accurate diagnosis of the pathological processes in endometriotic cysts in clinical practice.

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Introduction

Endometriosis, a prominent condition among women of reproductive age, causes pelvic pain and infertility and may be a risk factor for the development of ovarian cancer (1). Non-invasive approaches, such as multiple biomarkers, imaging techniques using ultrasound or magnetic resonance, and questionnaires have been implemented in clinical practice as screening, diagnosing and triage tests for endometriosis. Multiple databases of relevant articles have revealed that a subset of blood biomarkers, such as CA125 can be a non-invasive test to accurately diagnose or distinguish endometriosis from other benign ovarian masses (2). However, currently available evidence is insufficient to draw any clinically meaningful conclusions (2). Furthermore, a score based only on a questionnaire developed using the clinical and epidemiological data of patients may become an emerging diagnostic tool which can help clinicians to identify individuals who are at a high risk of developing endometriosis (3). The authors have been studying the association between cyst fluid components and clinical symptoms by focusing on ovarian endometrioma, which can easily be evaluated using ultrasound. The ovarian endometriotic cyst contains various iron-related compounds, such as hemoglobin, oxyhemoglobin, methemoglobin, heme iron and free iron (4). These compounds cause oxidative stress characterized by a redox homeostasis imbalance, and result in cell damage by affecting DNA, lipids and proteins, causing tissue injury and repair and pathological fibrosis (4,5). Of note, iron-related compounds are closely associated with the clinical manifestations of endometriosis (6,7). Iron in endometriotic cysts has been revealed as a predictive biomarker for the assessment of the severity of dysmenorrhea (7). Iron-induced oxidative stress plays a role in promoting neuropathic pain through nociceptor sensitization (7). Moreover, iron has been reported to be a useful predictor for infertility in women with endometriotic cysts, as excessive levels of reactive oxygen species are a main cause of female reproductive disorders (6,8). Indeed, the cyst fluid concentrations of iron have been found to be significantly higher in patients with infertility than in those with no infertility (median, 324.8 mg/l vs. 226.5 mg/l; P=0.019) (6). Additionally, the levels of iron-related compounds (e.g., total iron, heme iron and free iron) have been found to be markedly lower in patients with endometriosis-associated ovarian cancer than in women with endometriotic cysts, indicating that these compounds may predict the malignant transformation of endometriosis (9,10). A pulse oximeter can measure oxyhemoglobin and deoxyhemoglobin levels in arterial and venous blood. However, the cyst fluid in endometriosis does not contain deoxyhemoglobin and has a high methemoglobin concentration; thus, critical hemoglobin differences exist between cyst fluid and circulating blood (10). Therefore, no method has yet been established to non-invasively quantify methemoglobin and oxyhemoglobin concentrations, at least to the best of our knowledge. However, metallobiology technology has provided viable new solutions with which to resolve this challenge.

One of the most prominent, rapid and non-destructive testing methods for evaluating the external and internal quality attributes of various fruits and vegetables is the spectral imaging system using optical spectroscopy (11,12). Additionally, quantitative optical spectroscopic imaging is an attractive modality for various biomedical and clinical applications, and is a particularly promising technique for the non-invasive monitoring of patients in emergency clinical settings (e.g., ambulances, emergency rooms and operating rooms). This imaging technique has provided a quantitative, non-invasive and real-time monitoring of total hemoglobin and oxygen saturation (13). Optical spectroscopy has different measurement modes: Reflectance, transmittance and interactance spectroscopy (14). The most widely used reflectance spectroscopy detects changes in structural, biochemical and optical properties in samples that are close to the surface of biological tissue (e.g., leaf and skin), and also measures refractive indices (15). The reflectance method may be susceptible to threshold fluctuations caused by different spatial, temporal and physiological variations between subjects (e.g., variations in the color, tone and blood flow of the vaginal surface, and the thickness of the intervening vagina, muscle and fat layer) (16). Furthermore, transmittance spectra penetrate deeper compared to reflectance spectra; however, transmittance spectroscopy has the disadvantage of providing information only when the incident light can pass through the object. Over the past decade, the authors have investigated non-invasive methods which can be used to quantify iron or hemoglobin concentrations in endometriotic cyst fluid (10,16,17). The authors previously developed the near-infrared reflectance and transmittance spectroscopy system that non-invasively measures the hemoglobin and iron content of human endometriotic cyst fluids in *ex vivo* studies (10,16,17). However, the previous optical method (reflectance and transmittance spectroscopy) reduced the stability, reproducibility, sensitivity and accuracy of the measurements when the cyst fluid sample was covered with raw pork, beef, or chicken with a thickness of ≥5 mm (10,16). Conversely, unlike reflectance and transmittance spectroscopy, interactance spectroscopy collects more information from light that interacts with the internal elements of the sample, including the morphological, biochemical and biophysical composition (18). There is recent evidence to indicate that the interactance-based evaluation is an effective and non-destructive method which can be used to quantify deeper tissue components of a variety of crops, fruits and vegetables, and to conduct quality assurance (11,18).

Therefore, the authors aimed to develop non-invasive methods which can be used to estimate the hemoglobin concentrations using the interactance method. The present study aimed to evaluate whether interactance spectroscopy can accurately predict the hemoglobin concentration in endometriotic cyst fluid in an *ex vivo* experiment.

Materials and methods

Study population. A list of patients with adnexal masses who were treated at the Department of Gynecology, Nara Medical University Hospital, Kashihara, Japan, was generated from the institutional registry from January, 2008 to December, 2020. All cyst fluid samples collected from the patients intraoperatively were immediately aliquoted and frozen at -80˚C. The patients signed written informed consent forms for the use of their clinical data for research. They were also invited to provide biobanking consent for future research. The Institutional Review Board and the Research and Ethical Committee of Nara Medical University Graduate School of Medicine approved the study (approval no. 3377). Additionally, informed consent for the secondary use of biospecimens (measuring biomarkers in stored frozen serum samples) was obtained using an opt-out approach in all included patients. The present retrospective study analyzed prospectively collected data. The inclusion criteria were the following: i) Patients with a sufficient cyst volume to measure hemoglobin levels; ii) patients undergoing surgery that involved lesion removal for histological evaluation; and iii) patients with histologically proven ovarian endometrioma. The criteria for exclusion were as follows: i) Patients currently receiving hormone therapy or had received therapy within the previous 6 months; ii) those who had a history of malignancies; iii) pregnancy; iv) concomitant severe comorbidities; and v) incomplete, inadequate or missing data. Additionally, the following clinicopathological, hematological and imaging data were obtained from medical records of the patients: Age, body mass index (kg/m^2), parity, menopausal status, tumor size and histology. The present study only included patients with ovarian endometrioma (n=22) and excluded patients with superficial peritoneal endometriosis or deep infiltrating endometriosis.

Setup for interactance spectroscopy. The authors previously developed reflectance spectroscopy as a non-invasive near-infrared spectroscopic approach to quantify hemoglobin and total iron concentrations, which are determined by the absorption and scattering coefficients (16). The present study assessed the feasibility of visible and near-infrared interactance spectroscopy as an alternative to the reflectance and transmittance methods (18). The system is composed of a light stimulator (a halogen lamp), a measuring instrument, photoelectric detection, an image acquisition module and image signal processing (Fig. 1) (10). The source fiber (the illuminator and incident light) and detector fiber (the interactance spectra) were placed directly onto the pork that covers a cuvette containing the sample during the measurement. The present study used commercially available sliced pork, beef,

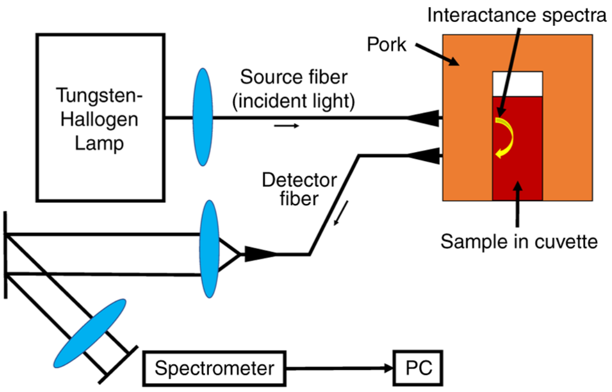

Figure 1. Simplified schematic diagram for the interactance spectroscopy setup. The end faces of a pair of optical fibers were placed on the surface of the sliced pork that covers a cuvette containing hemoglobin solution or endometriotic cyst fluid. The prepared cuvettes were covered with either 5-mm- or 10-mm-thick commercial sliced pork to mimic the anatomical barrier structures.

or chicken, used for human consumption. The center-to-center distance of the two fibers was maintained constant at 5 mm on the meat. The present study used 5-mm- or 10-mm-thick sliced commercially available pork-wrapped glass cuvettes in place of the intervening vaginal wall, fat and cyst surface layers to mimic human ovarian endometriomas. The amount of light that returned to the detector after scattering in the sample was measured. This light was coupled into a spectrometer (Ocean Optics USB4000, 600-1,000 nm, OptoSirius Corporation), which recorded the light on a computer. Matlab simulations (MathWorks Inc.) were used to analyze the interactance spectral data. The USB4000 enabled the capture and storage of a full spectrum in memory every millisecond. Spectra exported into Matlab were reconstructed onto the calibrated wavelength axis. Quantitation was completed from a calibration regression using the interpolation method.

Sample preparation. The present study used adjusted hemoglobin solutions and human endometriotic cyst fluids. First, hemoglobin was dissolved in phosphate-buffered saline containing 5 g/dl albumin (Nacalai Tesque, Inc.) and adjusted to 0, 0.5, 1.0, 2.0, 3.0 and 4.0 g/dl to create a standard curve. A total of 13 samples of each concentration were assigned to the calibration set (54 samples) and validation set (24 samples). This experiment was repeated with 5-mm (36 samples) and 10-mm (42 samples) thick pork slices, with a total of 78 samples. Second, a total of 22 endometriotic cyst fluid samples were obtained from biobanks. The demographics and baseline characteristics of the study cohort (n=22) at diagnosis were as follows: Mean age, 35.6 years (range, 29-44 years); parity, 0 (median; range, 0-3); tumor size, 61 mm (median; range, 40-93 mm); and CA125, 62.5 U/ml (median; range, 7.0-388.1 U/ml). Each frozen sample was divided into up to four equal aliquots. Of note, one aliquot contained 2 ml cyst fluid. Insufficient sample volumes were divided into at least two (9 patients) or three aliquots (8 patients). Overall, from a total of 22 frozen samples, 62 aliquots were originated so that 40 aliquots (first 65%) could be assigned to the calibration dataset and the remaining 22 aliquots (remaining 35%) for the validation dataset.

Modeling methods and assessment for predicting the hemoglobin concentration using the interactance measurement system. The Beer-Lambert law was used to calculate absorbance (19). Differences in the intrinsic viscosity of samples produce unwanted background (e.g., baseline offset, slope and shift) in the spectra that may adversely affect the creation of a calibration curve. Performing the pre-treatments of raw spectra using the second derivative prior to multivariate analysis is an effective strategy which can remove these effects (20). A second derivative has been reported to improve the analytical performance of the model (20). Multivariate analysis was then performed to model the association between spectral data and hemoglobin concentration. Partial least square (PLS) regression, a multivariate calibration technique, is a statistical method that extracts a set of factors used to identify predictors in the regression model, which allows for the quantitative analysis of spectral data (11,21,22). Therefore, the present study analyzed spectral data using the second derivative calculated from raw interactance spectra and then estimated the hemoglobin concentration using PLS regression.

Quantification of the hemoglobin concentration in endometriotic cyst fluid. The Sysmex automated hematology analyzer XN 330 (Sysmex UK Ltd.) was used to measure hemoglobin levels.

Statistical analysis. The proposed method consists of two steps. The first step compared measured and predicted hemoglobin concentrations using the calibration and validation sample sets. The second step compared the measured and predicted hemoglobin concentrations in stored endometrial cyst fluid to assess the feasibility of a clinical application. The second derivative and PLS regression were calculated and calibration and validation models were fitted using the software Unscrambler X (Ver. 10.5.1, CAMO Software) installed on a personal computer. P-values were not calculated.

Results

Visible and near-infrared spectral data obtained through the interactance measurement technique from solution samples with various hemoglobin concentrations. Raw interactance spectra in the visible wavelength region (500-800 nm) and near-infrared wavelength region (800-1,200 nm) for the hemoglobin solution are presented in Fig. 2. The interactance measurement technique was used to monitor spectral absorbances. The absorbance was measured using the 10-mm-thick sliced pork-wrapped glass cuvettes that contain various hemoglobin concentrations. The interactance technique demonstrated the change in the absorbance curve on visible and near-infrared spectra in response to the hemoglobin concentration. The spectra had typical peaks for hemoglobin at 600-650 nm and 900-1,000 nm. The interactance spectra demonstrated marked differences in the absorbance value among various hemoglobin concentrations; however, all spectra exhibited a similar pattern along the wavelength range. Replacing pork as the anatomical barrier with beef or chicken did not alter the spectral pattern (data not shown).

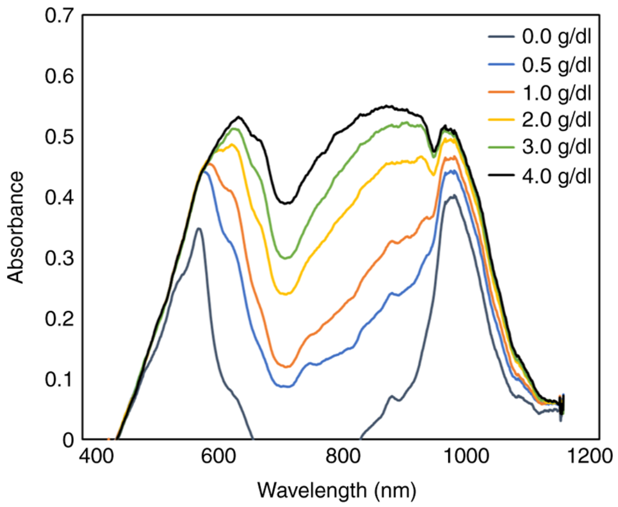

Figure 2. Visible and near-infrared spectral data obtained through the interactance measurement technique from solution samples with different hemoglobin concentrations. This figure shows the absorbance peaks and valleys of the raw interactance spectra corresponding to the variation of chromophore hemoglobin concentrations. Each sample contained various hemoglobin concentrations (0, 0.5, 1, 2, 3 and 4 g/dl). The Beer-Lambert law was used to calculate the absorbance.

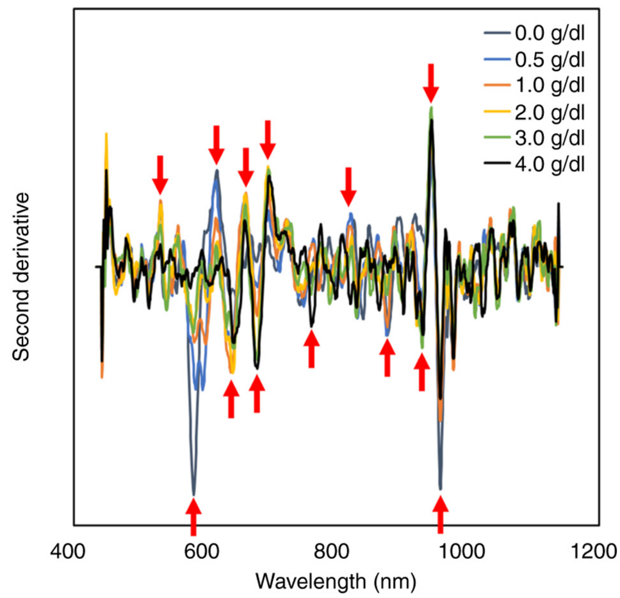

Figure 3. Second derivative spectra calculated from raw interactance spectra.

Second derivative spectra calculated from raw interactance spectra. The second derivative spectra calculated from the raw interactance spectra of various hemoglobin concentrations are presented in Fig. 3. The second derivative spectra demonstrated at least 13 sharp peaks, as indicated by red arrows. The hemoglobin concentration was statistically calculated using the multivariate analysis of these peaks.

Association between the actual and predicted hemoglobin concentration in solution samples with various hemoglobin concentrations using the calibration and validation sample sets. The PLS models were used following the second derivative spectra for predicting the hemoglobin concentrations. The calibration and prediction curves were initially created using a total of 78 solutions that contained various hemoglobin concentrations. The samples were assigned to calibration sets (i.e., evaluation sets, n=54) to create calibration plots and validation sets (i.e., prediction sets, n=24) to validate equations. The correlation between the actual and predicted hemoglobin concentrations is presented in Fig. 4. The prepared cuvettes were covered with either 5-mm-(Fig. 4A) or 10-mm-thick (Fig. 4B) commercial pork slices (9). As shown in Fig. 4A, the model achieved satisfactory predictions for the calibration (R^2=0.978) and validation sets (R^2=0.930). Additionally, the calibration (R^2=0.931) and validation sets (R^2=0.913) yielded satisfactory predictions even with the 10-mm pork thickness (Fig. 4B). Both results produced linear calibration curves.

Association between the actual and predicted hemoglobin concentration in endometriotic cyst fluids using the calibration and validation sample sets. The present study then investigated the hemoglobin concentration prediction in endometriotic cyst fluids using the interactance method. The aliquot samples were assigned to calibration sets (n=40) and validation sets (n=22). The calibration and validation data (Fig. 5) estimated against the measured hemoglobin concentrations in the cystic fluid, as well as the data obtained from the cuvettes covered with 5- and 10-mm-thick pork slices, respectively. The model for 5-mm-thick pork slices achieved satisfactory predictions for the calibration (R^2=0.977) and validation datasets (R^2=0.874) (Fig. 5A). The calibration (R^2=0.979) and validation (R^2=0.580) data for the 10-mm-thick pork slices estimated against the measured hemoglobin concentrations (Fig. 5B). The predicted concentration tended to reach a plateau at higher hemoglobin levels (>8 g/dl).

Discussion

Recent studies have revealed that iron or hemoglobin levels in endometriotic cyst fluid are associated with the malignant transformation of endometriosis and the severity of dysmenorrhea and infertility outcomes (6-8). Currently, investigations such as laparoscopy and fine-needle aspiration biopsy, are required to measure endometriotic cyst fluid concentrations of these parameters. Such techniques are considered invasive, costly and unsuitable as population-wide screening tools. Therefore, the authors investigated the feasibility of non-invasive optical diagnostics using the interactance method to predict the hemoglobin concentration of endometriotic cyst fluid in an *ex vivo* experimental model. The hemoglobin concentration was estimated using PLS regression based on the second derivative using visible and near-infrared interactance spectroscopy. The present study first used hemoglobin solutions with various concentrations (Fig. 4) and then endometriotic cyst fluids as samples for measurements (Fig. 5). The validation sets (R^2=0.913) produced a satisfactory prediction in hemoglobin solution samples even with a 10-mm pork thickness (Fig. 4B), indicating a strong correlation between the actual and predicted hemoglobin concentrations. Additionally, selecting cyst fluid samples at a 5-mm pork thickness demonstrated satisfactory discrimination in the validation cohorts (R^2=0.874) (Fig. 5A). However, the correlation between the actual and predicted

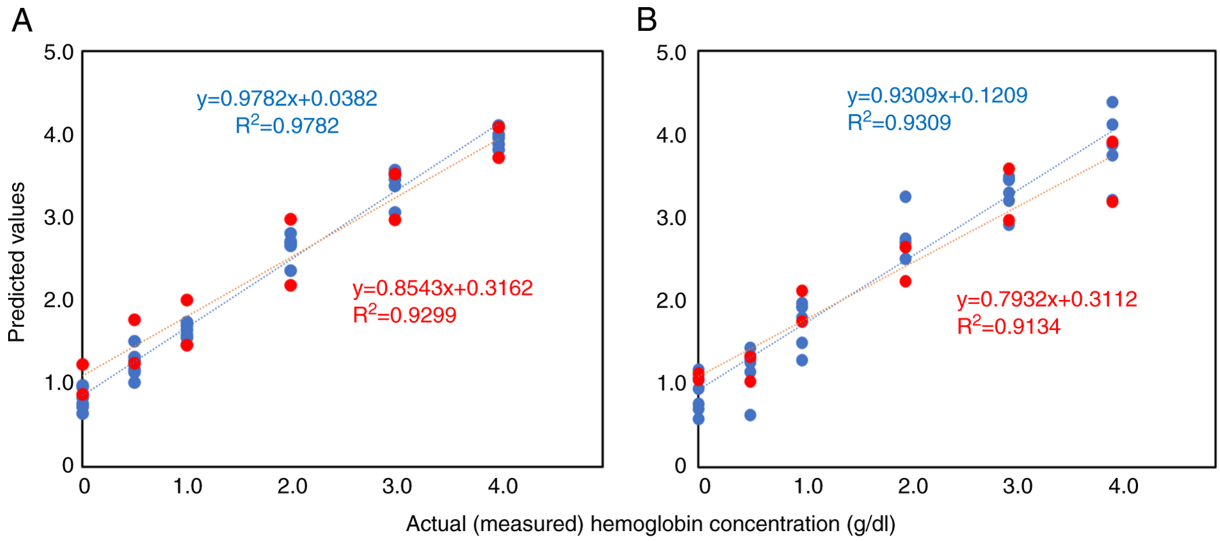

Figure 4. Scatterplots for the actual vs. the predicted hemoglobin concentrations in solution samples with various hemoglobin concentrations in the calibration and validation sets. The association between the actual and predicted hemoglobin concentrations in hemoglobin solutions containing various hemoglobin concentrations via the interactance technique. Scatterplots show the actual vs. the predicted hemoglobin concentrations in the calibration (blue) and validation (red) sets. (A) A cuvette covered with a 5-mm-thick pork slice. A total of six samples of each concentration were assigned to the calibration set (four samples of each concentration, blue circle) and validation set (two samples of each concentration, red circle). (B) A cuvette covered with a 10-mm-thick pork slice. A total of seven samples of each concentration were assigned to the calibration set (five samples of each concentration, blue circle) and validation set (two samples of each concentration, red circle).

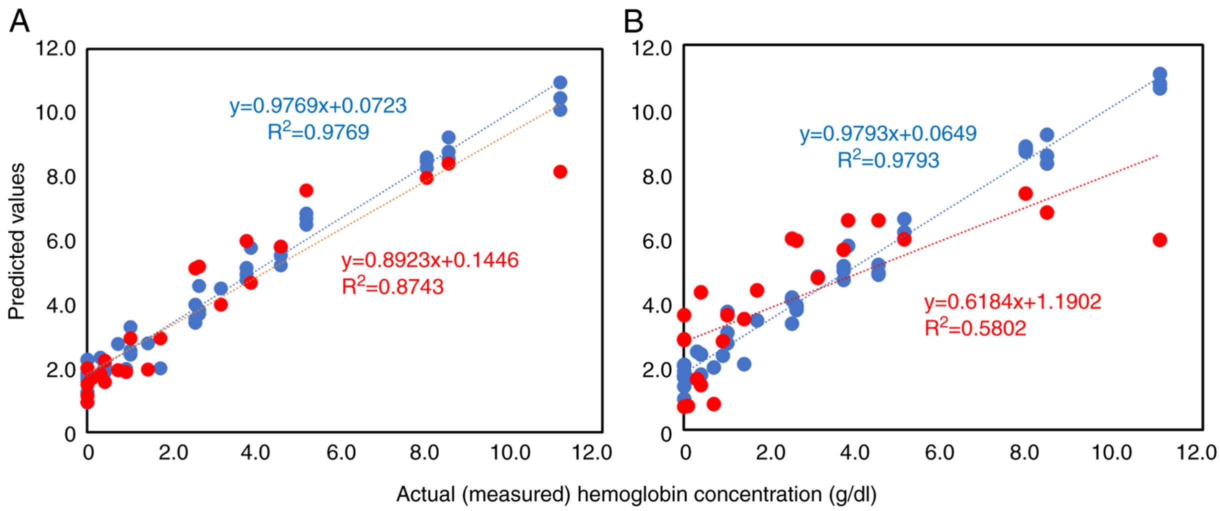

Figure 5. Scatterplots for the actual vs. the predicted hemoglobin concentrations in endometriotic cyst fluids in the calibration and validation sets. The figure illustrates the association between actual and predicted hemoglobin concentrations in the cystic fluid using the calibration (blue) and validation (red) sets. (A) A cuvette covered with a 5-mm-thick pork slice; and (B) a cuvette covered with a 10-mm-thick pork slice. A total of 40 cyst fluid samples were assigned to the calibration set and 22 to the validation set.

concentrations in the cystic fluid decreased slightly at a thickness of 10 mm, which may underestimate the hemoglobin concentration of >8 g/dl [i.e., validation (R^2=0.580); Fig. 5B]. These preliminary results indicate the clinical usefulness of the interactance method when the intervening vaginal and fat layer thickness is 5 mm.

A previous *ex vivo* study revealed that the reflectance method attenuated the power to predict the hemoglobin concentration when the sample was covered with a 5-mm-thick meat slice (10). By contrast, unlike the reflectance method, the interactance method has the advantage of being less susceptible to the various physiological variations between subjects (e.g., variations in vaginal surface tone and intervening vagina and fat layer thickness) (14). This may be as more interactions occur between the scattered light and substances in the cyst fluid when incident light passes through a certain distance within the cyst, and the interactance spectra provide more useful information than the reflectance spectra (14). This fundamental principle of the interactance method underlying the light behaviors facilitates further development of instruments for predicting hemoglobin concentration. Moreover, the present study utilized PLS regression based on the spectral preprocessing method, which is the second derivative, rather than the raw spectral data to improve the analytical performance of the model. The second derivative spectra reported better results than the raw spectra (20). This technology helps in the production of innovation and quality control in many fields of not only medicine, but also those of agriculture, chemistry and industry (e.g., the detection of adulteration, predicting chemical and nutritional properties of fermented barley, or identification of pharmaceutical ingredients). In particular, an interactance spectroscopy approach is currently a powerful tool for quantitative analysis for estimating the degree of red coloration and then grading and sorting systems for apples in the fruit industry (11,18). This technique has also been applied to identify fruits and vegetables that have been physically damaged internally (14,23). Therefore, the interactance method has been used for the non-destructive evaluation of the quality characteristics of fruits, vegetables, beverages, pharmaceuticals, etc., and is useful in our daily lives (11,14,18,23-26).

Finally, the feasibility and future perspectives of interactance technology in clinical practice is discussed. Transvaginal ultrasound (TVS) and magnetic resonance imaging (MRI) are currently available non-invasive imaging modalities for diagnosing and managing endometriosis. TVS, MRI and interactance technology have their advantages and limitations. TVS and MRI are powerful tools which can be used to discriminate ovarian cancer from benign pelvic mass and detect anatomical changes (e.g., the appearance of papillary projections, solid components and abnormal ascitic fluid), enabling the assessment of the malignant transformation of endometriosis. In particular, these imaging modalities cannot predict malignant transformation without the appearance of anatomical distortions and structural abnormalities. By contrast, interactance technology has provided valuable biochemical information that significantly differs from the morphological information obtained by conventional imaging modalities. A previous study revealed that cyst fluid iron levels predicted malignant transformation with a sensitivity of 90.9% and a specificity of 100% for women with endometriosis (9). Therefore, this technology may be one of the reliable modalities for predicting the clinical outcomes of patients with endometriosis. Moreover, iron levels have been shown to be associated with the severity of endometriosis-related infertility (6) and dysmenorrhea (7). However, other than the iron concentration, factors, such as anatomical distortions, immunological disturbances and endocrine abnormalities, may also contribute to the etiology of endometriosis-related dysmenorrhea and infertility (27). Therefore, whether the hemoglobin concentration alone can accurately estimate the severity of endometriosis remains unclear. To date, research has aimed to discover reliable non-invasive biomarkers, including serum markers or imaging modalities, for the early prediction and management of endometriosis (28). The interactance technology is a non-invasive, real-time, low-cost and reliable tool for rapidly and accurately predicting the hemoglobin content of endometriotic cysts. It is considered that conventional imaging modalities, in addition to the interactance method, provide complementary data that may aid in the diagnosis and monitoring of not only women with suspected malignant transformation, but also of women with pelvic pain and infertility. However, hemoglobin concentrations >8 g/dl are expected to be underestimated with the increased distance of the cyst from the vaginal surface. The distance from the optical fiber surface may affect the measurement of the hemoglobin concentration based on the interactance method, thereby limiting its use in clinical practice. Furthermore, new devices, including conventional TVS and interactance spectroscopy, are required for clinical use in an office setting. These devices may potentially extend the interactance spectroscopy system to clinical applications in other fields. In particular, this technology may help predict fetal anemia or estimate hemoglobin concentrations at sites of intracerebral hemorrhage in newborns. Future studies are required to further explore these challenges.

In conclusion, PLS regression based on the second derivative using visible and near-infrared interactance spectroscopy may be used to estimate the hemoglobin concentration in endometriotic cyst fluid. The interactance method accurately predicted the hemoglobin concentration when the anatomical barrier covering the cyst was <10-mm-thick, e.g., 5 mm. This technology may be potentially applied to diagnostics for predicting the severity of clinical manifestations in women with ovarian endometrioma. Further studies are required however, to assess the usefulness of the interactance method in clinical applications for diagnosis, patient stratification for specific treatment, or therapy monitoring.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HK conceptualized the study, and was involved in the study methodology and provided software. HK was also involved in the writing, reviewing and editing of the manuscript and in visualization. SI and HK were involved in data validation and curation. SI was involved in the formal analysis, data investigation, in the provision of resources, and in the writing and preparation of the original draft, as well as in funding acquisition. SI and FK were also involved in the conception and design of the study. FK was involved in the study supervision and project administration. SI and HK confirmed the authenticity of all the raw data. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki, and was approved by the Institutional Review Board of the Nara Medical University (approval no. 3377). Written informed consent was obtained from all subjects involved in the study. The opt-out form was provided through the institutional homepage.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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