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Original Article

Ultrasonographic quantification of the endometrium during the menstrual cycle using computer-assisted analysis

Szu-Yuan Chou [a], Chih-Yen Chen [b], Hung-Wen Su [a], Ming-I Hsu [a], So-Rong Liang [a], Chun-Sen Hsu [a,*]

[a] *Department of Obstetrics and Gynecology, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan*
[b] *Instrument Technology Research Center, National Applied Research Laboratories, Hsinchu, Taiwan*

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Abstract

Objective: Sonographic gray-scale histogram is used to assess the endometrial changes in the different phases of the menstrual cycle. The objective was to examine the usefulness of a gray-scale histogram and computer-assisted image analysis software in assessing normal physiologic states of the endometrium with sonography.
Materials and Methods: Thirty-eight patients, who visited the Taipei Medical University-Wan Fang Hospital and matched the eligibility criteria, were categorized into one of three groups: (1) menstrual phase; (2) follicular phase; and (3) luteal phase of the menstrual cycle. Ultrasonography of the uterus was performed on each patient and the endometrium was analyzed with ImageJ image analysis software.
Results: A statistically significant difference in signal intensity scores of the gray-level histogram, represented as m_j, was found among the three groups.
Conclusion: Sonographic images analyzed by using computer-assisted image analysis software and gray-level histogram are proven to be useful in assessing the physiological state of the endometrium.

Keywords: Computer-assisted analysis; Endometrium; Gray-level histogram; Menstrual cycle; Ultrasonography

Introduction

The different phases of the monthly menstrual cycle are attributed to the interaction between sex hormones and endometrial reactions. Endometrial abnormalities are frequently evaluated by sonography [1]. Ultrasonographic examination of the endometrium is invariably needed to evaluate various potential uterine abnormalities related to patients' irregular cycles, amenorrhea, or infertility. The use of this widely available diagnostic assessment of morphology for entire organs, such as the uterus or the ovaries, depends largely on a labor-intensive operator-dependent subjective grading system of echogenicity patterns rather than absolute quantification. The advent of commercially available computer-assisted image analysis (CAIA) software for three-dimensional ultrasonography has allowed highly reliable measurements of uterine structures and endometrial conditions [1]. An accurate measurement of endometrial thickness is important for the evaluation of various uterine physiologic and pathologic conditions [2,3].

ImageJ is a Java-based CAIA public domain software developed by the National Institutes of Health (Bethesda, MD, USA) [4]. The efficacy of ImageJ in image processing and analysis has been demonstrated with breast cancer, adipose tissue, lung cancer, cell adhesion dynamics, cyclic uterine changes, and pelvic organ perfusion [5−10]. In this study, we examined the usefulness of ImageJ software in analyzing ultrasonographic images of the uterus to assess physiologic

* Corresponding author. Department of Obstetrics and Gynecology, Taipei Medical University-Wan Fang Hospital, No. 111, Section 3, Hsing Lung Road, Taipei City 116, Taiwan.
E-mail address: morritw@yahoo.com.tw (C.-S. Hsu).

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Table 1
Descriptive statistics for intensity score m_j of menstrual phase, follicular phase and luteal phase

Descriptive statistics	Menstrual phase ($n = 7$)	Follicular phase ($n = 14$)	Luteal phase ($n = 17$)	p
Median (interquartile range)	131,574 (108,460–153,173)	293,561 (216,433–467,682)	490,757 (343,641–637,573)	<0.0007

All multiple analyses (menstrual phase *vs.* follicular phase *vs.* luteal phase) are significant at the 0.05 level using Kruskal-Wallis test.

states of the endometrium in normal menstrual cycles. Our study was designed to evaluate ultrasonographic quantification of the endometrium using ImageJ software in those women with a normal menstrual cycle.

Methods

This study was conducted between January and December in 2008 at the Taipei Medical University-Wan Fang Hospital and consisted of 38 female patients from the ultrasound registry of the Department of Obstetrics and Gynecology (most of them are from health examination patients) who fit the criteria of normal menstrual cycle: never pregnant; between 20 and 40 years of age; regular menstrual cycles, lasting 26–32 days with 3–8 days of bleeding, accompanied by only mild menstrual pain; nonhormonal contraception for the previous 2 months; no cycle disturbances for at least 1 year; and no previous major gynecological surgery. We divided our patients into the following groups: (1) menstrual phase, Day 1–8 of the menstrual cycle with vaginal bleeding; (2) follicular phase, Day 5–14 of the menstrual cycle without vaginal bleeding; (3) luteal phase, Day 15 to the day before menstruation. This study was approved by the ethics committee of our institution.

Ultrasonography was performed for the patients with normal menstrual cycles by a sonographer who had had gynecological ultrasonographic practice for more than 3 years. All data were acquired with a color Sonoace (SA)-8000 PRIME ultrasound system (Medison Co Ltd, Seoul, Korea) equipped with an 8.5 MHz curvilinear transvaginal transducer. Identical ultrasound settings, that is, magnification, focus line, gain, time gain compensation, were used for each examination. All of the two-dimensional longitudinal images were reviewed by one of our authors (SYC) who analyzed all of the ultrasonography in the PC computer.

Image gray-level histogram quantification was made by ImageJ software (available at http://rsb.info.nih.gov/ij/). The software was employed to measure the pixel value and area of the user-defined region (endometrium) in the ultrasonographic image. The quantitative ultrasonographic criteria for signal intensity of the gray-level histogram of the endometrium was analyzed for each of the three patient groups, where $p\ (x_{ij})$ represents the frequencies of the histograms, i represents gray-scale levels, and j represents the participants of one group: Signal intensity score of gray-level histogram: m_j

$$m_j = \sum_{i=0}^{255} x_{ij} \times p\left(x_{ij}\right)$$

where $i = 0, 1, 2, 3, \ldots 255$, $j = 1, 2, 3, \ldots 38$.

Once the signal intensity scores were obtained, a Kruskal-Wallis test (applied as a nonparametric analysis of variance test) was performed by the skewed data to evaluate the statistical differences between the three groups. In addition, the signal intensity scores derived at two different times by the same author (SYC) were compared by using Bland-Altman analysis. Statistical analysis was performed using SPSS, version 12.0 for Windows (SPSS Inc., Chicago, IL). A p value less than 0.05 was considered statistical significance.

Results

Thirty-eight patients were categorized into three groups: (1) menstrual phase, $n = 7$; (2) follicular phase, $n = 14$; (3) luteal phase, $n = 17$. Descriptive statistics for intensity score m_j were summarized using the median (50th percentile) and 25th and 75th percentiles in Table 1 are shown as a scatter plot in Fig. 1. The correlation of signal intensity scores measured at two different times and a Bland-Altman plot are shown in Fig. 2. The results indicate no significant difference between the two measurements. Statistically significant differences were obtained ($p < 0.05$) among the menstrual phase, follicular phase, and luteal phase. Representative ultrasonographic examinations before and after using ImageJ image analysis are shown in Figs. 3 and 4. The suspicious lesions can be identified by signal intensity scores according to different phases,

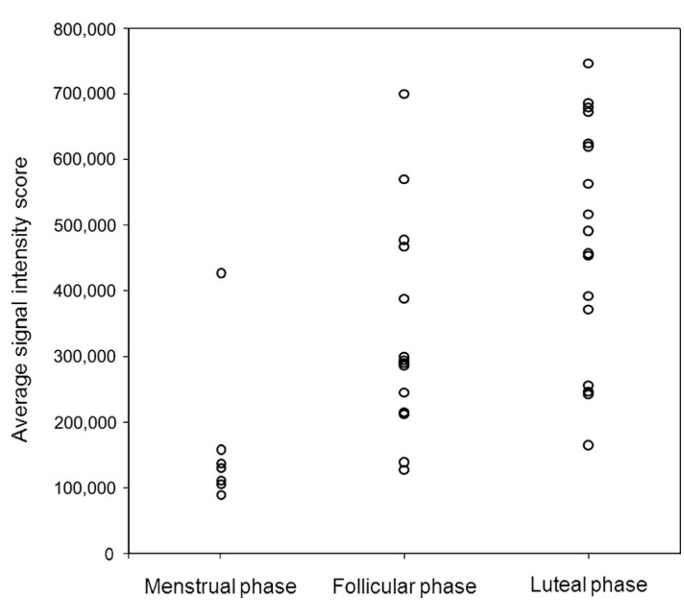

Fig. 1. Signal intensity score of the gray-level histogram, m_j, for the three groups studied. All multiple analyses (menstrual phase *vs.* follicular phase, follicular phase *vs.* luteal phase, and luteal phase *vs.* menstrual phase) were statistical significance at the 0.05 level.

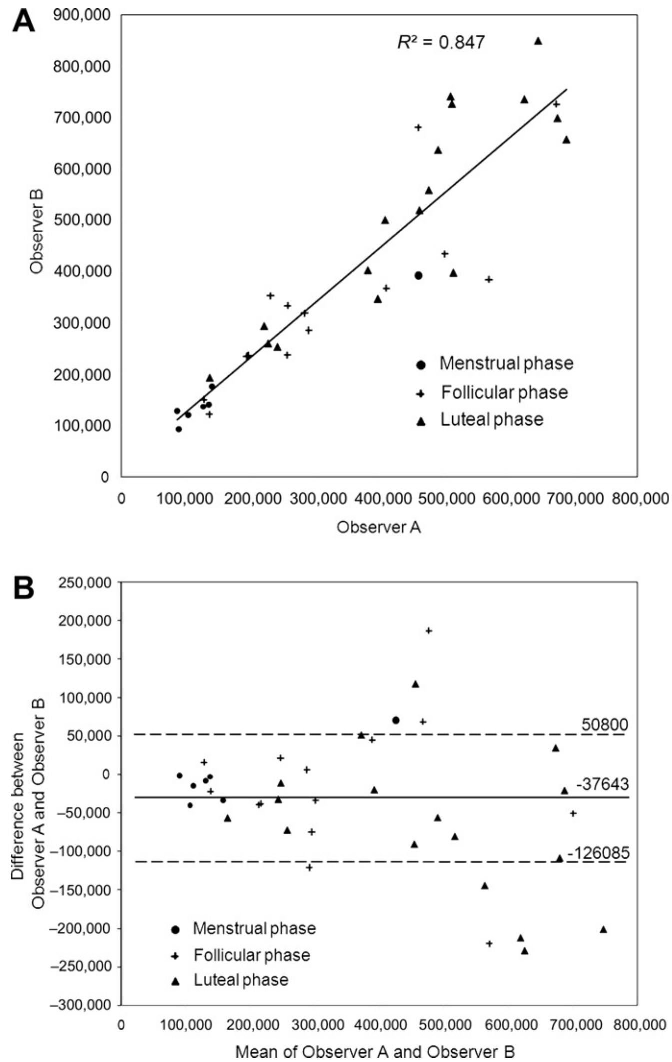

Fig. 2. Signal intensity scores were measured twice by the same observer to evaluate intraobserver reproducibility. (A) Plot of correlation between the two measurements ($r = 0.847$, $p < 0.05$). (B) Bland-Altman plot. Solid line and dashed lines represent mean difference and 95% confidence interval, respectively.

such as less than 158,200 during menstrual phase, the follicular phase in the range between 158,200 and 456,373, and the luteal phase larger than 456,373.

Overall, the thickness of the endometrium was approximately less than 7 mm in the menstrual phase group, 20 mm in the follicular phase group, and 25 mm in the luteal phase group.

Discussion

ImageJ software is commonly used in the postprocessing of images for immunochemistry staining and previous studies have examined its application for gynecological tissues [5,11,12]. Wilson et al [12], in a study of 38 patients suspected of having ovarian tumors, found ImageJ image analysis of frequency-based ultrasonography was 72% sensitive and 76% specific for the diagnosis of malignancies. The authors

Fig. 3. The thresholded image of the user-defined region of endometrium before ImageJ image analysis. Note the highly reflective hyperechoic surrounding border and brightness of proliferative endometrium.

concluded that although a deviation in interobserver agreement was not observed, the application of ImageJ analysis to quantify areas of interest identified with ultrasonography was only marginally useful in determining tumor vascularity.

CAIA software makes it is easy to obtain the gray-scale histogram of the endometrial area defined by the user in an ultrasonographic image. The information allows a more detailed investigation of the endometrium because it evaluates not only the thickness of the endometrium [9,10] but also the number of pixels, the brightness (intensity), and the gray scale of user defined area. In this study, signal intensity scores were shown reproducibility and reliability through the evaluation of intraobserver measurements.

In one study, endometrial evaluation was performed with ultrasonography without using ImageJ analysis in participants undergoing hormone therapy, an endometrial thickness greater than 4.5 mm had a positive predictive value of 47% for the presence of polyps, hyperplasia, and endocavity fibroids [11].

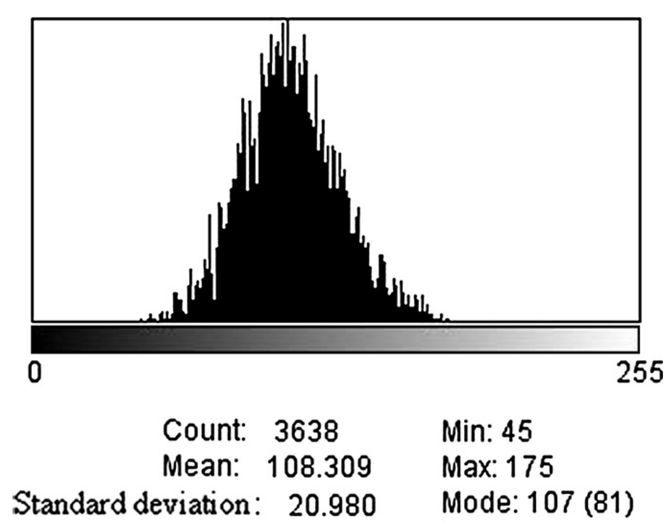

Fig. 4. The histogram of the user-defined region by ImageJ analysis.

In a study of participants with incomplete abortions, patients treated with Mifepristone had a wide range of endometrial thickness, suggesting that the use of any standardized cutoff value for endometrial thickness to define incomplete abortion is not a clinical option [3].

In the normal menstrual cycles, monthly changes were observed in the internal echo and the endometrial thickness. In our study, we divided the histogram based on the image echogenicity into 0–255 gray scales: (1) hyperechoic state (the gray scale being close to 255); (2) hypoechoic state (the gray scale close to 0); and (3) isoechoic state (the gray scale being similar to the tissue of comparison). The luteal phase is more hyperechogenic than the follicular phase, and during the menstrual phase the pixel number is smaller in comparison with the luteal and follicular phase.

The results of this study present a statistically significant difference in the signal intensity score of the gray-level histogram, m_j, of the endometrial scans performed with ultrasonography and investigated with ImageJ software.

Our finding may assist physicians in determining the phase of the menstrual cycle, thus reducing invasive procedures to evaluate the status of the endometrium [13]. To further examine this technique in clinical practice, we are conducting another study of patients with malignant versus benign intrauterine pathology.

The limitations of this technique involve the region of choice, and the signal intensity score of the gray level histogram on which quantification is based are subjective criteria defined by the user. ImageJ analysis cannot distinguish artifactual regions and thus requires human intervention to postprocess images to remove artifacts. These findings may outweigh the economic aspect of ImageJ as freely accessible image analysis software when compared with other solutions that contains postprocessing algorithms (e.g. virtual organ computer-aided analysis). Additionally, interobserver variability was not evaluated as one physician analyzed all of the image scan data and signal intensity scores.

Finally, we can conclude that a statistically significant difference in the signal intensity score of a gray-level histogram, m_j, of endometrial scans performed with ultrasonography and analyzed with ImageJ software were found between women with different phases of the normal menstrual cycles. Analysis of ultrasonographic images in different phases of the endometrial cycle with CAIA software may be useful and helpful before appropriate treatment.

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