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Title

MR imaging of uterine morphology and dynamic changes during lactation

Abstract

Purpose: To investigate the influence of lactation and ovarian hormones on uterine morphology and function by comparing uteruses of lactating women with nulliparous women on MRI.

Materials and Methods: Sagittal T2WI and cine MR images were obtained with 1.5T and 3T scanner from 22 lactating women and 16 nulliparous women as a control group. The lactating group was further divided into amenorrhea and menorrhea subgroups. Uterine area, endometrial thickness, junctional zone (JZ) thickness, relative signal intensity (rSI) of the JZ and of the outer myometrium (OM), were measured on T2-weighted fast spin echo images as static image parameters. Frequency of peristalsis (/3minutes), degree of endometrial transformation, subendometrial conduction, outer myometrial (OM) conduction, and sporadic myometrial contraction were evaluated using cine MR images. The above image parameters were compared between the lactating group and the control group, and between the lactational amenorrhea group and the

lactational menorrhea group as a sub-analysis.

Results: A significant difference was observed in all the static image parameters and in three of the five cine image parameters between the lactating group and the control group ($p < 0.01$). In sub-analysis, a statistical significance was found between lactational amenorrhea group and lactational menorrhea group in area of the uterus and both endometrial and JZ thickness ($p < 0.05$), but not in cine image parameters ($p = 0.682$, $p = 0.096$, $p = 0.191$, $p = 0.939$, $p = 0.289$, respectively).

Conclusion: Uterine appearance and peristalsis were different between lactating and nulliparous women. The morphological differences were pronounced between lactating amenorrhea and menorrhea women.

Keywords

Uterus, peristalsis, postpartum, lactation

INTRODUCTION

Uterine morphology and function are known to vary under the influence of hormonal fluctuations, such as during the menstrual cycle, pregnancy, delivery, nursing and menopause (1, 2). On static MR images, endometrial thickness and signal intensity of the uterine myometrium will vary depending on the phases of the menstrual cycle or upon use of oral contraceptives (2, 3, 4). After the menopause and pre-menarche, the uterine size is small with a thin endometrium because of the absence of effect from ovarian hormones (2). The frequency of uterine peristalsis increases during the follicular phase, probably due to the effect of estrogen, and decreases during the mid-luteal phase due to the effect of progesterone (5).

Similarly to menarche or menopause, childbirth is accompanied by dramatic fluctuations in levels of hypothalamic, pituitary and ovarian hormones. Morphological changes in the uterine body and cervix are prominent within thirty hours to one week post-delivery (6). Uterine involution occurs after delivery accentuated by uterine contractions induced by nursing (7). Subsequent breastfeeding activity or lactation also induce hormonal changes which may also impact uterine morphology and function, including uterine peristalsis and contraction (8).

Hence, the aim of our study was to investigate the influence of lactation and female hormones on uterine appearance and function using MRI by comparing lactating with nulliparous women.

MATERIALS AND METHODS

Study Population

This prospective study was approved by our institutional review board and written informed consent was obtained from all subjects.

The study population consisted of two groups: lactating women prospectively recruited after delivery (lactation group) and healthy nulliparous volunteers (control group). Lactating women were recruited between October 2010 and March 2012 and Nulliparous volunteers were recruited between December 2007 and March 2012. A part of nulliparous volunteers also participated in a prior study (9). In total, thirty-eight healthy women (22 women in the lactation group, 16 women in the control group) were recruited for the study. The exclusion criteria were: use of oral contraceptive pills (OCs), insertion of intrauterine contraceptive devices (IUD), or known müllerian duct anomalies, since the use of OCs and IUD is indeed known to affect uterine peristalsis (3, 10). In the lactation group, 22 women were included and all of them were in a postpartum

uninterrupted lactation period (7-54weeks postpartum). The lactation group was further divided into two subgroups. The first subgroup was composed of lactating women presenting amenorrhea (n=15), i.e., who did not show resumption of menses after delivery and designated lactational amenorrhea group. The second subgroup included women with menorrhea (n=7), i.e., who showed resumption of menses and designated lactational menorrhea group. The menstrual cycle phase was evaluated as follows: the next expected menstrual day (cycle date 1) was estimated by calculation from the usual menstrual cycle. In other words, the ovulation date was assumed to be 14 days before the first day of the next menstrual cycle. The time interval between delivery and MR examination was 178.1 ± 92.1 days (52-383 days) in amenorrhea group and 229.1 ± 71.0 days (131-379 days) in menorrhea group.

As the control group, 16 women were recruited and none of them was excluded from the study. All the volunteers were nulliparous with regular menstrual cycles.

MR scanning protocols

MR examination was carried out twice in the lactating group, just prior to and after lactation, and performed once in the control group regardless of the menstrual phase. In our study, the time interval between lactation ended to the post-lactation imaging was

around ten minutes. The menstrual period was avoided for the MR examination, since it has been reported that uterine zonal anatomy is often indistinct during the menstrual period (11).

MR imaging examination was performed with a 1.5 T (EXCELART Vantage, Toshiba Medical Systems, Otawara, Japan) and 3T scanner (Toshiba Medical Systems, Otawara, Japan). On the 1.5T scanner, T2-weighted fast spin-echo images (TR/TE = 4000/80 msec, FOV of 260×195, matrix of 512×384, slice thickness of 5 mm) were acquired in the sagittal and transverse planes. T2-weighted fast-advanced spin echo (FASE) images (TR/TE = 3000/130 msec, FOV of 300×300 mm, matrix of 256×256, slice thickness of 5 mm) were acquired in sagittal plane. On the 3T scanner, T2-weighted fast spin-echo images (TR/TE = 5400/80 msec, FOV of 260×195, matrix of 512×384, slice thickness of 4 mm) were acquired in the sagittal and transverse planes. T2-weighted fast-advanced spin echo (FASE) images (TR/TE = 3000/130 msec, FOV of 300×300 mm, matrix of 256×256, slice thickness of 5 mm) were obtained in sagittal plane. In the midsagittal plane of the uterus, 60 serial FASE images were acquired to create cine MR images with quiet respiration. An AVI movie was created with Sante DICOM viewer FREE® (Santesoft LTD, Athens, Greece) so as to display 5 frames per second (i.e. 15× faster than the real-time speed). A T1-weighted spin-echo (TR/TE = 500/20 msec, FOV of

260×195, matrix of 512×384, slice thickness of 5 mm on 1.5T scanner, TR/TE =550/12, FOV of 260×195, matrix of 512×384, slice thickness of 5 mm on 3T scanner) and images were acquired in the sagittal plane as part of the pelvic screening. Image parameters were also represented in Table 1.

Image analysis

Evaluation on static images

To perform a quantitative evaluation, the area of the uterus, endometrial thickness, junctional zone (JZ) thickness, relative signal intensity (rSI) of the JZ and of the outer myometrium (OM) were measured on midsagittal T2-weighted fast spin echo images using Osirix® (version X v.7.0.1 64-bit, Pixmeo, Genova, Switzerland) by two radiologists independently (S.D. and A.K., with 13 and 19 years of experience in diagnostic imaging). The area of the uterus was measured by marking the outline of the uterus polygonally. Endometrial and JZ thickness were measured at the thickest region of the uterus. A rSI of the JZ and the OM were calculated using the following formula:
$$rSI = (\text{mean SI of uterine region} - \text{mean SI of paraspinal muscle}) / (\text{mean SI of fat} - \text{mean SI of paraspinal muscle}) \times 100 \quad (12)$$
 . Regions of interest (ROIs) were placed on the junctional zone and the outer-myometrium, and drawn as large as possible. Reference ROIs were

drawn in the paraspinal muscle and adipose tissue of the gluteal region. (Fig.1). All the results of each radiologist were averaged and used for the following analyses.

Evaluation of cine MRI

Frequency of peristalsis (/3minutes) and other peristaltic features were evaluated by two radiologists independently (S.D., A.N. with 18 years of experience in diagnostic imaging) using cine MR images. Uterine peristalsis was defined as the wave conduction in the longitudinal axis within the junctional zone, in line with previous reports (5). The degree of endometrial transformation, subendometrial conduction, outer myometrial (OM) conduction, and sporadic myometrial contraction were evaluated on a five-point grading scale: 0: not present, 1: present, but weak, 2: mild, 3: moderate and 4: marked. Endometrial transformation was defined as subtle wavelike configuration with or without identifiable direction (5, 9). Subendometrial conduction was defined as conduction of low signal intensity within the subendometrial myometrium with or without identifiable direction (5, 9)- OM conduction was defined as the conduction of low signal intensity toward the outer myometrium (9). Myometrial contraction was defined as transient focal masses of low signal intensity on T2WI, bulging into the endometrium (13) . The qualitative evaluation was carried out in the following manner: two radiologists

independently evaluated the images in a random order, and consensus was subsequently reached when the scores were not in complete agreement. When no agreement could be reached at the consensus reading, the averaged scores of the two radiologists obtained during the re-evaluation were taken into account.

Statistical Analysis

Statistical analysis was performed using by MedCalc® (version 12.7.2.0, MedCalc Software, Ostend, Belgium).

Concordance between two readers in static image evaluation was analyzed by intraclass correlation coefficient (ICC). Concordance of the two readers' results in cine MR images in both the original and the consensus were measured by the weighted-kappa coefficient. A κ value less than 0.00 signified poor agreement; 0.00-0.20, slight agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; 0.81-1.00, almost perfect agreement (14).

Time interval between delivery and MR examination was compared between the lactating groups using unpaired Student's t-test. For the comparison between follicular and luteal phase of each lactational menorrhagia group and control group, unpaired Student's t-test for quantitative analysis and Mann Whitney and Wilcoxon rank sum test for qualitative analysis were used.

In advance, the comparison was made about the factors in either static or cine images between women in the follicular phase and luteal phase within lactational menorrhagia group and control group. After that, to investigate the influence of lactation, a

comparison of quantitative and qualitative parameters was made between lactation and control group. Next, to evaluate influence of the resumption of menstruation, lactation group was sub-divided into two groups: lactational amenorrhea group and lactational menorrhea group. Comparison between the two subgroups was performed as a sub-analysis. In addition, to investigate the influence of nursing, the same parameters between pre- and post-lactation was also compared.

The comparative analysis involving quantitative parameters including area of the uterus, endometrial and JZ thickness, rSI of JZ and OM, and peristaltic frequency was performed using the unpaired Student's t-test. For qualitative parameters of the peristaltic features, Mann Whitney and Wilcoxon rank sum test were used. The comparison between pre- and post-lactation, paired t-test was used for quantitative parameters and Wilcoxon signed rank test was used for qualitative parameters. A p value inferior to 0.05 was considered statistically significant.

RESULT

As none of the recruited women fit into any of the exclusion criteria, all of them, 22 lactating women (29.0 ± 3.2 years old) and 16 control women (32.4 ± 4.3 years old), were included in this study. In lactational menorrhoea group, MR examination was performed in follicular phase in two women and in luteal phase in five women. In control group, nine of 16 women performed MR examination in follicular phase and seven women in the luteal phase. In relation to the comparison between pre- and post-lactation, T2WI were obtained from 22 women pre-lactation and from 19 women post-lactation. Cine MR images were obtained from 22 women pre-lactation and from 21 women post-lactation. The reasons for incomplete images in post lactation were: insufficient T2WI images (n=2), and sick baby (n=1).

Interobserver agreement in static image evaluation was substantial for rSI of JZ and OM, almost perfect for area of uterus, thickness of endometrium and JZ. Interobserver agreement for cine MR image was moderate for sporadic myometrial contraction, substantial for frequency, endometrial transformation, subendometrial conduction and almost perfect for OM conduction in original data (Table 2). Upon consensus reading, substantial agreement was obtained for sporadic myometrial

contraction, and an almost perfect agreement for the other four parameters (Table 2)

There was no statistical significance in the time interval between delivery and MR examination between the lactational amenorrhea and menorrhea groups ($p=0.22$).

Similarly, there was no statistical significance in any of the factors described in either static or cine images between women in the follicular phase and luteal phase within lactational menorrhea group and control group.

Upon comparison between the lactating group and the control group, a significant difference was observed in all the parameters obtained from the static images and in three out of five parameters from the cine images (Table 3). These results indicate that the uterus of lactating women tend to show a smaller and thinner endometrium, a lesser JZ thickness, but a higher relative signal intensity in the JZ and the OM in comparison to nulliparous women. In relation to the uterine peristalsis, the frequency of peristalsis was inferior and was associated with a weaker endometrial transformation and sporadic myometrial contraction in the lactating group compared to the control.

There was a significant difference between the two lactation subgroups, amenorrhea vs. menorrhea, in area of uterus, both endometrial and JZ thickness in the static images, but no differences were found in the cine images (Table 4).

When comparing pre- and post-lactation, there was significant difference only in outer myometrial conduction (Table 5). In relation to other parameters, there was no significant difference whether in the static or the cine MR images.

Representative uterine images taken from lactational amenorrhea, lactational

menorrhea and control women were shown in Figure 2. Descriptive features on the cine MR image were shown in Movie 1 and 2 (supplemental material).

DISCUSSION

In this study, we investigated the influence of lactation on uterine morphology and function by comparing lactating women to nulliparous women using MRI analysis. The result accords with our hypothesis that lactation and female hormone influences uterine morphology and function.

However, there seemed to be a variation of the data in area of the uterus, thickness of endometrium and junctional zone, frequency of peristalsis among lactation group. Our sub-analysis revealed significant differences between lactational amenorrhea group and lactational menorrhea group morphologically. The serum levels of estrogen and progesterone decrease abruptly after delivery (8). In addition, ovarian activity of lactating women is suppressed during lactational amenorrhea (15). Therefore, morphology of uterus in amenorrhea group may reflect suppression of ovarian activity. As for the uterine function, there was no significant difference in any parameter between lactational amenorrhea group and menorrhea group, suggesting that recovery of uterine morphology precedes resumption of uterine function.

In comparison between pre- and post- lactation, there was a significant difference only in outer myometrial conduction. The hormone that changes before and after lactation

is oxytocin and its serum level elevates by suckling (16) . Oxytocin act on myoepithelial cells in lactiferous ducts to make contraction and eject milk (17). Oxytocin also has an effect on the contraction of uterine myomerium (18). The half-life of oxytocin is 6 min 53 seconds in female (19). The remaining effect of oxytocin might have caused significant difference in outer myometrial conduction.

The appearance of the uterus during lactation period shown in this study has potential implications for the clinical diagnosis of female pelvic MRI. It had been reported about the normal appearance of uterus in reproductive age and postmenopausal women. The rise of maternal age (20) and the decrease of the affected age of gynecological disease (21) imply overlapping these generations. As a result, it is expected increasing lactating women who undergo MRI. We should be cautious about that the existence of disease, size and extent of the lesion may be indistinct in small uterus with thinner layer structures during lactational amenorrhea.

There are several limitations in this study. Firstly, the study population, especially proportion of lactating amenorrhea women, was small, and comparison among variable hormonal status were limited Post-partum women without lactation and menopausal women were not include in this study for comparison. Further investigations on a larger population with variable hormonal state may be necessary to further support

our results. Secondly, menstrual cycle of lactation menorrhea group and control group was not separated for evaluation. Since the uterine peristalsis changes according to the cycle, the MR images had better to be obtained in the same cycle for comparison. However, since the number of subjects was small and the range of cycle date was wide, we have merged the two phases and confirmed there was no significance in every data analyzed between the cycle phases in our study. Thirdly, the serum level of hormones was not measured. On the basis of the correlation between lactation and menstruation, hormonal levels are indeed an important contributing factor. However, because the study included volunteers in both the control and lactating women groups, invasive methods such as blood sampling, would be difficult to perform. Comparison between the hormonal serum levels of control subjects compared to lactating amenorrhea and menorrhea women may help to understand the changes specific to each group and require further investigations. Fourthly, measurement of the uterine volume may have allowed for a better distinction between groups more accurately though in our study, the area of the uterus was measured.

In conclusion, uterine appearance and peristalsis were different between lactating and nulliparous women as visualized by MR imaging. The morphological differences were pronounced between lactational amenorrhea and menorrhea women.

Our results suggest that ovarian hormones may impact on uterine appearance more than hormonal influences associated with lactation, such as oxytocin.

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Tables

Table1 Image Parameter of MRI

	1.5T			3T		
Sequence	T2 FSE	T2 FASE	T1WI	T2 FSE	T2 FASE	T1WI
TR/TE(ms)	4000/80	3000/130	500/20	5400/80	3000/130	550/12
FOV	260×195	300×300	260×195	260×195	300×300	260×195
Matrics	512×384	256×256	512×384	512×384	256×256	512×384
Slice thickness(mm)	5	5	5	4	5	5

FSE: Fast spin-echo, FASE: Fast-advanced spin echo

Table2 Concordance Between the Two Readers

Parameter	Intraclass Correlation Coefficient	
Area of Uterus	0.81	
Endometrial thickness	0.98	
Junctional zone thickness	0.92	
rSI of junctional zone	0.74	
rSI of outermymetrium	0.78	

Parameter	κCoefficient in original data	κCoefficient after consensus reading
Frequency of Peristalsis	0.68	0.98
Endometrial Transformation	0.70	0.88
Subendometrial Conduction	0.76	0.81
OM Conduction	0.83	0.85
Sporadic Myometorial Contraction	0.60	0.67

Table3 Comparison Between Lactating Group and Control Group

Static image evaluation			
	Lactation (n=22)	Control (n=16)	p value
Area of the uterus (mm²)	2250.5±611.1	2914.6±642.4	p<0.01*
Endometrial thickness (mm)	3.9±4.6	9.4±2.4	p<0.01*
JZ thickness (mm)	2.2±1.7	4.5±1.3	p<0.01*
rSI of JZ	43.1±15.6	15.6±8.3	p<0.01*
rSI of OM	68.1±18.5	47.3±12.4	p<0.01*
Cine evaluation			
	Lactation (n=22)	Control (n=16)	p value
Frequency of peristalsis (/3min)	2.8±1.9	6.3±1.8	p<0.01*
Endometrial transformation (4-0)	0.8±1.0	2.5±1.2	p<0.01*
Subendometrial conduction (4-0)	2.1±1.3	2.3±0.6	1.00
Outermymetrial conduction (4-0)	0.9±1.1	1.4±1.3	0.082
Sporadic myometrial contraction (4-0)	0.0±0.2	1.0±1.2	p<0.01*

Table4 Comparison Between Lactational Menorrhea Group and Lactational Amenorrhea Group

Static image evaluation			
	amenorrhea(n=15)	menorrhea(n=7)	p value
Area of the uterus (mm²)	2047.8±535.4	2684.9±531.7	0.032*
Endometrial thickness (mm)	1.8±1.1	8.5±5.8	0.030*
JZ thickness (mm)	1.5±1.4	3.6±1.5	0.016*
rSI of JZ	47.4±15.1	34.0±12.4	0.057
rSI of OM	70.3±19.1	63.4±16.1	0.425
Cine evaluation			
	amenorrhea(n=15)	menorrhea(n=7)	p value
Frequency of peristalsis (/3min)	2.7±1.6	3.0±2.6	0.682
Endometrial transformation (4-0)	0.5±0.6	1.5±1.3	0.096
Subendometrial conduction (4-0)	2.4±1.2	1.5±1.4	0.191
Outermymetrial conduction (4-0)	0.8±1.0	0.9±1.1	0.939
Sporadic myometrial contraction (4-0)	0±0	0.1±0.3	0.289

Table5 Comparison Between Pre-lactation and Post-lactation

Static image evaluation			
	Pre-lactation(n=19)	Post-lactation(n=19)	p value
Area of the uterus (mm2)	2343.5±591.2	2332.1±569.7	0.763
Endometrial thickness (mm)	3.9±4.6	4.0±4.7	0.571
JZ thickness (mm)	2.5±2.0	2.6±2.1	0.518
rSI of JZ	37.4±16.0	34.5±16.8	0.492
rSI of OM	61.9±18.3	66.2±15.2	0.292
Cine evaluation			
	Pre-lactation(n=21)	Post-lactation(n=21)	p value
Frequency of peristalsis (/3min)	2.8±2.0	2.8±2.1	0.979
Endometrial transformation (4-0)	0.8±1.0	0.8±1.0	0.799
Subendometrial conduction (4-0)	2.1±1.4	2.0±1.3	0.717
Outermymetrial conduction (4-0)	0.9±1.1	1.4±1.6	0.017*
Sporadic myometrial contraction (4-0)	0.0±0.2	0.3±0.7	0.108

Figure Legends

Figure 1

Midsagittal T2 weighted image of the uterus of a 36-year old woman, 10 months after delivery with amenorrhea. ROIs were drawn on the JZ(a), the outer myometrium(b), the adipose tissue in the gluteal region(c) and the paraspinal muscle(d) as per indicated by white /black circles.

Figure 2

Midsagittal T2 weighted images showing representative uterine appearance of each group.

(a) 35-year old lactating woman presenting amenorrhea with a small uterus, thin endometrium, and indistinct junctional zone. Frequency of peristalsis was three /3min.

(b) 23-year old lactating women presenting resumption of menses in follicular phase. The layered structure of the uterus was distinct from that of a nulliparous woman. Frequency of peristalsis was 2.25/3min.

(c) 27-year old nulliparous woman in follicular phase. Endometrium, junctional zone and outer myometrium are clearly visible and frequency of peristalsis was equal to nine/3min.

Movie 1 (supplemental material)

A case of 30-year old lactating woman with resumption of menstruation in follicular phase.

Subendometrial conduction which is conduction of low signal intensity at subendometrial myometrium surging from cervix to fundus was shown. In addition, amorphous low signal intensity area is moving from inner-myometrium toward outer-myometrium, corresponding to outermymetrial conduction.

Movie 2 (supplemental material)

A case of 30-year old woman of lactational amenorrhea.

Sporadic myometrial contraction which is transient focal low signal intensity was seen at uterine fundus. Endometrial transformation which is endometrial wave-like movement with subendometrial conduction can be observed.

Figure1

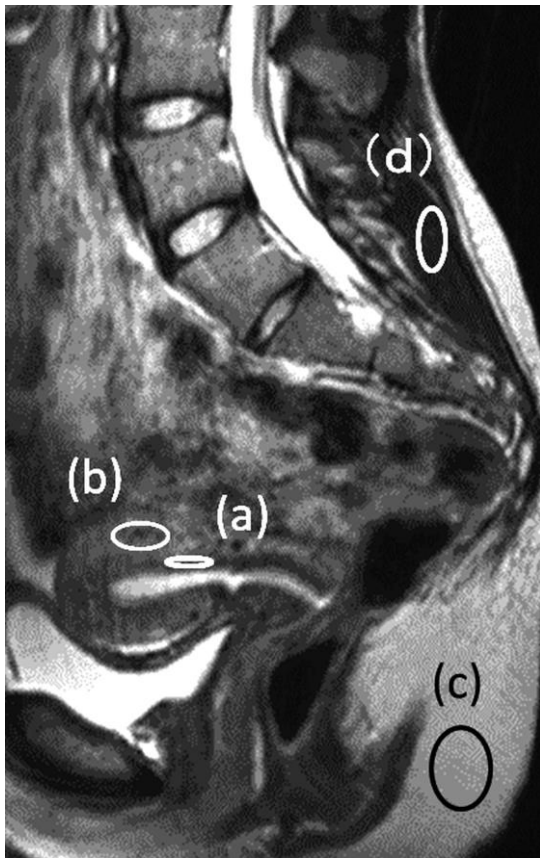


Figure2

