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Transvaginal ultrasound and lactate dehydrogenase isoenzyme activity profile in uterine aspirate for diagnosis of endometrial carcinoma in women with postmenopausal bleeding

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Abstract

Postmenopausal patients with vaginal bleeding (n=72) were evaluated with the combination of transvaginal ultrasonography (TVS) and analysis of the lactate dehydrogenase (LD) isoenzyme activity profile in uterine fluid aspirates. TVS evaluation of the endometrium was classified as < 5 mm, ≥ 5 mm, or poorly defined. The LD isoenzyme activity profile was characterized as abnormal or normal. Pathological findings were further evaluated with diagnostic curettage. TVS found the endometrium to be ≥ 5 mm or poorly defined in 44 patients (61%). Endometrial carcinoma was found in 6 out of 72 patients (8 %). They appeared in the TVS groups endometrium > 5 mm (n=2) and endometrium poorly defined (n=4), but not in the endometrium < 5mm. The LD isoenzyme activity profile was abnormal in the 6 malignant cases and in 10 benign cases. Thus, the need for further evaluation with hysteroscopy and curettage was reduced to 16 cases. Since TVS had 100% sensitivity but only 42% specificity, it is suitable for first level examination in patients with postmenopausal bleeding. The second level method should have similarly high sensitivity, but much higher specificity. The LD isoenzyme activity profile in uterine fluid aspirates had 100% sensitivity and 85% specificity. Another important feature is that the method is not sensitive to endometrial thickness, amount of sample, sampling device or dilution. Thus, it is more reliable than aspiration histology. For every hysteroscopy or curettage that can be replaced by LD analysis, the cost is reduced by approximately EUR 720 or 540, respectively.

Introduction

Postmenopausal bleeding is a common complaint. The incidence is up to 40% in the second year after menopause and it is subsequently reduced to about 4% in the fourth and later years after menopause (1). Bleeding occurring soon after menopause is more likely to have hormonal background, whereas that occurring later is more likely to have organic background. Generally, non-organic reason for postmenopausal bleeding has been estimated to 60% (2,3). In the work-up of patients with postmenopausal bleeding, an office method which can reduce the need for hysteroscopy and curettage, with minimal risk of false negative result, is much wanted.

Transvaginal ultrasonography (TVS) has become a common method to monitor endometrial thickness, in particular in cases of postmenopausal bleeding. A large study clearly demonstrated a relation between thickness and pathology in the endometrium (4). However, a fairly high proportion of patients present with either thick endometrium or inconclusive findings, and these patients need further evaluation.

Endometrial processes, malignant as well as inflammatory, are reflected in the uterine fluid. We have recently reported that the lactate dehydrogenase (LD) isoenzyme activity profile in uterine fluid aspirates identifies endometrial malignancy with 100% sensitivity and 100% negative predictive value (5). Most importantly, accuracy of this test is not dependent on endometrial thickness. LD is a crucial glycolytic enzyme in all human tissues. It is present in five isoforms, which are assembled as homo- or heterotetramers of two different subunits. These are the products of two different genes, LD-A and LD-B (6). LD isoenzyme-1, which consists of four LD-B subunits, has the highest electrophoretic mobility and is mainly active during anaerobic conditions. In contrast, LD-5, which consists of four LD-A subunits, has the lowest mobility and is active in aerobic conditions. Tumor tissue predominantly expresses

LD-4 and LD-5, which enables it to produce lactate under aerobic conditions, in contrast to normal cells (7). Since, however, the LD-A subunit is the main product also in granulocytes, the pattern seen in inflammatory processes mimics that in malignant tissues (8). Endometrial cancer, like most other malignant tumors, releases predominantly LD-4 and LD-5 (9,10). In vaginal fluid the LD activity indicates inflammation (11).

The purpose of this study was to compare sensitivity, specificity, and predictive values between TVS and the LD-isoenzyme activity profile, and also to evaluate LD analysis as a second level method applied after TVS, in postmenopausal women with a history of bleeding.

Patients and methods

Patients

TVS and uterine aspiration was performed as office examinations in 72 postmenopausal patients, who had experienced at least one bleeding episode. Postmenopausal bleeding was defined as a bleeding after six or more months of amenorrhea. The examinations were performed as soon as possible after the bleeding, usually within 2 weeks, and no attempt was made to postpone the examinations even though an ongoing bleeding can give false positive results (5). The group of patients was intended to represent non-selected postmenopausal women with bleeding. Consecutive patients were included in the study during the period 1995-2000. They were examined by a gynaecologist (ON) with experience of endometrial diagnosis with TVS as well as the aspiration method. Sixty-six of the 72 patients in this study, including all cases with endometrial cancer, have been presented in an earlier study in which, however, the results of TVS were not reported (5). Another patient with endometrial cancer presented in that study was excluded from the present study because of cervical stenosis and aspiration for LD analysis done at the time of curettage. The median age was 65 (range 46–

89) years. Patients using hormone replacement therapy (n=33) had either systemic estriol (n=13), sequential combined (n=8), or continuous combined (n=12) therapy.

Transvaginal ultrasonography (TVS)

All ultrasound examinations were performed with a Sonoace 5000 Kretz-Medison scanner with a 6.5 MHz vaginal transducer (Medison Inc., Seoul, Korea). Thickness of the endometrium (double layer) was measured using the built-in calipers in the ultrasound longitudinal section of the uterus where the endometrium was thickest. The results were classified according to thickness of the endometrium as ≥ 5 mm or < 5 mm. In cases where the examination was inconclusive, i.e., where the border between the endometrium and myometrium could not be clearly identified, the endometrium was classified as "poorly defined". Both endometrium ≥ 5 mm and endometrium poorly defined were considered pathological findings.

Uterine fluid sampling and processing

Uterine fluid samples were aspirated with an Endorette™ uterine sampling device (Medscand, Malmö, Sweden) (12). The Endorette instrument was designed for simplest possible endouterine aspiration. Furthermore, it permits injection of contrast fluid like saline for hydrosonography, which allows diagnosis of e.g. endometrial polyps. The aspirate was transferred to a test tube containing 5 mL saline and allowed to sediment. The supernatant was removed, centrifuged at 700 g for 5 minutes, and used for LD analysis. The sediment was fixed in formaldehyde 4% and used for histopathologic diagnosis, when the amount of tissue allowed.

LD isoenzyme activity profile

The isoenzymes were electrophoretically separated in agarose gel in a weak alkaline buffer system and reacted with substrate to be visualized (13,14). The activity of individual isoenzyme fractions was quantified by visual estimation by one investigator (GS). Visual estimation was based on previous experiences with densitometric scanning. Samples were not frozen since freezing markedly reduces LD activity, in particular that of LD 4 and 5 (10). Samples can be stored at room temperature for at least one week without detectable deterioration of activity for any of the isoenzymes. Hemolysis, which might release LD 1-3, was minimal and did not necessitate correction. The activity of LD 1 was set to 1.0 and used as an internal standard. An abnormal LD profile was defined as LD1 = 1.0, LD2 ≥ 1.5, LD3 ≥ 2.5, LD4 ≥ 2.6, and LD5 ≥ 3.8 (5).

Histology

Those cases, where the amount of tissue was insufficient for histological evaluation, were assumed to have atrophic endometrium, and were classified as benign. Patients with abnormal histology in the Endorette aspirate or with abnormal LD isoenzyme profile were further evaluated with diagnostic curettage.

Finally, The Regional Cancer Register was checked 4-9 years after the bleeding episode for all 72 patients.

Results

TVS

Forty-four patients (61 %) had endometrium either ≥ 5 mm (n=20) or poorly defined (n=24). Only 28 women (39 %) had endometrial thickness < 5 mm. The patients with endometrial cancer (n = 6) had endometrium either ≥ 5 mm (n=2) or poorly defined (n=4) (Table 1). With the present definition of a pathological finding, sensitivity as well as negative predictive value was 100%, whereas specificity was only 42% and positive predictive value 14%. Among patients with hormone therapy (n = 33) fourteen had endometrium ≥ 5 mm, and twelve had poorly defined endometrium. Two out of six cases with malignant endometrium were found in the hormone therapy group (endometrium ≥ 5 mm and poorly defined respectively).

LD isoenzyme activity profile

The LD isoenzyme activity profile was abnormal in 16 patients. Six of them had endometrial carcinoma and 10 of them had benign histology, including two cases with endometritis. All cases with endometrial carcinoma were found in the abnormal LD profile group, and all cases with normal LD profile (n=56) had benign histology. Thus, sensitivity as well as negative predictive value of the LD profile was 100%, specificity was 85%, and positive predictive value 38%. In the group with abnormal LD profile and benign histology (n=10), the endometrium was ≥ 5 mm in seven patients and poorly defined in 3 patients. The aspirate was insufficient for histology in 5 patients, and 7 patients had hormone therapy.

Combining TVS and LD analysis

When LD analysis was applied in the group of patients with abnormal endometrial finding at TVS (n=44), the number of patients who had persisting suspicion of malignancy and needed further evaluation with hysteroscopy/curettage could be brought down to 16, i.e. about one third, and still maintaining 100% sensitivity. Thirteen of these patients had curettage, while

three were re-evaluated with repeated TVS and LD analysis because of age or poor physical condition.

Histology

Altogether 6 patients (8%) had malignant histology both in the aspirate and at diagnostic curettage. One additional case had suspected malignant histology in the Endorette aspirate, but the following curettage showed endometrial atrophy. The LD isoenzyme profile was normal in this case. Among the 6 cases with endometrial carcinoma 4 received hormone replacement therapy. There was no case with atypical hyperplasia in this group of patients. Aspiration samples were insufficient for histology in 39 patients (54%).

Follow-up of all 72 patients in the The Regional Cancer Register 4-9 years later revealed only those six women already diagnosed with endometrial cancer.

Discussion

A shift in the LD isoenzyme activity profile in tissues and body fluids to slowly migrating fractions can be regarded as a general marker of malignancy as well as inflammation. This is true also for the endometrium, and the LD isoenzyme activity profile of uterine fluid reflects endometrial processes. We have previously shown that the LD isoenzyme activity profile of uterine fluid is able to detect malignant as well as inflammatory processes (10), and the LD activity of vaginal fluid is an indicator of inflammation (11). Moreover, a normal distribution of the isoenzyme activity profile in uterine fluid excluded significant endometrial pathology, i.e. the negative predictive value was 100% (this study and 5).

In our earlier studies of postmenopausal women the LD activity, total and isoenzyme, were operationally defined, the critical limit for “malignant” being the mean value + 2 standard deviations of the proliferative phase (10). However, it became apparent that the relative distribution of the isoenzyme activities associated with endometrial pathology was always dominated by the slow migrating isoenzymes, i.e. LD4 and LD5. Based on this understanding we focused on the isoenzyme profile. The activity level of LD1 was set at 1.0 and was used as an internal standard within each sample. The relative activity level of each isoenzyme fraction was subsequently calculated. In uterine aspirates obtained from a set of 11 patients with endometrial cancer, the cut-off levels for isoenzyme fractions LD 2-5 were subsequently established as the lowest level in any of these cases (5). It is possible that even lower cut-off levels for each isoenzyme could have been used, since a gradual increase in LD activity i.e. 1<LD 2<LD 3<LD 4<LD 5 is pathological.

TVS is a widely used diagnostic method for endometrial carcinoma in postmenopausal patients. Generally, it is imperative that a first level examination method has close to 100% sensitivity for the specific condition it is designed to detect. In fact, TVS has an excellent sensitivity, provided that a wide definition of "not normal" is applied (4). Since a large fraction of the malignant tumors appears as inconclusive findings, this study took a liberal approach to classify the endometrium as poorly defined. Specificity of the TVS is low, partly because of other reasons for thick endometrium like hormone replacement therapy, and partly because the endometrium might not be clearly identified due to myometrial echoes.

The high percentage of false positive findings calls for a second diagnostic level in order to reduce the need for hysteroscopy and/or curettage. One such diagnostic measure, endometrial biopsy taken as an office procedure, has been widely used over the past 30 years and a large

number of biopsy techniques have been devised. In a meta-analysis of endometrial sampling studies, the best instrument, Pipelle de Cornier, had a sensitivity of 99.6, and the number of insufficient samples varied from 0 to 54% (15). This is in accordance with our present study, which found sensitivity of aspiration histology to be 100% and aspiration samples to be insufficient for histology in 54%

Discrepancy between histology obtained with aspiration vs. curettage has been reported, and seems to occur preferentially in cases with endometrial thickness ≥ 6 mm (16). Since the second level diagnostic method must have close to 100% sensitivity as well as negative predictive value, regardless of thickness of the endometrium and the sample size, aspiration biopsy does not recommend itself. We have shown that the LD isoenzyme activity profile has 100% sensitivity and 100% negative predictive value for endometrial malignancy (this study and 5). The present study furthermore showed that the LD profile reliably detects endometrial malignancy also in cases with endometrial thickness ≥ 5 mm. Thus, this method is insensitive for endometrial thickness, amount of sample, sampling device and dilution. Furthermore, intrauterine manipulation of the instrument, in order to obtain tissue samples for histology, is reported to be painful (16). Based on our experience such manipulation is not necessary in order to obtain an adequate sample for LD isoenzyme determination. It is of course crucial to make sure that the aspiration catheter is located in the uterine cavity, if present cervical stenosis may present an obstacle for the aspiration catheter. Disagreement between uterine length measured by ultrasound and insertion length of the catheter should alert the examination.

Based on the above results as well as reports in the literature, we recommend that TVS is used as the first line examination method. The LD isoenzyme activity profile fulfils the criteria for

the second line method, i.e., 100% sensitivity and 100% negative predictive value, and a reasonably high specificity in order to reduce the number of patients requiring further evaluation with hysteroscopy and/or curettage. If only TVS had been used in the present study, suspicion of malignancy had persisted in 44 of the 72 patients (endometrium ≥ 5 mm or poorly defined). The LD analyses brought this figure down to 16 patients.

The cost – benefit ratio was calculated for LD analysis versus hysteroscopy or curettage. Actual cost for the LD isoenzyme activity analysis is EUR 27, and for the Endorette instrument just EUR 2. The costs for hysteroscopy and curettage (anaesthesia and postoperative care included) are EUR 750 and 570, respectively. Finally, the cost for histological examination is EUR 15 regardless of sampling techniques. Thus at least 20 LD analyses can be performed to the cost of one curettage. This is of economical importance, since we need to apply a wide definition for "abnormal TVS", and we need to utilize second level diagnostic procedures with lower costs. In this study, the LD analysis brought down the need for curettage to one third of the original.

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Table 1.

Distribution of the findings at transvaginal ultrasonography (TVS) and lactate dehydrogenase (LD) isoenzyme activity profile analysis related to histology of the endometrium in 72 postmenopausal women with uterine bleeding

	Histology		
	Malignant	Benign	**Total**
TVS			
Endometrium ≥ 5 mm	2	18	20
Endometrium poorly defined	4	20	24
Endometrium < 5 mm	0	28	28
Total	6	66	72
LD profile			
Abnormal	6	10	16
Normal	0	56[a]	56
Total	6	66	72

[a] In one case histology of the Endorette aspirate suspected malignancy, but histology of the subsequent curettage was benign.