Original Article The effects of delayed formalin fixation on endometrial pathology in hysterectomy specimens

Murad Alturkustani¹, Haneen Al-Maghrabi^{1,2}

¹Department of Pathology, King Abdulaziz University, Jeddah, Saudi Arabia; ²Department of Anatomic Pathology, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia

Received May 26, 2019; Accepted July 22, 2019; Epub August 1, 2019; Published August 15, 2019

Abstract: Opening hysterectomy specimens for pathological assessment can be performed before or after formalin fixation. The former method, preferred by most pathologists, limits the autolytic changes of the endometrium, but it may result in a distortion of the uterine wall as a result of the contraction of the myometrium. This may interfere with the assessment of the degree of tumor extension to the uterine wall. The latter method, which is less common, preserves the uterine wall but may limit the assessment of the endometrial glands due to autolytic changes, and the effect of nuclear features is unknown. In this study, we assessed 78 hysterectomy specimens opened by the second method for these changes. The autolytic changes were present in all cases, but they didn't limit the pathologist's assessment to reach the final diagnosis in both the benign and malignant cases. Although, it was difficult to determine which of the nuclear changes was caused by delayed fixation, we found nuclear rounding and prominence of the nucleoli were not features of autolytic changes. Conditions with nuclear features that may mimic mild nuclear atypia were common in these specimens, but not severe atypia. We concluded that a delayed opening of the uterus is an acceptable procedure in dealing with these specimens.

Keywords: Hysterectomy, uterus, formalin fixation, specimen handling, nuclear atypia

Introduction

The products of hysterectomy are common specimens received in the anatomical pathology laboratory. There is a debate about when to open the uterus in these specimens. The most common accepted method is to open the uterus as soon as possible to facilitate the formalin penetration through the myometrium and endometrium [1, 2]. This method should allow for better formalin fixation to avoid autolysis and fixation artifacts that may lead to false interpretations. The major disadvantage of using this method is the distortion in the uterine wall as result of the contraction of the myometrium. This distortion may interfere with the assessment of the degree of tumor extension to the uterine wall, which may alter the stage of endometrial carcinoma [3].

The less commonly-used method is to immerse the hysterectomy specimens in formalin for fixation and postpone opening the uterus until the next day in the pathology department. This method will preserve the uterine wall without distortion but results in a delayed fixation of the endometrium. The delayed fixation may result in autolytic changes which could alter the normal nuclear features (i.e. shape, chromatin pattern, and nucleoli). Nuclear atypia is an important parameter to asses in endometrial pathology, but it is not known if nuclear changes in delayed fixation will result in the appearance of atypical nuclei. Another modification of this method is to inject the endometrial cavity with the fixative through the cervix to allow better endometrial fixation and prevent distortion of the uterine wall [4].

In this study, we assess the result of delayed fixation on the pathological assessment of these specimens. This is done by reviewing the pathologist final diagnosis on these specimens. We also study the nuclear changes in these specimens to assess whether the delayed fixation results in nuclear atypia.

Materials and methods

Ethical approval to perform this study was obtained from the King Abdulaziz University Hospital's ethics committee. All microscopic slides for hysterectomy specimens for a oneyear period were retrieved along with the signed pathological reports. The usual practice in dealing with these specimens during that period was: the unopened hysterectomy specimen was placed in the container in the surgical operative room and the 10% formalin solution was added to the container. The specimen was then transferred to the pathology department. This transfer could be completed on the same day or the next morning. This usually resulted in delaying opening the hysterectomy specimen for 16 hours for most of the specimens, but sometimes longer if the specimen was received over the weekend. In the pathology department, hysterectomies were opened using proper techniques. In general, the sections for the histological examination for benign diseases included two sections from the endometrium and underlying myometrium (unless a gross lesion was seen) and two sections from the transformation zone of the cervix, one from the anterior and one from the posterior lip. For malignant lesions, more samples were obtained according to the size of the neoplasm and the recommended sampling. The final diagnosis reports were reviewed and the histopathological examination of the endometrial sampled sections for all cases were examined by the 2 authors. The purpose of the review and the examination were to determine the effect of the delayed opening of the hysterectomy specimen (i.e. delayed fixation) on the following: 1) The ability to reach to a final diagnosis. 2) The presence of autolytic features. 3) Nuclear changes (nuclear shape and size, regularity of the membrane, pleomorphic changes, chromatin pattern and prominence of the nucleoli) in the epithelial and stromal component. We used the following definitions in the pathological assessment of these parameters. Autolytic features were defined as separation of the epithelium from the stroma by clear spaces (autolysisinduced retraction artifact), collapse of the glands, and epithelial sloughing [5]. For nuclear atypia, we did not find a clear definition adopted by all pathologists, which does reflect the different threshold among pathologists. We used Clement's definition and criteria for this

purpose [6]. Nuclear atypia was defined as changes in nuclear shape (rounding, enlargement, pleomorphism, and irregular borders), changes in nuclear chromatin (hyperchromasia, coarse chromatin and condensation along the nuclear membrane), and by the presence of prominent nucleoli. These were divided into mild, moderate, and severe and into focal and diffuse. These atypical changes were usually associated with increased cytoplasm volume and eosinophilia.

Results

We examined 78 hysterectomies that were received and opened after fixing the uterus in formalin for at least 16 hours. The patients' ages ranged from 30-76 years, with an average age of 52.4 years. The final diagnoses were reached on all of these specimens, and none of the reports indicated any limitation due to delayed formalin fixation. These cases included 71 benign and 7 malignant cases. These were distributed as following: proliferative endometrium (8), weakly proliferative endometrium (4), secretory endometrium (6), inactive endometrium (15), atrophic endometrium (15), disordered proliferative endometrium (15), hyperplasia without atypia (4), atypical hyperplasia (4), endometrioid endometrial carcinoma (4), endometrial serous carcinoma (2), and mixed endometrioid and serous carcinoma (1). The autolytic changes as defined in the methods were present in all the cases and range from mild to moderate and from focal to diffuse (Figure 1A-F). None of these cases was so badly autolyzed to the degree that it prevented the assessment of the endometrial pathology even in cases with the most prominent autolytic changes (Figure 1E, 1F). For nuclear changes, we had studied whether nuclear atypia was present in these specimens and when present if it delayed fixation resulting in these changes. For the first question, we found that at least focal nuclear atypia was present in all specimens. However, in most cases these changes could be attributed to mimickers of atypia rather than true nuclear atypia as we will discuss later. For the second question, we found a great variability of nuclear changes in the same case and even in the same glands. It was difficult to decide which of these changes were caused by delayed fixation, and which represented genuine nuclear changes after excluding the mimickers of the



Figure 1. Endometrial gland changes with delayed fixation. (A) Separation of the glands from the stroma and epithelial sloughing/falling out in the space. There are no changes or breakdown in the stroma. The nuclei are elongated, hypochromatic with no prominent nucleoli. (B) Similar changes as in (A) but the nuclei are oval with open chromatin and visible nucleoli. Stromal cells appear round with no prominent nucleoli. (C & D) The nuclear features in these glands show similar features to (B) but in addition show elongated nuclei and stromal nuclei with open chromatin and hyperchromasia. (E & F) The case with 2 days fixation showed more pronounced autolytic changes, but the nuclear details in (F) did retain elongated nuclei and did not become rounded and there are no prominent nuclei. All figures are stained by hematoxylin and eosin (H&E). Original magnification: (A-D) 400^{\times} , (E) 20^{\times} , (F) 600^{\times} .

nuclear atypia. However, these changes were not diffuse, and the nuclear changes in the glands and adjacent stroma were not similar.

In glands with early autolytic changes in the form of separation of the entire glands from the stroma, some of the nuclei were elongated and had pale chromatin but showed no significant pleomorphism or any prominent nucleoli (**Figure 1A**). The pathologist's diagnosis in this case was proliferative endometrium without atypia, and we concur with this diagnosis. Other cases with early autolytic changes showed some atypical features in the form of oval nuclei and visible nucleoli (Figure 1B). The adjacent stroma in these cases did not show similar changes to those in the glands, even in areas with more advanced autolytic changes (Figure 1C, 1D). This also was not considered atypical by the signing off pathologist, although it would be considered mild atypia by the criteria used here. In the case with most autolytic changes, most of the glands were detached and fragmented, which interfered with the assessment of the architecture (Figure 1E). Although the pathological assessment was limited, the separation between the glandular spaces was interpreted as "negative for hyperplasia and malignancy." The detached fragmented glandular epithelium did show elongated nuclei, with mild pleomorphism, visible nucleoli in only a few cells and mild nuclear irregularity. So even in these areas, there was no diffuse rounding of the nuclei and no prominence of the nucleoli, which supports our finding that delayed formalin fixation does not result in these changes. One of the pitfalls of nuclear atypia is to exclude the mimickers (Figure 2A-E) before diagnosing it as such. We found the following mimickers as the most common causes of such changes: tubal metaplasia

(Figure 2A), nuclei of secretory glands (Figure 2B), nuclear changes in stratum basalis glands (Figure 2C-E), and cystic atrophy. These changes were comparable to the genuine nuclear atypia (Figure 2F).

Discussion

There is more than one way to deal with hysterectomy specimens. The less common way, which we studied here, is to fix the uterus first and then open it the next day. Many pathologists do not prefer this way as it will delay fixation of the endometrium and result in artifac-



Figure 2. Mimickers of nuclear atypia. Tubal metaplasia with round nuclei, open chromatin and cilia. (B) Secretory endometrial glands with round nuclei but secretory cytoplasmic blebs and the stroma showed many apoptotic bodies. (C-E) The endometrial glands in the stratum basalis show round nuclei with prominent nucleoli as compared to the nuclei of the glands in the functionals. (F) Atypical hyperplasia shows endometrial glands with atypical nuclear features. All figures are stained by hematoxylin and eosin (H&E). Original magnification: (A, B) 400×, (C) 10×, (D) 20×, (E, F) 40 Tubal metaplasia with round nuclei, open chromatin and cilia. (B) Secretory endometrial glands with round nuclei, but the secretory cytoplasmic blebs and the stroma showed many apoptotic bodies. (C-E) The endometrial glands in the stratum basalis show round nuclei with prominent nucleoli as compared to the nuclei of the glands in the functionals. (F) Atypical hyperplasia shows endometrial glands with atypical nuclear features. All figures are stained by hematoxylin and eosin (H&E). Original magnification: (A, B) 400×, (C) 10×, (D) 20×, (E, F) 40×.

tual changes in the architectural and cytological features of the endometrial glands. However, it is not known, to our knowledge, if the cytological changes will result in nuclear atypia. We found that a delayed opening of the uterus will result in autolytic changes in all the specimens. Although this may limit the assessment of the endometrial pathology, the pathologists were able to reach a diagnosis in all of the examined cases. For nuclear changes, we found that delayed fixation did not result in a rounding of the nuclei or a prominence of the nucleoli (i.e. important features for atypia). We were less certain about other nuclear changes like nuclear size and shape, and chromatin intensity. Finally, many of the nuclear changes that could be interpreted as atypical in these specimens were attributed to the mimickers that should be excluded before adding this objective to the nuclear changes.

Pathological assessment depends on the staining characteristics of well-fixed tissue, and autolytic changes may interfere with this assessment. For this reason, most pathologists prefer to open the uterus as soon as possible to facilitate the formalin penetration and fixation of the endometrium and limit autolytic changes [1, 2, 4]. In this study, we concur with this observation, as autolytic changes were present in all cases with delayed fixation but did not completely limit the pathological assessment of the endometrium. The pathological diagnoses on most cases, even with the presence of some autolytic changes, were straightforward, especially in the benign cases. This is consistent with a previous study which found none of the 98 hysterectomy specimens was badly autolyzed to prevent

the assessment of the endometrial pathology [3]. For hyperplastic and malignant endometrial cases, there was a potential for some major limitations that may affect the diagnosis. The detachment and sloughing of the endometrial glands would interfere with the assessment of the percentage of the solid areas in the endometrioid carcinoma (i.e. affect the grading), and nuclear changes with a delayed fixation may be interpreted as atypical (an important feature in

the pathological assessment of the endometrial pathology). Nuclear atypia represents the difference between atypical and non-atypical hyperplasia, and if they are severe enough in the endometrioid carcinoma, the grade will be upgraded. The threshold for diagnosing nuclear atypia in the endometrial gland is different among pathologists [7, 8]. In general, the most common used features include: loss of polarity, nuclear pleomorphism and irregularity, rounding of the nuclei, and prominence of the nucleoli, and these can be graded into mild, moderate, and severe [6]. Nuclear changes are known to occur with delayed fixation, but it is not known if these changes will result in atypicallooking nuclei. Few studies describe the nuclear changes with delayed fixation. Delayed fixation may result in indistinguishable nuclear chromatin, and the nucleoli are sometimes not visualized [9]. Although we could not determine the effect of delayed fixation on nuclear features, we concluded that two of the important atypical features (i.e. nuclear rounding and prominence of the nucleoli) were not caused by delayed fixation. This conclusion is the result of the following observations: 1) The nuclear changes in the examined cases were not diffuse, as not all nuclei had similar effects. 2) The nuclear changes in the endometrial glands did not match the immediately adjacent stromal nuclei. 3) Even in the most autolytic glands, there were elongated nuclei and no prominence of nucleoli. We could not reach a firm conclusion of chromatin changes, but there was a general tendency to show pale chromatin in both glandular and stromal nuclei. The nuclear shape and size were also difficult to assess, but there was a general, mild enlargement of the nuclear size. These possible alterations will not result in severe atypia but may overlap with mild atypia. So we concluded that these changes would not result in severe nuclear atypia to upgrade the endometrioid carcinoma. For hyperplasia, pathologists could interpret mild atypia differently, so this is a possible limitation in the assessment of specimens with delayed fixation of the endometrium.

The more common way is to open the hysterectomy specimens as soon as they are received for a better fixation of the endometrium. The major disadvantage of using this method is the distortion in the uterine wall as result of the contraction of the myometrium [3]. This distortion may interfere with the assessment of the degree of tumor extension to the uterine wall, which may alter the stage of endometrial carcinoma, [3] and, even in these cases, endometrial autolytic changes are not uncommon. Another proposed method is to fix the uterus before opening but inject the endometrial cavity with the fixative through the cervix to allow better endometrial fixation and prevent the distortion of the uterine wall [4]. In our laboratory, we mainly open the hysterectomy specimens after fixation. This is mainly because we receive most of these specimens after hours or the next morning in the pathology department. We do not have major limitations to enforce a change in our method of dealing with these specimens, and as we discussed here, both methods have their limitations. The major limitation of this study is the lack of a comparison to a cohort of uteri opened immediately before fixation, as most of our specimens were opened after fixation.

An important pitfall in assessing nuclear atypia is to exclude the mimickers of nuclear atypia. These represent mimickers, as a rounding of the nuclei and the prominences of the nucleoli are a common finding in these benign changes. The most common in the examined cohort were nuclear changes in the stratum basalis glands, tubal metaplasia, nuclei of secretory glands, and cystic atrophy. The stratum basalis glands are basally-located glands and not commonly sampled in endometrial biopsy, but they are always present in sampling from hysterectomy specimens. These glands appear more crowded as they may branch. The nuclei are commonly round with open chromatin, and sometime the nucleoli are visible. These can be easily mistaken as atypical glands if the locations of these glands are not taken in consideration. The other clue for their recognition is that they have a different stroma than the functionalis layer. Tubal metaplasia is a very frequent physiological change found in the endometrium [10]. Metaplastic glands have 3 different cells, and ciliated cells can be round and sometime have prominent nucleoli. If cilia are not visualized, these cells can be called atypical. Other common causes include other epithelial metaplasia, pregnancy-related changes, hormonal changes and postmenstrual changes. All of these changes will lead to a disorganized, compact glandular growth with a mild degree of nuclear

atypia. Cystic atrophy, usually seen in elderly women, are composed of fibrotic stroma with cystically dilated glands lined by a single layer of flattened epithelium, so these cells might show the features of a rounding of the cells and chromatin clearing [7]. Usually no mitotic activity is seen.

In conclusion, we found that a delayed opening of hysterectomy specimens did not completely limit the examination of the endometrial pathology. Delayed formalin fixation of the endometrium is associated with autolytic changes and may change the nuclear features of the endometrial glands. However, it does not cause atypical round nuclei and prominent nucleoli. Finally, endometrial changes in hysterectomy specimens usually have many mimickers of nuclear atypia that could be misinterpreted.

Disclosure of conflict of interest

None.

Address correspondence to: Murad Alturkustani, Department of Pathology, King Abdulaziz University, Abdullah Sulayman, PO Box 80205, Jeddah, Saudi Arabia. Tel: +966500936683; Fax: +966 012-6048433 (hosp); E-mail: alturkustani.murad@gmail. com

References

 Lester SC. Gynecologic and Perinatal Pathology. In: manual of surgical pathology. 3rd edition. Elsevier Health Sciences; 2010. pp. 423-472.

- [2] Rosai J. Appendix H. Guidelines for handling most common and important surgical specimens. In: Ackerman's surgical pathology. 8th edition. St Louis: Mosby; 1996.
- [3] Heatley MK. The effect of prior fixation on the distortion of uterine specimens opened after receipt in the pathology laboratory. Pathol 2006; 38: 539-540.
- [4] Houghton J, Roddy S, Carroll S and McCluggage W. A simple method for the prevention of endometrial autolysis in hysterectomy specimens. J Clin Pathol 2004; 57: 332-333.
- [5] Reichert RA. Pathology of the Uterine Corpus. Diagnostic gynecologic and obstetric pathology: an atlas and text. 1st edition. Lippincott Williams & Wilkins; 2012. pp. 168-306.
- [6] Clement PB and Young RH. Endometrial hyperplasia and carcinoma. In: editors. atlas of gynecologic surgical pathology. 3rd edition. Elsevier Health Sciences; 2013. pp. 161-193.
- [7] McCluggage W. My approach to the interpretation of endometrial biopsies and curettings. J Clin Pathol 2006; 59: 801-812.
- [8] Owings RA and Quick CM. Endometrial intraepithelial neoplasia. Arch Pathol Lab Med 2014; 138: 484-91.
- [9] Rastogi V, Puri N, Arora S, Kaur G, Yadav L and Sharma R. Artefacts: a diagnostic dilemma-a review. J Clin Diagn Res 2013; 7: 2408.
- [10] Simon RA, Peng SL, Liu F, Quddus MR, Zhang C, Steinhoff MM, Lawrence WD, Sung CJ. Tubal metaplasia of the endometrium with cytologic atypia: analysis of p53, Ki-67, TERT, and longterm follow-up. Mod Pathol 2011; 24: 1254-61.