

## RESEARCH ARTICLE

# Endometrial Intraepithelial Neoplasia (EIN) in Endometrial Biopsy Specimens Categorized by the 1994 World Health Organization Classification for Endometrial Hyperplasia

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### Abstract

Our study is to determine the presence of endometrial intraepithelial neoplasia (EIN) in endometrial biopsy specimens classified by the 1994 World Health Organization (WHO) criteria for endometrial hyperplasia. Endometrial biopsy specimens that were stained with hematoxylin and eosin (HE) were examined and categorized by the WHO 1994 criteria and for the presence of EIN as defined by the International Endometrial Collaborative Group.  $\beta$ -catenin expression was examined by immunohistochemistry. A total of 474 cases of HE stained endometrial biopsy tissues were reviewed. There were 379 cases of simple endometrial hyperplasia, 16 with simple atypical endometrial hyperplasia, 48 with complex endometrial hyperplasia, and 31 with complex atypical endometrial hyperplasia. Among the 474 endometrial hyperplasia cases, there were 46 (9.7%) that were classified as EIN. Of these 46 cases, 11 (2.9%) were classified as simple endometrial hyperplasia, 1 (6.3%) as simple atypical endometrial hyperplasia, 6 (12.5%) as complex endometrial hyperplasia, and 28 (90.3%) as complex atypical endometrial hyperplasia. EIN was associated with a higher rate of  $\beta$ -catenin positivity than endometrium classified as benign hyperplasia (72% vs. 22.5%, respectively,  $P < 0.001$ ), but a lower rate than endometrial adenocarcinoma (72% vs. 96.2%, respectively,  $P < 0.001$ ). In benign endometrial hyperplasia, high  $\beta$ -catenin expression was noted in the cell membranes, whereas in EIN and endometrial adenocarcinoma high expression was noted in the cytoplasm. In conclusion, EIN is more accurate than the WHO classification for the diagnosis of precancerous lesions of the endometrium.

**Keywords:** Endometrial hyperplasia - endometrial intraepithelial neoplasia - beta-catenin - World Health Organization

*Asian Pac J Cancer Prev*, 14 (10), 5935-5939

### Introduction

Endometrial hyperplasia (EH) is characterized by endometrial glands of irregular size and shape due to non-physiological proliferation of the endometrium (Horn et al., 2007). The 1994 World Health Organization (WHO) guidelines classified EH as simple non-atypical hyperplasia, complex non-atypical hyperplasia, simple atypical hyperplasia, and complex atypical hyperplasia (Tavassoéli and Devilee, 2003). Complex EH with atypia has been regarded as a precancerous lesion of endometrial adenocarcinoma, with the progression of the aforementioned 4 categories to endometrial carcinoma of < 1%, 3%, 8%, and 29%, respectively (Kurman et al., 1985). However, this classification is limited by subjectivity and the lack of objective features for diagnosis, and has been found to be poorly reproducible (Kendall et al., 1998; Bergeron et al., 1999). Moreover, the 4 classification system is not reliable for predicting therapeutic options, i.e., observation, hormonal treatment, or hysterectomy (Gültekin et al., 2009).

The endometrial intraepithelial neoplasia (EIN) classification system was introduced in 2000 by the

International Endometrial Collaborative Group and defines 3 disease categories: benign hyperplasia, a polyclonal hormone dependent diffuse lesion; EIN, a neoplastic monoclonal lesion that can be localized initially but later become diffuse; and cancer (Mutter et al., 2000a; Baak and Mutter, 2005). The EIN classification system relies on objective criterion and has been shown to be highly reproducible (Mutter et al., 2000b; Baak and Mutter, 2005; Mutter et al., 2007; Zhou et al., 2008; Salman et al., 2010). Moreover, the 3 categories correspond to treatment options (Gültekin et al., 2009).

The purpose of this study was to determine the presence of EIN in endometrial biopsy specimens that were classified by the WHO criteria.

### Materials and Methods

In this study, we retrospectively reviewed endometrial curettage specimens which had undergone hematoxylin and eosin (HE) staining collected from the Department of Pathology of the General Hospital of Tianjin Medical University from 2008 to 2010. All specimens have been confirmed as endometrial hyperplasia and classified by the

**Table 1. WHO and EIN Classification in Cases of Endometrial Hyperplasia**

	Total	Simple Endometrial Hyperplasia	Simple Atypical Endometrial Hyperplasia	Complex Endometrial Hyperplasia	Complex Atypical Endometrial Hyperplasia
Number	474	379	16	48	31
EIN cases	46 (9.7)	11 (2.9)	1 (6.3)	6 (12.5)	28 (90.3)
Numbers of cases with follow-up	13 (2.7)	5 (1.3)	1 (6.3)	1 (2.1)	6 (19.4)
Number of patients who received hysterectomy	3 (0.6)	0 (0)	0 (0)	0 (0)	3 (9.7)

Data are presented as number (percentage); EIN, endometrial intraepithelial neoplasia

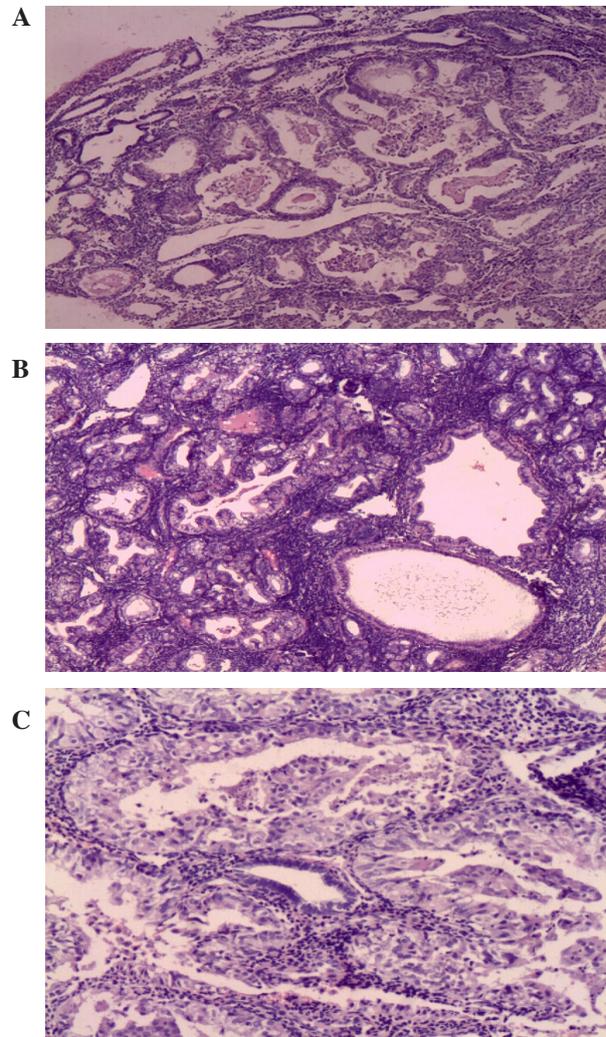
WHO 1994 criteria. They were also classified according to the EIN system and the results were compared. Clinical data of patients were also collected. This study was approved by the Institutional Review Board of the hospital, and because of the retrospective nature of the study the requirement for informed patient consent was waived.

The diagnostic criteria for EIN developed by International Endometrial Collaborative Group were used in this study (Mutter et al., 2000b; Baak and Mutter, 2005). These criteria emphasize the importance of size, structure and cytological features endometrial glands, and in brief are as follows. 1) EIN is characterized by crowding of glands to a point where the area of glands exceeds that of stroma in the architectural changes. 2) The cytological features of EIN can differentiate EIN from the concomitant benign endometrial glands. EIN usually occurs in the background of benign endometrial hyperplasia, but there is no transition or clear boundary apparent on histological examination. The features of EIN include round nuclei, evident nucleolus, and uneven and coarse granular distribution of nuclear chromatin. However, not all EIN presents with these features, and atypical features in cytology can not be used for the diagnosis of EIN. 3) The diameter of lesions is >1 cm. 4) Benign endometrial changes similar to those in EIN should be excluded (such as artifacts, responsive changes, menstrual endometrium, endometrial polyps, and endometrial changes after steroid treatment. 5) Endometrial adenocarcinoma should be excluded.

To detect the expression of  $\beta$ -catenin, sections were preincubated with 10% normal rabbit serum in PBS (pH7.5) and then incubated with anti- $\beta$ -catenin (Neomarker, Inc.) antibody in 10% normal serum in PBS (pH7.5). On the following day, sections were washed in PBS and incubated with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature. Immunoreactivity was detected using the Vectastain Elite ABC kit (Vector Laboratories).

#### Statistical analysis

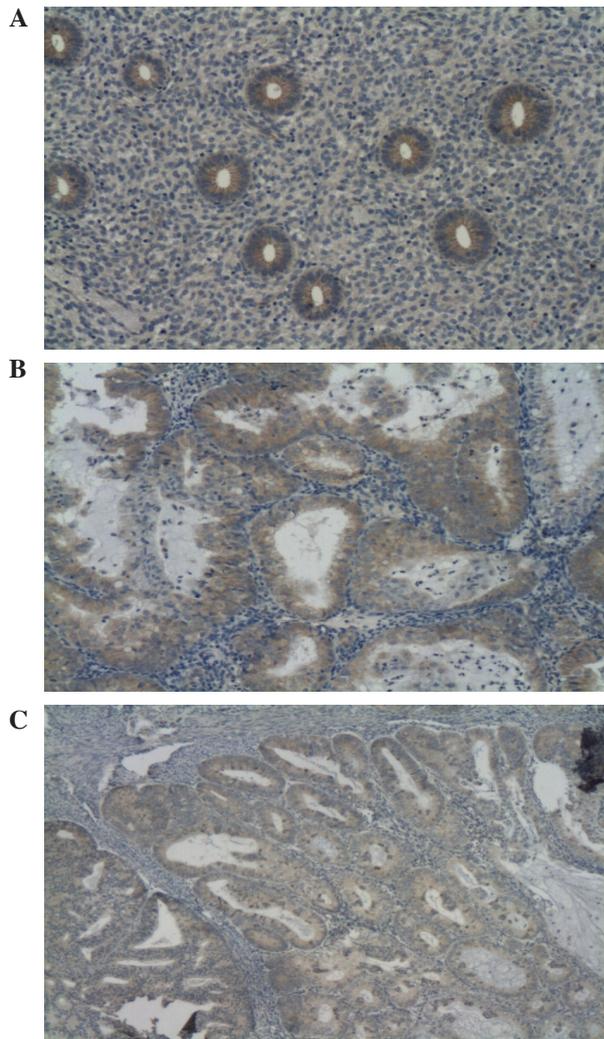
General data were expressed as number and (%) for given types of endometrial hyperplasia. The  $\beta$ -catenin expression was also represented as number for cases with negative and positive expression. The positive rate of  $\beta$ -catenin expression was compared between endometrial hyperplasia types using Fisher's exact test. Statistical assessments were two-tailed and a value of  $P < 0.05$  was considered to indicate statistical significance. Statistical analyses were performed using SPSS 15.0 statistics software (SPSS Inc, Chicago, IL, USA).



**Figure 1.** A) EIN lesion in a case with simple endometrial hyperplasia. 40 X. B) EIN lesion in a case with complex endometrial hyperplasia. Left, EIN lesion; right, glands with complex hyperplasia. 40 X. C) EIN lesion in a case with simple endometrial hyperplasia. 100 X. A-C) Hematoxylin and eosin staining

#### Results

A total of 474 cases of HE stained endometrial biopsy tissues were reviewed. There were 379 cases of simple endometrial hyperplasia, 16 with simple atypical endometrial hyperplasia, 48 with complex endometrial hyperplasia, and 31 with complex atypical endometrial hyperplasia. Among the 474 EH cases, there were 46 (9.7%) that were classified as EIN. Of these 46 cases, 11 (2.9%) were classified as simple endometrial hyperplasia, 1 (6.3%) as simple atypical endometrial hyperplasia, 6 (12.5%) as complex endometrial hyperplasia, and 28



**Figure 2.  $\beta$ -catenin Expression.** A) In benign endometrial hyperplasia, high expression in the cell membrane was noted. Magnification: 40 X. B) In EIN, high expression in the cytoplasm (abnormal expression) was noted. Magnification: 100 X. C) In endometrial adenocarcinoma, high expression in the cytoplasm (abnormal expression) was noted. Magnification: 40 X

(90.3%) as complex atypical endometrial hyperplasia. Complete follow-up data were available for 13 patients (length of follow-up, 1-3 years), and 3 (0.6%) with complex atypical endometrial hyperplasia received hysterectomies (Table 1)

In simple EH, EIN lesions presented with focal distribution, which was previously diagnosed as simple EH with focal gland crowding or epithelial eosinophilic changes. In complex EH, EIN lesions presented with a focal or diffuse distribution. Endometrial glands with complex hyperplasia showed irregular expansion and were found to be “back-to-back,” stratified epithelium was present in the glands, but these epithelial cells were similar in morphology, and clear boundaries were not noted. Of the cases classified as simple atypical EH, one was diagnosed as EIN. In this case, most of the glands were similar, several atypical glandular cells were present, and crowding of the endometrial glands was noted. Of the 31 cases with complex atypical EH, 28 were diagnosed with EIN in which a focal and diffuse distribution of the lesions was found in 25 and 3 patients, respectively (Figure 1).

**Table 2.  $\beta$ -catenin Expression**

Histological Type	Total Number	$\beta$ -catenin		Positive rate <sup>a</sup>
		-	+	
EIN	25	7	18	72%
PE	10	10	0	0%
BH	40	31	9	22.5%*
EA	26	1	25	96.2%*

EIN, endometrial intraepithelial neoplasia; PE, proliferative endometrium; BH, benign hyperplasia; EA, endometrium adenocarcinoma; <sup>a</sup>Positive rate was calculated as the percentage of cases with +  $\beta$ -catenin expression; \* $P < 0.001$ , indicates significant difference as compared with EIN type by Fisher's exact test

The  $\beta$ -catenin expression is presented in Table 2. EIN was associated with a higher rate of  $\beta$ -catenin positivity than endometrium classified as benign hyperplasia (72% vs. 22.5%, respectively,  $P < 0.001$ ), but a lower rate than endometrial adenocarcinoma (72% vs. 96.2%, respectively,  $P < 0.001$ ). In benign endometrial hyperplasia, high expression in the cell membrane was noted, whereas in EIN and endometrial adenocarcinoma high expression in the cytoplasm (abnormal expression) was noted (Figure 2).

## Discussion

The results of the present study comparing the diagnosis of endometrial changes by the WHO classification and the presence of EIN showed that 90% of cases classified as complex atypical hyperplasia were determined to be positive for EIN, and importantly, almost 3% of simple endometrial hyperplasia cases were found to have a diagnosis of EIN. In addition, EIN was associated with a significantly higher rate of  $\beta$ -catenin positivity than endometrium classified as benign hyperplasia, but a lower rate than endometrial adenocarcinoma.

The WHO classification (1994) for EH is highly subjective and exhibits poor reproducibility (Kendall et al., 1998; Bergeron et al., 1999). Since the introduction of the WHO classification and the development of the EIN classification system by the International Endometrial Collaborative Group, a large amount of knowledge has been gained with respect to the molecular pathogenesis of endometrial hyperplastic and neoplastic lesions (Moreno-Bueno et al., 2003; Norimatsu et al., 2007; Doll et al., 2008; Jeong et al., 2009; Pavlakis et al., 2010). The International Endometrial Collaborative Group scheme divides EH into benign hyperplasia, EIN, and cancer (Baak and Mutter, 2005). Benign hyperplasia and EIN represent 2 distinct diseases with different pathogeneses. Benign hyperplasia is a polyclonal hormone dependent diffuse lesion, whereas EIN is a monoclonal neoplastic disease that can initially be localized and in later stages diffuse (Tavassoéli and Devilee, 2003). Although this classification is not approved by the WHO, it is increasingly being accepted by clinical investigators and experts in the field. EIN is regarded as a precancerous lesion of endometrial adenocarcinoma, and the progression from normal

hyperplasia to precancerous lesion and subsequently to cancer is implied. The EIN classification provides objective measures for the examination and diagnosis of proliferative endometrial lesions, and the classification has high diagnostic accuracy, good repeatability, and favorable predictive value for endometrial cancer (Francz, 2008).

The concept of EIN does not aim to simply re-classify endometrial proliferative lesions, but to confirm the presence of precancerous lesions of endometrial adenocarcinoma on the basis of histopathology. EIN usually presents with focal neoplastic proliferation, and is a proliferative disease significantly different from benign endometrial hyperplasia. EIN represents an intraepithelial tumor that has genetic features consistent with some malignancies (Norimatsu et al., 2007; Jeong et al., 2009; Pavlakis et al., 2010). There is evidence showing that EIN and cancer can occur simultaneously, and the risk of developing cancer in EIN patients is significantly higher than in patients with benign endometrial hyperplasia (Mutter et al., 2000a). EIN is not further subdivided or graded, which is different from classifications of other intraepithelial tumors; nevertheless, EIN is clearly distinguished from benign EH.

Our follow-up results showed that the risk for developing endometrial cancer was increased in patients who were diagnosed with EIN, and almost 3% of patients with simple EH were found to have EIN. Although this indicates a small likelihood of EIN in patients with simple EH, patients with EIN have a high likelihood of developing endometrial cancer and is thus of important clinical value. In addition, 6.3% and 12.5% of patients with simple atypical EH and complex EH, respectively, were found to have a diagnosis of EIN, another finding that highlights the value of using the EIN classification system. Yang et al. (2012) compared EIN diagnosis and the WHO classification for the prediction of coexistent carcinomas in biopsy specimens and reported that the incidence of coexisting carcinoma in EIN cases was similar to that in atypical EH cases, but for the exclusion of coexisting carcinomas the EIN criteria for benign lesions was more predictive than that of the WHO criteria for non-atypical simple and complex hyperplasia.

The WHO criteria for EH are difficult to master and there is overlap between neoplastic and non-neoplastic lesions, and as such the WHO classification has poor reproducibility, especially when there are atypical cells. The EIN classification defines intraepithelial neoplasia on the basis of structure and cytology, and thus exhibits good reproducibility, which is crucial for pathological diagnosis and treatment. Atypical hyperplasia in the WHO classification may be attributed to cytological changes due to exogenous steroids, whereas a diagnosis of EIN is supported by the cytological and structural features, which make the EIN classification system more reliable and reproducible (Mutter et al., 2000a; Mutter et al., 2000b; Baak and Mutter, 2005).

$\beta$ -catenin has been shown to be closely related to the occurrence and development of cancers (Park et al., 2001; Shun et al., 2001; Wong et al., 2001). Bound  $\beta$ -catenin mediates adhesion among cells of the same type, and free  $\beta$ -catenin is involved in the Wnt signaling pathway.

Abnormal activation of the  $\beta$ -catenin-mediated Wnt signaling pathway is an early event in the occurrence of some cancers, and  $\beta$ -catenin-related dysfunction of the E-cadherin/catenin complex may cause invasion and metastasis of cancer cells, which are late events (Li and Ji, 2003; Moreno-Bueno et al., 2003; Duan et al., 2004). Correlation has been observed between early and late events in the development of malignancies, in which  $\beta$ -catenin serves as a bridge (Moreno-Bueno et al., 2003). Abnormal  $\beta$ -catenin protein expression has been found in some malignancies, including endometrial cancer (Li and Ji, 2003; Moreno-Bueno et al., 2003; Duan et al., 2004; Pavlakis et al., 2010). In the normal endometrial epithelium,  $\beta$ -catenin is expressed on the cell membrane (Norimatsu et al., 2007). However, when the APC gene and/or  $\beta$ -catenin gene are mutated,  $\beta$ -catenin is found to be aggregated in the cytoplasm or nucleus (Norimatsu et al., 2007; Doll et al., 2008). Immunohistochemistry results of this study showed that in benign endometrial hyperplasia  $\beta$ -catenin was primarily located in the cell membrane whereas in cases of EIN and endometrial adenocarcinoma it was primarily located in the cytoplasm. These results suggest mutation of the  $\beta$ -catenin gene in EIN and endometrial adenocarcinoma. There are limitations of this study that must be considered. First, the number of cases with a diagnosis of EIN was relatively small. Additionally, follow-up data were only available for a small number of patients.

In summary, EIN can be observed in all categories of the WHO classification of EH. Importantly, EIN can be found in cases categorized as endometrial biopsy specimens that are categorized as simple hyperplasia by the WHO scheme. These results suggest that EIN is more accurate for the diagnosis of precancerous lesions of the endometrium.

## Acknowledgements

The author(s) declare that they have no competing interests.

## References

- Baak JP, Mutter GL (2005). EIN and WHO94. *J Clin Pathol*, **58**, 1-6.
- Bergeron C, Nogales FF, Masseroli M, et al (1999). A multicentric European study testing the reproducibility of the WHO classification of endometrial hyperplasia with a proposal of a simplified working classification for biopsy and curettage specimens. *Am J Surg Pathol*, **23**, 1102-8.
- Doll A, Abal M, Rigau M, et al (2008). Novel molecular profiles of endometrial cancer-new light through old windows. *J Steroid Biochem Mol Biol*, **108**, 221-9.
- Duan GJ, Yan XC, Bian XW, et al (2004). The significance of  $\beta$ -catenin and matrix metalloproteinase-7 expression in colorectal adenoma and carcinoma. *Chin J Pathol*, **33**, 518-22. (in Chinese)
- Francz M (2008). The premalignant disease of the endometrium: endometrial intraepithelial neoplasia. *Magy Onkol*, **52**, 35-40. (in Hungarian)
- Gültekin M, Diribaş K, Dursun P, Ayhan A (2009). Current management of endometrial hyperplasia and endometrial intraepithelial neoplasia (EIN). *Eur J Gynaecol Oncol*, **30**,

396-401.

- Horn LC, Meinel A, Handzel R, Einkenkel J (2007). Histopathology of endometrial hyperplasia and endometrial carcinoma: an update. *Ann Diagn Pathol*, **11**, 297-311.
- Jeong JW, Lee HS, Franco HL, et al (2009). beta-catenin mediates glandular formation and dysregulation of beta-catenin induces hyperplasia formation in the murine uterus. *Oncogene*, **28**, 31-40.
- Kendall BS, Ronnett BM, Isacson C, et al (1998). Reproducibility of the diagnosis of endometrial hyperplasia, atypical hyperplasia, and well-differentiated carcinoma. *Am J Surg Pathol*, **22**, 1012-9.
- Kurman RJ, Kaminski PF, Norris HJ (1985). The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. *Cancer*, **56**, 403-12.
- Li YJ, Ji XR (2003). Relationship between the expression of  $\beta$ -cat, cyclin D1 and c-myc and the occurrence and biological behavior of pancreatic cancer. *Chin J Pathol*, **32**, 223-41. (in Chinese)
- Moreno-Bueno G, Hardisson D, Sarrio D, et al (2003). Abnormalities of E- and P-cadherin and catenin (beta-, gamma-catenin, and p120ctn) expression in endometrial cancer and endometrial atypical hyperplasia. *J Pathol*, **199**, 471-8.
- Mutter GL, Baak JP, Crum CP, et al (2000a). Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. *J Pathol*, **190**, 462-9.
- Mutter GL, Lin MC, Fitzgerald JT, et al (2000b). Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. *J Natl Cancer Inst*, **92**, 924-30.
- Mutter GL, Zaino RJ, Baak JP, et al (2007). Benign endometrial hyperplasia sequence and endometrial intraepithelial neoplasia. *Int J Gynecol Pathol*, **26**, 103-14.
- Norimatsu Y, Moriya T, Kobayashi TK, et al (2007). Immunohistochemical expression of PTEN and beta-catenin for endometrial intraepithelial neoplasia in Japanese women. *Ann Diagn Pathol*, **11**, 103-8.
- Park BH, Vogelstein B, Kinzler KW (2001). Genetic disruption of PPARdelta decreases the tumorigenicity of human colon cancer cells. *Proc Natl Acad Sci U S A*, **98**, 2598-603.
- Pavlikis K, Messini I, Vrekoussis T, et al (2010). PTEN-loss and nuclear atypia of EIN in endometrial biopsies can predict the existence of a concurrent endometrial carcinoma. *Gynecol Oncol*, **119**, 516-9.
- Salman MC, Usubutun A, Boynukalin K, Yuce K (2010). Comparison of WHO and endometrial intraepithelial neoplasia classifications in predicting the presence of coexistent malignancy in endometrial hyperplasia. *J Gynecol Oncol*, **21**, 97-101.
- Shun CT, Wu MS, Lin MT, et al (2001). Immunohistochemical evaluation of cadherin and catenin expression in early gastric carcinomas: correlation with clinicopathologic characteristics and Helicobacter pylori infection. *Oncology*, **60**, 339-45.
- Tavassoéli FA, Devilee P (2003). Pathology and Genetics Tumours of the Breast and Female Genital Organs. IARC Press, Lyon. pp 221-232.
- Wong CM, Fan ST, Ng IO (2001). beta-Catenin mutation and overexpression in hepatocellular carcinoma: clinicopathologic and prognostic significance. *Cancer*, **92**, 136-45.
- Yang YF, Liao YY, Peng NF, et al (2012). Prediction of coexistent carcinomas risks by subjective EIN diagnosis and comparison with WHO classification in endometrial hyperplasias. *Pathol Res Pract*, **208**, 708-12.
- Zhou JJ, Luo Q, Yan W (2008). Endometrial intraepithelial neoplasia: a clinicopathologic analysis of 53 cases. *Chin J Diag Pathol*, **15**, 438-40. (in Chinese)