PUB: 46/2011

Original Article

Significance of Apoptosis in Endometrial Hyperplasia and Carcinoma

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ABSTRACT

***Objectives** : Apoptosis has been observed at increasing levels in the hyperplasia – adenocarcinoma sequence in the endometrium. The aim was to study apoptosis in proliferative, hyperplastic and malignant endometrium and to evaluate its prognostic role in malignancy.*

***Methods** : The study included 57 endometrial biopsies in patients with abnormal uterine bleeding. Evaluation of apoptotic index (AI) and mitotic index (MI) within endometrial glands was performed using light microscopy on Hematoxylin and Eosin stained sections.*

***Results** : The mean apoptotic index increased progressively in proliferative , hyperplastic and malignant endometrium. The difference between proliferative and hyperplastic endometrium was significant ($P<0.001$). The difference between proliferative and malignant endometrium was significant ($P<0.001$). The AI/MI ratios were lowest in proliferative and highest in hyperplastic endometrium.*

***Conclusions** : Apoptosis can be fairly accurately assessed using light microscopy. In proliferating tissues, like endometrium, AI and AI/MI ratios are good indicators of progression of disease.*

***Key words :** apoptosis ; carcinoma ; endometrial hyperplasia*

INTRODUCTION

Endometrial carcinoma is one of the most common malignancies of the female genital tract, and its development is partially associated with the influence of unopposed estrogen, resulting in uncontrolled proliferation.[1]

Apoptosis is a physiologic mechanism of cell death that has been shown to play a role in the onset and development of cancer.[2] In general, the onset and development of cancer has been linked to a decrease in the rate of apoptosis. But in the endometrium, apoptosis is observed at increasing levels in hyperplasia, atypia and adenocarcinoma. Recently, histological methods that reveal parameters of cell proliferation and cell death have become important and might facilitate identification of individuals who are at high risk of developing carcinoma, besides having prognostic significance. Counting of apoptotic cells/bodies by light microscopy is feasible and has been used and described by various authors.[3,4]

MATERIAL AND METHODS

The study included 57 cases of endometrial biopsy in women with a history of abnormaluterine bleeding. The patient's age ranged from 23 to 57 years. Endometrial biopsies were processed and all sections were routinely stained with Hematoxylin & Eosin (H&E). Care was taken to have sections of uniform thickness (not greater than 5mm). The H&E sections were examined using x 400 magnification (high power).

Apoptotic cells/bodies were counted in the endometrial glands. Identification of apoptosis required a clearly defined rounded intraepithelial structure containing fragments of basophilic pyknotic chromatin material usually without

cytoplasmic remnant. Care was taken to exclude intraepithelial lymphocytes and equivocal morphological changes. Similarly identification of mitotic figures required clear separation of chromosomal material.

Apototic index (AI) was calculated by counting the number of apoptotic cells/bodies per 1000 cells counted in 10 randomly selected high power fields. Similarly mitotic index was calculated by counting the number of mitotic figures per 1000 cells counted in 10 randomly selected high power fields. The results were tabulated and the mean apoptotic index, mitotic index and standard deviation were calculated for each sub group.

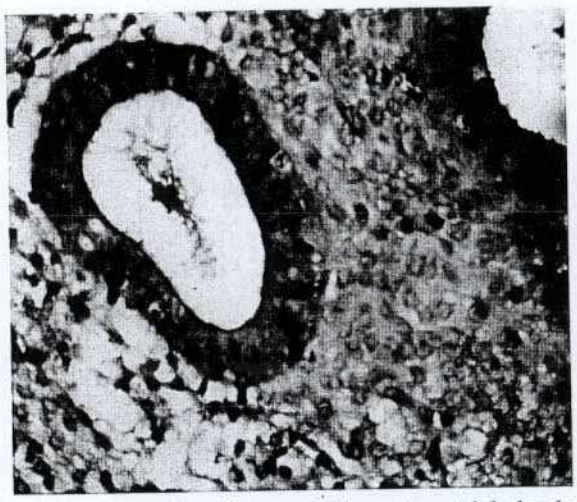

Fig: 1 Apoptotic cells seen in the endometrial glands. H & E x 400.

RESULTS

The apoptotic cells showed certain well defined features, which included cell shrinkage, condensation and deep eosinophilia of the cytoplasm, pyknotic, round to crescentic irregular nucleus [Fig:1]. Apoptotic bodies appeared as tiny, round and pyknotic nuclear fragments [Fig:2].

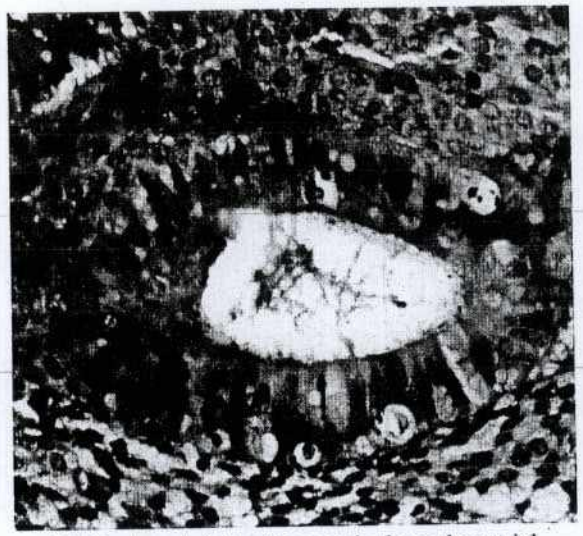

Fig:2 Apoptotic bodies seen in the endometrial glands. H& E x 400

We examined a total of 57 cases, including 10 cases of proliferative phase [PP] endometrium, 10 cases of disordered proliferative [DP], 18 cases of simple hyperplasia [SH], 9 cases of complex hyperplasia [CH] and 10 cases of endometrial carcinoma.

Apoptotic index was 0.7%, 1.63%, 1.11%, 1.22% and 3.42% in PP, DP, SH, CH and endometrial carcinoma.[$P<0.001$] as shown in Table:1. Mitotic index was 0.36%, 0.31%, 0.21%, 0.28%, and 0.69% respectively [$P < 0.001$] as shown in Table:2. The AI/MI ratio was lowest in proliferative phase and highest in simple hyperplasia.

DISCUSSION

Endometrium is a hormone sensitive tissue, and variation in the frequency of apoptosis, is known to occur due to hormonal changes in both physiological and pathological conditions.[2] Normally, apoptosis is more in the glandular epithelium in late secretory and menstrual phase when compared to proliferative phase. There is also strong evidence to suggest that the balance between mitotic activity and apoptosis, which is thought to regulate normal endometrial development, is subject to hormonal control.[5]

Endometrial hyperplasia is associated with abnormally high and prolonged estrogenic

Table:1 Apoptotic index in hyperplastic and malignant endometrium.

Category	Apoptotic index Mean% ± SD	Range Min-max
Proliferative phase (n-10)	0.07 ± 0.20	0.4 - 0.9
Disordered Proliferative (n-10)	1.63 ± 0.62	0.5 - 2.5
Simple hyperplasia (n-18)	1.11 ± 0.51	0.4 - 2.0
Complex hyperplasia (n-9)	1.22 ± 0.42	0.6 - 1.8
Endometrial carcinoma (n-10)	3.42 ± 0.91	2.5 - 5.0

Table: 2 Mitotic index in hyperplastic and malignant endometrium.

Category	Mitotic index Mean% ± SD	Range Min-max
Proliferative phase (n-10)	0.36 ± 0.18	0.1 - 0.6
Disordered Proliferative (n-10)	0.31 ± 0.12	0.2 - 0.6
Simple hyperplasia (n-18)	0.21 ± 0.12	0 - 0.5
Complex hyperplasia (n-9)	0.27 ± 0.08	0.2 - 0.4
Endometrial carcinoma (n-10)	0.69 ± 0.26	0.4 - 1.2

stimulation, unopposed by progesterone. Hence endometrial hyperplasia and carcinoma are often seen as points on a continum, which includes disordered proliferation, simple and complex hyperplasia, atypical hyperplasia and adenocarcinoma.

Our study is based on the evaluation of apoptotic index in 57 cases of endometrial lesions on light microscopy. Apoptotic bodies and mitotic index were counted using x 400 magnification (under high power). Mitotic figures were also readily identifiable and morphologically distinct. Soini et al [3] also observed that a fairly accurate assessment of apoptosis is possible on light microscopy. However, care must be taken to distinguish lymphocytes from apoptotic bodies and not to include neutrophils or other leucocytes in the count.

Various advanced methods have been developed to study apotosis, i.e electron microscopy, flow cytometry, electrophoresis, in situ end labeling offragmented DNA and terminal deoxy nucleotidyl transferase mediated d-UTP biotin nick end labeling technique[TUNEL]. The TUNEL technique is now the most commonly used method for evaluating apoptosis, but it is expensive and its use in developing countries like India is not feasible. Moreover, it is a specialized technique and so its setup and standardization is not available at every institute.

In the present study, apoptotic index was increased in hyperplasia and malignant endometrium, when compared to normal proliferative endometrium. In contrast, mitotic indices were seen to be decreased in hyperplasia as compared to proliferative

Endometrium carcinoma. This suggests that ratio of AI/MI may serve as a better prognostic marker than mitotic index alone in endometrial carcinoma.

Ioffe et al [6] in their study, showed that apoptotic index was approximately 2 fold higher in simple hyperplasia, 2.5 fold greater in complex hyperplasia and 3 fold higher in endometrial adenocarcinoma, when compared to normal proliferative phase. Mitotic indices in their study was seen to be decreased in hyperplasia as compared to proliferative endometrium and carcinoma.[6]

Kokawa et al[7] showed that apoptosis increases in atypical hyperplasia and markedly increases in Endometrioid adenocarcinoma, thus suggesting that the occurence of apoptosis may play a critical role in carcinogenesis and differentiation in endometrioid adenocarcinoma. Heathley et al[8] showed a 2 fold higher AI in poorly differentiated carcinoma, 3 fold higher AI in undifferentiated carcinoma compared to well or moderately differentiated endometrial carcinoma.

In the present study apoptosis was also increased in disordered proliferative endometrium, which could be associated with unopposed estrogen stimulation. Thus there is an important etiological and functional overlap between the neoplastic sequence and disordered proliferative endometrium, although the duration and pattern of unopposed stimulation varies.

Apoptosis is a type of programmed cell death which controls normal cell homeostasis. It also plays an important role in carcinogenesis and is controlled by several genes. Bcl-2 is an important anti-apoptotic gene, belonging to the Bcl-2 gene family, which has the ability to block a wide variety of apoptotic signals. Bax is a pro-apoptotic gene (another member of the Bcl-2 family), but in contrast it has an apoptosis stimulating function. Hence, the cellular ratio of Bcl-2/Bax is important in regulation of apoptosis. A high Bcl-2/Bax ratio makes cells resistant to apoptotic stimuli, while a low ratio induces cell death.

Studies have shown that there is increased Bcl-2 protein expression in simple and complex hyperplasia without atypia, but weaker staining in approximately half of atypical hyperplasia and further reduced Bcl-2 expression in adenocarcinomas, consistent with the findings of increased apoptosis in both atypical complex hyperplasia and endometrial carcinoma.[9] In contrast expression of Bax was increased in endometrioid adenocarcinoma and decreased in endometrial hyperplasia. Thus overexpression of Bax may lead to progression of endometrial hyperplasia to carcinoma.[9]

Hence, increasing frequency of apoptosis is seen in the hyperplasia – adenocarcinoma sequence of the endometrium and is likely associated with genetic alterations driving progression towards the neoplastic pathway.

The present study highlights the morphological assessment of apoptotic index in endometrial tissue on light microscopy. Further studies showing the expression of these proteins- Bcl-2 and Bax in the endometrium will reflect the role of these factors in regulation of apoptosis in carcinoma.

CONCLUSIONS

Apoptotic bodies can be readily and accurately assessed on routine H&E sections, provided there is strict adherence to established criteria for recognition and counting of apoptotic bodies. In actively proliferating tissues and hormonally controlled tissues like endometrium, apoptotic index and AI/MI are better indictors of disease progression than mitotic indices alone.

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Source of Support : Nil **Conflict of Interest** : Nil