ORIGINAL ARTICLE

Apoptosis in Endometria of Dysfuntional Uterine Bleeding Women

K Rekha, MD*, A Malini, MD*, R Xavier, PhD, K Baba, MD****

*Department Pathology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, Madras University, Chennai-113, India, ** Faculty of Medicine and Applied Sciences, AIMST, Sungai Petani, Kedah, Malaysia

Summary

The study of apoptosis in endometrium of women with irregular uterine bleeding and its predictive value in endometrial malignancy. Analyze apoptotic and mitotic indices and their relevance in irregular uterine bleeding. To determine the expression of Bcl-2 oncoprotein in endometrial glands from patients with irregular uterine bleeding. Department of pathology in a Government Hospital serving a varied socio-economic population in Chennai. Random samples of endometrial currettings from dysfunctional uterine bleeding (DUB) patient who underwent endometrial curettage as therapeutic and diagnostic procedure during the year 2000. Of 50 cases of endometrial samples from patients diagnosed as cases of DUB, the apoptotic and mitotic indexing was carried out and histological categorization revealed 13 cases as Anovulatory, 14 as simple hyperplasia, 5 as early secretory endometrium, 4 as mid secretory and 4 as late secretory endometrium and 7 as endometrium showing features of hormonal imbalance. Three cases were not included, due to sub-optimal processing. A good correlation of the Bcl-2 expression and the apoptotic cell morphology/indices, in the different categories of the endometria of DUB cases is observed. This preliminary study gives an insight to the existence of a correlative pattern of apoptosis in DUB cases. A prospective study on a larger number of cases may substantiate the hypothesis that the Apoptotic and Mitotic indices are useful screening methods with predictive values on development of endometrial carcinoma. It is observed that an increased apoptotic index correlating with high Bcl-2 expression, reflecting the actual cell burden. This prolonged cell survival resisting cell deletion is associated with irregular uterine bleeding endometria.

Key Words: Dysfunctional Uterine Bleeding, Apoptosis, Bcl-2.

Introduction

Apoptosis is a physiological cell suicide program, which is critical for the development and maintenance of healthy tissues. The counterpart of apoptosis - mitosis or cell birth has been well studied for over a century, while Apoptosis was first observed and reported by authors[1] and its concept gained significance nearby 10 years later. Proteins that are involved in the regulation of apoptosis are currently of great biological interest and many would make attractive therapeutic targets. In fact Nobel Prize for the year 2002 had been awarded to work done on Apoptosis! Concerns about abnormal menstrual bleeding are a common reason for women to consult a primary care physician. One of the commonly diagnosed entities is Dysfunctional Uterine Bleeding. It is defined, as excessive, prolonged, unpatterned bleeding from the endometrium that is unrelated to structural or systemic disease. The significance of Apoptosis has been widely studied in the normal endometrium and other entities like endometrial carcinoma and endometriosis. Dysfunctional uterine

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Corresponding Author: Kota Rekha, Department of Pathology, AIMST, No. 2, Persiaran Cempaka, 08000 Sungai Petani, Kedah

bleeding a rather nebulous term for a variety of menstrual abnormalities has been a gray zone. Inspite of a lot of work being done they have been rather inconclusive. But work done thus far does prove a fact that increased apoptosis is noted in patients with dysfunctional uterine bleeding. Though the significance of this finding as mentioned earlier is yet unknown, it may serve as a morphological marker of abnormal endometrial development.

To summarize, morphological features of Apoptotic cells can be divided into specific stages. Firstly there is cell shrinkage: cell size becomes smaller, cytoplasm dense and cell organelles become more tightly packed. Chromatin Condensation: The most important feature observed, the chromatin aggregates peripherally under the nuclear membrane into well-delimited dense masses of various shapes and sizes. Formation of cytoplasmic blebs and apoptotic bodies: Extensive surface blebbing is seen which then undergoes fragmentation into a number of membrane bound apoptotic bodies. Phagocytosis of apoptotic cells by adjacent parenchymal cells or macrophages: They are rapidly degraded by lysosomes and adjacent cells migrate or proliferate to replace the space of the dead apoptotic cell. On routine H & E sections, apoptosis is seen involving a single cell or small clusters of cells. The cell appears as a round or oval mass with an intensely eosinophilic cytoplasm with dense nuclear chromatin fragments.

Materials and Methods

Samples from women with irregular uterine bleeding problem were collected and formalin fixed. Routine processing of same to paraffin and four micron thick sections screened after being stained with H&E was carried out.

Immunohistochemistry
All the cases were subjected to Immunohistochemical staining for expression of Bcl-2 oncoprotein; placental tissue was taken as control. Method used: LSAB technique Antibody: Bcl-2 (Dako, clone 124)

Evaluation of Bcl-2 expression:
In our study 35 cases out of 50 were subjected to Immunohistochemical staining for Bcl-2 expression. A section of placenta was used as a control which always had an intensity of grade "4+". The positive cases were picked up and Bcl-2 expression was recorded based on the intensity of staining. We followed the grading used by authors[2] in their study of endometrial hyperplasia and carcinoma. The intensity of staining was recorded separately for cytoplasmic and nuclear expression as given below[3]:

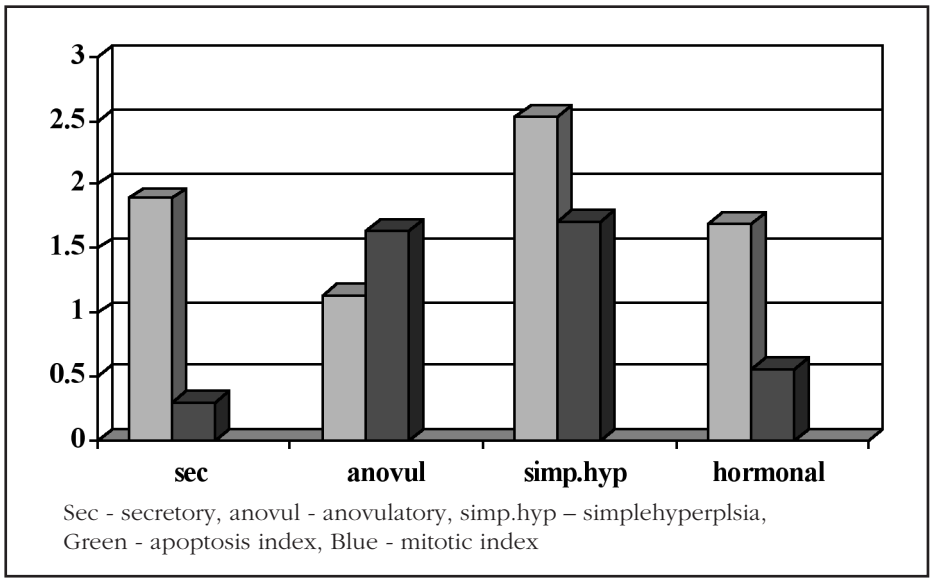
Grading of Bcl-2 expression with intensity of staining

1	Absent	-
2	Weak	1+
3	Moderate	2+
4	Strong	3+
5	Very strong	4+

Results

In our study of 50 cases of endometrial currettings (from patients who presented with irregular menstrual bleeding), the apoptotic and mitotic indexing was done. Of these 13 were categorized as Anovulatory, 14 as simple hyperplasia, 5 as early secretory endometrium, 4 as mid secretory and 4 as late secretory endometrium and 7 as endometrium showing features of hormonal imbalance (due to both exogenous and endogenous hormones). Three cases were not included, due to sub-optimal processing.

Apoptotic index (A.I) and Mitotic Index (M.I):
Apoptotic index was calculated by counting the number of apoptotic cells/ bodies per 1000 cells counted in 10 randomly selected high power fields. Similarly the Mitotic index was calculated by counting the number of mitotic figures per 1000 cells counted in 10 randomly selected high power fields. The results are tabulated and mean A.I and M.I, standard deviation were calculated for each sub-group and depicted in the histogram (Fig 1).



Sec - secretory, anovul - anovulatory, simp.hyp – simplehyperplsia,
Green - apoptosis index, Blue - mitotic index

Fig 1: Irregular Bleeding Uterus- Apoptosis and Mitosis

Discussion

Apoptosis in Dysfunctional Uterine Bleeding

Variation in the frequency of apoptosis in the hormone sensitive tissues like the endometrium has been well studied and established. Following these studies deranged apoptosis is being studied in other endometrial pathological conditions such as Dysfunctional Uterine Bleeding, Endometrial carcinoma and endometriosis. DUB is caused by anovulatory cycles or irregular cycles with an inadequate luteal phase and reflects prolonged excessive estrogenic stimulation of the endometrium by estrogens coupled with an absolute or relative lack of an opposing progestational phase[4].

In anovulatory cycles there is no formation of a corpus luteum, and consequently no progesterone production. The endometrium fails to undergo glandular and stromal differentiation which usually occurs in the secretory phase. The estrogen-producing follicle may present or regress resulting in declining estrogen concentrations. In irregular cycles with an inadequate luteal phase there is poor progesterone output by the corpus luteum. Although some studies have shown snapshot measurements of estrogen and progesterone levels within the normal ranges in some patients with DUB, hormonal output in many cases of DUB is irregular with falling levels. Decreasing estrogen concentration fail to maintain the endometrium and this leads to irregular shedding of the endometrium. Further more changing patterns of expression of the endometrial estrogen and progesterone receptors have been observed[2].

In such a hormonally changeable environment the potential for increased apoptosis associated with falling estrogen levels is evident perhaps as a precursor to endometrial shedding. However heterogeneity of hormonal patterns amongst DUB cases is likely to be reflected in heterogeneity in the frequency of endometrial apoptosis,[5] much work has been conducted on hormonal changes in endometria from cases with DUB. They found that apoptosis appears to be increased in histologically normal proliferative phase endometria of DUB, being approximately twice as common as cycle matched controls. The significance of increased apoptosis in biopsies from patients with abnormal uterine bleeding is unknown. Various abnormalities of local clotting mechanisms within the endometrium have been demonstrated and these are thought to account in some cases for the disturbance in uterine bleeding. It seems possible that any changes in the control of glandular proliferation could be a secondary effect of abnormal cytokine production or of endometrial repair and regeneration

following menstruation. However, increased apoptotic cell death within glands could directly contribute to an abnormal endometrial milieu [5]. The product of the Bcl-2 gene is known to exert an anti-apoptotic effect in a variety of tissues including endometrium. It has been suggested that persistent Bcl-2 expression by glands in the zona basalis may provide a protective effect against menstrual phase apoptosis [6]. Attention has also been drawn to the presence and possible function of T lymphoid aggregates that are characteristic of normal zona basalis. The release of lymphoid cytokines such as TNFα and γ-interferon which has been immuno-localised within the endometrium[7] influence glandular apoptosis and account for local variation in endometrial development.

Apoptosis in endometrial hyperplasia and endometrial carcinoma

Endometrial hyperplasia and endometrial carcinomas are often viewed as points on a continuum, which includes disordered proliferation, simple and complex hyperplasia, atypical hyperplasia and adenocarcinoma. Endometrial hyperplasia is also associated with abnormally high and prolonged estrogenic stimulation of the endometrial epithelium, unopposed by progesterone; this is considered to generate the above-mentioned spectrum. Thus there is an important etiological and functional overlap between the neoplastic sequence and DUB, although the duration and pattern of unopposed estrogenic stimulation differs. Apoptosis is observed at increasing levels in the hyperplasia, atypia, adenocarcinoma sequence. In contrast, interestingly mitotic indices were seen to be decreased in hyperplasia as compared to proliferative endometrium and endometrial adenocarcinoma[3]. The mitotic rates being lower; there is no regular shedding of the tissue; the net result is glandular crowding with increased possibility of subsequent malignant transformation. One other explanation for the fact the mitotic indices are lower (than apoptotic) could be that there is significant difference in the persistence of apoptotic bodies compared to mitotic figures that only last for seconds to minutes [8].

In this study an analysis of fifty samples of endometria from women who had presented with complaints of irregular menstrual bleeding and diagnosed as Dysfunctional Uterine Bleeding was done, which included morphological classification, apoptotic and mitotic indexing. The results revealed that the mitotic index was slightly higher than apoptotic index in anovulatory endometrium it was reversed in cases of simple hyperplasia as also observed by authors[3] as early as 1998. The apoptotic indices in the secretory phases early, mid and late were significantly higher than mitotic indices, correlating with existing literature. Cases showing effects due to hormonal, both exogenous and endogenous, imbalance also showed increased apoptotic activity over mitotic activity. In the endometria showing morphological changes due to hormonal imbalance, apoptotic index was more than mitotic index.

Conclusion

Morphological identification and quantification of glandular apoptosis on routine hematoxylin and eosin stained sections with acceptable accuracy is possible, it could be adapted as a morphological parameter in assessing endometria from cases of irregular uterine bleeding. This study showed increased apoptotic index correlating with high Bcl-2 expression. These apoptotic and mitotic indices in endometria of irregular uterine bleeding reflect the actual cell burden that may play a causative role in bringing about the hyperplastic states of the endometrium. The high Bcl-2 expression in the endometrial glandular epithelium reflects the prolonged cell survival resisting cell deletion. This is expressed morphologically as a hyperplastic state of the endometrium and is associated with irregular uterine bleeding. A prolonged hyperplastic state in coordination with other physiological, hormonal and genetic factors may hypothetically enhance the probabilities of the endometrium going into a state of dysplasia or malignancy. Thus apoptotic and mitotic indices of dysfunctional uterine bleeding endometria may be of value in predicting the endometrial malignancy.

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