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ORIGINAL ARTICLE

Nuclear Morphometry of Perimenopausal Endometrium in Nepali Population

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Abstract: Cytoarchitecture of the endometrium is an important tool for the diagnosis of precancerous state to carcinoma. In which, one of the most significant feature is nuclear morphology and morphometry. Present study was aimed to report the morphology and morphometry of perimenopausal endometrium because this age group is vulnerable to precancerous state to carcinoma. 204 cases were studied. Our study result showed, mean nuclear diameter was 5 µm, proliferative phase 4.75 µm and secretary phase 5 µm. There was much variation observed in the secretary phase endometrium: early-6 µm and both mid and late stage observed 5 µm. Morphology of endometrium reflected the precancerous state in sixteen cases.

Keywords: Endometrium, Perimenopausal age, Nucleus, Morphometry

Introduction

The perimenopausal transitional period may be defined as the time of life from regular ovulatory periods to the prior of cessation of menses. The onset varies from 39 to 51 year of age in 95% women and average duration is 05 years [1]. Menstrual irregularities are more common in perimenopausal transitional period of life [2]. Therefore, periodic cytoarchitecture assessment of endometrium is necessary to over rule pre-cancerous state, specially when patient complaints regarding irregular menstruation. Hence nuclear morphology and morphometry gained importance over the decades to diagnose various statuses of endometrial glands. Baak, et al, (1977) [3], mentioned that morphometry is important to quantify pathological alteration of the tissue microscopically. However, according to Maksem et al (2007) [4], cytoarchitecture is an effective tool for the assessment of benign, premalignant state to malignant endometrium. For the differential diagnosis of perimenopausal endometrium, frequently imposed happens specially between proliferative phase and simple hyperplasia [5-6].

There are various studies on endometrial morphology in the available literatures. [4, 7-14]. Though on nuclear morphometry and morphology of perimenopausal endometrial gland is scanty and rare. The present study was aimed to report nuclear morphometry and morphology of endometrial glands in reference to perimenopausal age group of Nepali population. The study was a retrospective in nature and no where research work was needed direct patient involvement; therefore it was not needed to obtain ethical committee permission of the Institute.

Material and Methods

Present study was conducted at Manipal College of Medical Sciences and Manipal Teaching Hospital, Pokhara, Nepal in the department of Anatomy with the assistance of Pathology department. Patients of perimenopausal age group were admitted to the hospital with the history of irregular bleeding and undergone diagnostic dilatation and curettage. The tissue was sent to pathology department for diagnostic purpose. Patient's history was obtained from hospital record. The cytology slides were procured from the department of Pathology. Two hundred four (204) patients tissue was collected form the pathology laboratory for the study at the department of anatomy. The nuclear morphology and morphometry were studied under light microscope with the help of ocular micrometer scale.

Morphology of all tissues was studied by the researcher and one pathologist. The study result of each tissue used to be submitted separately by each investigator to third investigator for correlation. In case of any controversy third investigator cross checked the tissue and conclusion was made by the third investigator. Each slide was studied in five fields, one at the centre, others were right upper and lower; left upper and lower corners to have unanimous status of nuclear character of the gland. On each field ten nuclear morphology and morphometry were studied. Individual nuclear diameter and morphology was recorded. The measurements of nuclei are studied by ocular micrometer scale; individual nuclear length and breadth were measured to calculate the nuclear diameter. The method of Palkovits and Fischer (1968) [15] was employed to determine the nuclear diameter.

D = L + B/2
L = Greatest diameter
B = Diameter of right angle to 'L'

Results

The mean nuclear diameter of the gland was 5µm. Whereas proliferative phase ranges from 4-6 µm and mean was 4.75 µm. The secretory glands showed maximum variation in their size, which ranges from 3-7 µm, mean was 5 µm. In which mean diameter of early secretory was 6 µm; mid secretory was 5 µm and latesecretory was 5µm.

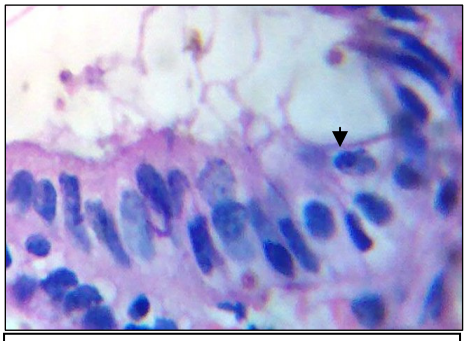

Fig-1: Pseudostratified appearance, Pleomorphic nuclei, Irregular nuclear membrane (arrow head); (Proliferative phase, 100X)

The morphology of endometrial gland cell nuclei of the age group were observed as follows: The proliferative phase glands' nuclei were predominantly basal in position, vesicular with nuclear pseudostratification in all cases (Fig.1). There were pleomorphic nuclei (Fig.1) in 92 cases, whereas only 04 cases exhibited monophesic, ovoid nuclei.

Regarding the nuclear membrane concerned, 72 cases were regular and 24 cases were irregular (Fig.1). There were hyperplasia of the glands observed in 16 cases with 5µm nuclear diameter. All nuclei were vesicular and predominantly basal in position with pseudostratification. There was presence of ovoid nuclei in 04 cases with irregular nuclear membrane. Presence of pleomorphic shape of nuclei were observed in 12 cases, of which, 08 cases nuclear membrane was irregular and regular in 04 cases.

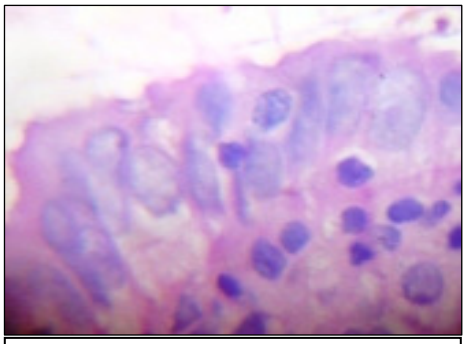

Fig-2: Pseudostratified appearance and Pleomorphic nuclei (Secretory phase, 100X)

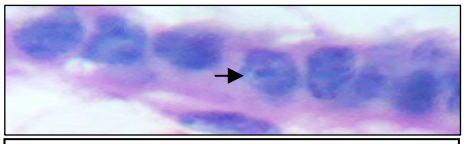

Fig-3: Simple epithelium, central nucleus.Arrow head: Irregular nuclear membrane
(Sect. phase, 100X)

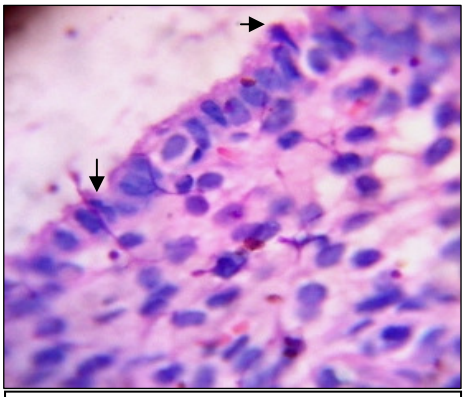

Fig-4:: ↓ Hyperchromatic nuclei;Arrow :Irregular nuclear membrane (Secretory Phase, 100X)

The secretory phase was divided into three subgroups: early phase showed maximum variation in their position (Table-II), all nuclei were vesicular and pleomorphic in shape. Pseudostratification was present in all cases.There were irregular nuclear membrane in 08 cases, whereas 04 cases exhibited regular nuclear membrane. Midsecretory phase gland nuclei in 44 cases were predominantly basal, 8 cases predominantly central and 4 cases central in position. There was presence of vesicular pleomorphic nuclei in all cases but pseudostratification (Fig.2) was observed in 52 cases. Whereas in 04 cases, it was simple and central in position (Fig.3). In 32 cases was exhibited regular nuclear membrane. However, there was presence of irregular nuclear membrane (Fig.4) in 24 cases.

Late secretory endometrial gland nuclei showed in all cases (Table-II) predominantly basal in position with pseudostratification. There was presence of hyperchromatic nuclei in 4 cases and in 24 cases vesicular. All the hyperchromatic nuclei were elongated (Fig.4), where as other 24 vesicular nuclei were exhibited pleomorphic shape. In 20 cases, there was presence of regular and irregular nuclear membrane in 8 cases.

Among the all cases of secretary glands only 4 cases exhibited atrophic glandular tissue whose nuclear diameter was 3 µm, central in position, vesicular and without pseudostratification. They were uniform in nature.

Table – I: Nuclear morphometry (n=204)			
Various stages of menstruation		Range in µm	Mean in µm
Proliferative		4-6	5
Secretory	Early	5-7	6
	Mid	3-7	5
	Late	4-6	5
Mixed stage (Proliferative +Secretory)		5-6	6

There were 12 cases exhibited both stages of glandular tissue and considered as mixed phase: of which, 08 cases were nuclei were predominantly central and in 4 cases basal position. There was presence of nuclear pseudostratifictaion in all cases. All nuclei were vesicular and pleomorphic in shape. 08 cases were exhibited regular nuclear membrane and in 4 cases were irregular nuclear membrane.04 cases showed hyperplasia of the glandular tissue and nuclear diameter was 6 µm. Nuclei were predominantly central in position with pseudostratification. All nuclei were vesicular, pleomorphic shape and regular nuclear membrane.

Table-II: Nuclear morphology (n=204)											
Endometrial gland		NUCLEUS									
Proliferative		Predominant Position			Type		Shape		Membrane		Apperance
		Basal	Central	Luminal	Vesicular	Hyperch-romatic	Pleo morphic	Mono phesic	Reg ular	Irre gular	
		96	00	00	96	00	92	04**	72	24	Pseudo srtatified
Secre-tory	Early	04	04	04	12	00	12	00	04	08	Pseudo stratified
	Mid	44	04* 08	00	56	00	56	00	32	24	04 (SC)† 52 (PS)††
	Late	28	00	00	24	04	24	04***	20	08	Pseudo srtatified
Mixed (Prolif. + sect.)		04	08	00	12	00	12	00	08	04	Pseudo Stratified
* All were central position; ** All were ovoid shape; *** All were elongated shape † SC – Simple cuboidal; †† PS - Pseudostratified											

Discussion

Endometrium undergoes structural changes in different phases of menstrual cycle and also menarche to menopause. Rock and Thompson (1997) [16] described, endometrium as an endocrine organ. The growth and maturation of the organ is under control of circulating estrogen and progesterone [17]. As age progress, normal menstrual cycle often turns to irregular due to various factors that may include normal physiology to malignancy. The malignancy of endometrium is the fifth common neoplasia but most common type of female genital tract [18-19]. The incidence increases as age progress, specially post menopausal group is more vulnerable [20].

Traditionally, morphological changes are being given importance to assess the status of endimetrium [7]. But in recent years, morphometric technique gained impotance

in the process of assessment of endometrium [3, 10]. Papaefthimious et al, (2005) [14] reported, nuclear feature is one of the most important indicator to diagnose physiological to pathological changes of endometrium [8]. The morphometry and morphology features of the various stages of the glands (Table I & II) are as follows:

Proliferative phase: The proliferative glandular epithelium shows nuclear psudostratification due to mild nuclear crowding and overlapping. Nuclei are mostly basal in position whereas actively nuclei are apical. The shape of nuclei was elongated, oval to cigar shaped and with smooth contour [4,21]. Incase of cytological atypia, there is increase in nuclear size, they become round and pleomorphic in nature, which are the features of malignancy [21]. Present study result showed the mean diameter of nucleus was 5µm, and ranges from 4-6 µm. As far as best of our knowledge, no such value is available in literature regarding any specific age group of Nepali population. The nuclei were predominantly basal in position, vesicular with nuclear pseudostratification in all cases. There were pleomorphic shape nuclei in majority of cases (Table-II) whereas only 4 cases exhibited uniform ovoid nuclei. In majority of cases nuclear membrane was regular. However, in 24 cases, irregular membrane was observed. The irregular nuclear membrane indicates the pathological change of the gland from precancerous to malignant stage. Present case study differ from the usual description is being described in the book [21].

Hyperplasia of the gland was observed in 16 cases with 5µm nuclear diameter. All nuclei were vesicular and predominantly basal in position with nuclear pseudostratification.4 cases, exhibited presence of ovoid nuclei with irregular nuclear membrane. Presence of pleomorphic shape nuclei observed in 12 cases, of which, in 8 cases, there were irregular nuclear membrane. However, regular membrane was observed in 4 cases. Our study result regarding the morphology of the gland exhibited significant changes in comparison with the earlier researchers [4, 21].

Secretory phase: The secretory glands are broadly divided into early, mid and late phases [21]. Maksem et al, (2007) [4] reported that gland epithelial cells exhibit "cytoplasmic dominance" in place of nuclear dominance. The earlysecretory gland epithelia are characterized by elongated or ovoid nuclei with nuclear pseudostratification due to nuclear crowding and overlapping [4, 21]. The midsecretory nuclei are of oval to round, vesicular and basal in position. Moreover nuclei are uniform in nature [4, 21]. The latesecretory nuclei are characterized by hyperchromasia and exhibit pleomorphism.[21] Maksem et al, (2007) [4] reported, as the glands are exhausted and stop their secretory activities, therefore, nuclei are approximated with each other without variation in their size. The most important was absence of nuclear pseudostratification. The secretary glands nuclei showed maximum variation in their size, which ranges from 3-7 µm. The maximum size was observed in the mid secretory phase may be due to active phase of the gland and declining of the size was observed in late secretory phase (Table-1).

The morphology of secretory glands nuclei were as follows: early phase showed maximum variation in their position (Table-II), all nuclei were vesicular with pleomorphism in shape. Pseudostratification was present in all cases with irregular

nuclear membrane in majority of cases. However, regular nuclear membrane observed in 4 cases. Midsecretory phase gland nuclei, in majority cases (Table-II) were predominantly basal. Whereas in 8 cases, were predominantly central and 4 cases central in position. The significant observation was presence of vesicular, pleomorphic shape nuclei in all cases. Nuclear pseudostratification was exhibited in majority of cases. However, simple central position nuclei were observed in 4 cases. Nuclear membrane was regular in majority of cases though 24 cases, showed irregular nuclear membrane. Latesecretory stage nuclei showed in all cases (Table-II) predominantly basal in position with pseudostratification. There was presence of hyperchromatic nuclei only in 4 cases but majority of cases nucleibwere vesicular in nature. All the hyperchromatic nuclei were elongated. However, in 24 cases, there was presence of vesicular and pleomorphic shape nuclei. Nuclear membrane concern, only 8 cases showed irregular nuclear membrane. Among the all cases of secretory glands, only 4 cases exhibited atrophic glandular tissue and nuclear diameter was 3 µm, central in position, vesicular and without pseudostratification. They were uniform in nature. Our study result is quite significant in comparison of earlier workers [4, 21].

In 12 cases, glandular tissue were in proliferative phase as well as in secretory phase, therefore considered as mixed phase. In 8 cases nuclei were predominantly central and 4 cases predominantly basal in position. Nuclear pseudostratifictaion was observed in all cases. All nuclei were vesicular and pleomorphic in shape. 8 cases were exhibited regular and 4 cases irregular nuclear membrane. 4 cases showed hyperplasia of the glandular tissue and nuclear diameter was 6 µm. Nuclei were predominantly central in position with pseudostratification. All nuclei were vesicular, pleomorphic in shape and regular nuclear membrane.

Baseline morphometry study may be useful to evaluate early carcinoma in-situ where increased nuclear size, irregular nuclear membrane, pleomorphic nuclei are well known features of neoplasia. Our study result suggests that mild increase in sizes of nuclei with variation in shape could form a clue to detect early neoplasia.

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