Material & Method
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Present study was carried out in the department of Obstetrics and Gynaecology in Maharani Laxmibai Medical College and Hospital, Jhansi in the period of twelve months.

Selection of Cases

Study group - Further divided into

(A) Reproductive age group with complaints.

(B) Menopausal age group with complaints.

Patient being the following complains were studied

(i) Excessive discharge per vagina

(ii) Pain in lower abdomen or back.

(iii) Irritation or itching vulva.

(iv) Suspicious naked eye appearance of cervix.

(v) History of Known diethyl stil boesterol exposure.

(vi) Patients in whom a suspicious or positive smear is obtained.

(vii) Menstrual abnormalities.

- Menorrhagia - Post menopausal bleeding

- Polymenorrhoea - Post coital bleeding

- Metrorrhagia - Intermenstural bleeding

- Continuos bleeding -
3. Control group - follow up cases of normal delivery abortions and sterilization.

**Clinical examination.**

(1) History

Detailed history of presenting complaints was taken along with duration of complaints, obstetrical history under the headings of gravida, parity, year, month, date of last delivery and number of abortion noted. The menstrual history was taken under the headings cycle which include the number of days the flow lasted and the duration of menstrual cycle the amount of flow and the date of the last menstrual period. enquiry about family history of diabetes and cancer cervix was also made along with history of any venereal disease, pelvic inflammatory disease or drug in take in the past.

(ii) *General examination*

Thorough general examination was done with special alteration as regards to general condition, pulse, blood pressure, anemia, jaundice and weight of the patient.

(iii) *Per vaginum examination*

Per vaginal examination was done to know the size and consistency of the uterus, the condition of the tubes and ovaries and any evidence of pelvic inflammation, any type of bleeding was also noted.

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(iv) *Per speculum examination.*

Per speculum examination was done to inspect the condition of cervix and vagina, types of discharge and to see whether the bleeding was from the os, or from other areas in the vagina or polyp or growth or erosions and discharge.

**VAGINAL SMEAR**

*Guidelines.*

For preparing smears for gynecological cytology ayre's spatula is used and the material is collected from the following sites.

(A) lateral vaginal wall  
(B) Posterior fornix  
(C) Ectocervix  
(d) Endocervix

For diagnosis of premalignant and malignant for lesions of the cervix ecto and endocervical scrapes obtained with ayre's spatula are the best, posterior vaginal material usually contain desquamated cells which may be degenerated. therefore for only detection of cervical cancer it is imperative to scrape ecto and endocervix with ayre's spatula.

Ayres spatula is made of wood having two ends was used for taking vaginal and cervical smears, with flat end vaginal
smear was taken from the posterior fornix and with the hooked end cervical scraping was taken, cervical punch biopsy forcep was used for taking cervical biopsy.

A uterine cannula made of steel and 20a glass syringe were used for aspiration from the endometrial cavity and on endometrial curette having serrations at proximal end a wire inserted through the distal end was used for taking the biopsy from the endometrial cavity.

**Preparation of the patient**

A written consent of the patient was taken, she was asked to evacuate her bladder. Cases who were unable to evacuate the bladder was catherized, no sedition was given to the patient before doing vaginal, cervical and endometrial aspiration cytology, 10 mg diazepam was given doing endometrial & cervical biopsy.

Patients were made to lie down in lithotomy position.

**Technique of cytology.**

*Vaginal and cervical cytology.*

Patient was made to the lie in lithotomy position. posterior vaginal wall speculum or sims speculum was applied and vaginal smear was taken from the posterior fornix with the flat end of the ayre's spatula and it was spreaded on the slide and

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immediately fixed in cytology solution. The cervix was then visualized with the help of the anterior vaginal wall retractor and cervical smear was taken by scraping the squamo-columnar junction of the cervix with Ayres spatula under direct vision by rotating it through 360°. The material was spreaded on a glass slide which was immediately fixed in a glass bottle containing equal amount of ether and 80% alcohol.

**Endometrial Aspiration**

The vulva was painted with antiseptic situation and vagina was cleaned by antiseptic lotion the part was draped by a cutsheet, vaginal examination was done to confirm the previous findings. Posterior vaginal wall was separated by Sims speculum. The anterior vaginal wall retracted by anterior vaginal wall retractor, volsellum was used to hold the anterior lip of cervix. A uterine sound was passed to know the length of uterus and the patency of cervical os was determined. Endometrial aspiration cannula was passed into the uterine cavity negative pressure was created by a twenty ml syringe and negative pressure was maintained till the tip of the aspiration cannula had reach just beyond the level of the internal os and endometrial aspiration was taken.

A drop of the aspirated material was placed on a glass slide and a smear was made. These slides were immediately
fixed in glass bottle containing of equal amounts of ether with 80\% alcohol.

**TECHNIQUE OF BIOPSY**

**Endometrial biopsy**

An endometrial biopsy curette was introduced and curettage of uterine cavity was done. The curettings obtained were placed in a bottle containing formalin.

**Cervical Biopsy**

A cervical punch biopsy was taken and a portion of the diseased cervix was taken and the piece was placed in a glass bottle containing formalin.

The tissue was then processed and sections were cut of five micron thickness. These section were placed on slides which were stained by haematoxylin and eosin staining. Then the slides were viewed under microscope.

**Interpretation of Cytology Slides:**

A. Normal: (Pap I) - Cells are with normal healthy appearance

B. Inflammatory:(pap II)- (Atypical squamous cells of undetermined significance)

Inflammatory smear shows increase in number of inflammatory cells predominantly polymorphs type in
acute infections and lymphocytes and occasionally plasma cells in chronic infection.

Increase in parabasal cells in young women and intermediate and superficial cells in older women.

Specific infestation of protozoan trichomonalis or fungus monilia or viral papilloma virus or herpes.

C. Mild Dysplasia (papIII) - (CIN I) (Low SIL) (Low equomous intra epithelial lesions)

Cells are generally of the intermediate or superficial type There is slight nuclear enlargement with mild hyperchromasia with the capacity for normal squamous differentiation.

D. Moderate Dysplasia - (CIN II) (H-SIL) (High grade squamous intraepithelial lesions)

Cells are of parabasal type showing an increased nuclear cytoplasmic ratio. The nucleus is irregular with coarse chromatin pattern & scanty or only a ring cytoplasm is seen

E. Severe Dysplasia - (CIN III) (H-SIL) (High grade squamous intra epithelial lesion)

Cells are of parabasal type showing an increased nuclear cytoplasmic ratio. The nucleus is irregular with coarse chromatin scanty or only a rim of cytoplasm is seen.
F. Cardinomo-in-situ (H-SIL) (high grade squamous intraepithelial lesion)

Parabasal cells have very large nuclei with irregularly clumped chromatin, Nuclear and cytoplasm ratio is markedly increased.

G. **Invasive Carcinoma (Pap V)**

A large nucleus with irregular nuclear membrane and irregularly clumped chromatin is present in keratinized squamous cell of different shape and size. There may also be malignant naked nuclei showing marked pleomorphism.

**Endometrial Aspiration**

The slides reveal outstanding cellular characteristics of various types of endometrial cells. Two main types of cells are recognized epithelial and stromal cells. Endometrial epithelial cells are present in aggregates or clumps. These cells are small and columnar to cuboidal in shape, with finely vacuolated cytoplasm and eccentrically placed nucleus having fine uniform chromatin.

Endometrial stromal cells can be derived from spongiosa or compact layer of functional endometrium. They may be shed in groups or singly. These cells are small, round or irregular and showing variation in size. Nucleus is eccentric, round and has prominent granular chromatin pattern.
The cellular diagnosis of proliferative endometrium is made by presence of an active chromatin nuclear pattern and absence of cytoplasm vacuolization is endometrial epithelial cells.

Diagnosis of secretory endometrium by cell study depends on comparatively less activity of nucleus and some evidence of vacuolization of cytoplasm in endometrial epithelial cells.

Diagnosis of cystic and stromal hyperplasia is based on presence of large numbers of hyperchromatic endometrial stromal cells usually clumped or in extensive sheets. Cellular smears of adenomatous hyperplasia tend to have typical clusters or groups of endometrial epithelial cells and may be difficult to differentiate from cells noted, in cases of endometrial polyps.

Malignant cells from endometrium (Adenocarcinoma) may desquamate in groups or less frequently as isolated cells. The malignant endometrial cell is larger and round oval or columnar. The nucleus is round, oval showing pleomorphism or deformed and pushed aside by cytoplasmic vacuolation. The chromatin is irregularly distributed, hyperchromasia is usually moderate. Nuclear hypertrophy is a fairly content characteristic. The cytoplasmic borders are usually indistinct when intact this cytoplasm is pale and eosinophilic and exhibited one or several large vacuoles or it may be foamy due to numerous microvacuoles.
Interpretation of Histology Slides:

Endometrial biopsy

(a) Proliferative

The stroma is dense and compact. The glands are tall rounded and lining is of tall columnar epithelial cells. There is no secretion in glands. The blood vessels are cork-screw shaped.

(b) Early Secretory Phase

A subnuclear vacuole appears and this gradually moves towards periphery of cell and is then extended into the lumen of the gland. The stroma is loose, the blood vessels are dilated.

(c) Late secretory phase:

The glands are filled with secretion. The luminal borders are frayed. The stroma is loose and shows decidual reaction. There is infiltration of leucocytes.

(d) Endometrial hyperplasias:

Silverberg classified the hyperplasias into four categories which is morder of increasing severity are called simple, cystic, adenomatous and atypical.

(i) Simple hyperplasia

There is increased thickness of endometrium, increased crowding of glands and evidence of estrogenic activity. The glands of simple hyperplasia are generally small round and regular and fail to show cellular atypia. The glands are
generally crowded and the morphology of the proliferative phase is universally encountered.

**(ii) Cystic Hyperplasia**

Co-existence of large cystically dilated glands and small round glands in a voluminous endometrial of gland. Glands are round in shape, neither any irregularity nor cellular a typical is encountered. There is stratified epithelium with proliferative activity seen in cystic hyperplasia.

**(iii) Adenomatous hyperplasia**:

Above findings plus irregularity and abnormal shape of the hyperplastic endometrial glands. A typical finding is the presence of small buds projecting from larger often cystically dilated glands. Amount of endometrium obtained at curettage is more voluminous in adenomatous hyperplasia than in the less severe forms.

**(iv) Endometrial Carcinoma**

Endometrial adenocarcinoma are usually glandular in pattern with a smaller percent being papillary. Increasing anaplasia of the tumor is manifested by a tendency to grow in solid nests or sheets with less formation of glands or papillae.

Grade I adenocarcinoma are composed entirely of tumor growing in glandular or papillary pattern. Grade II tumors here an intermediate pattern of growth there is moderate differentiation of tumor cells manifesting predominantly glandular elements but with a mixture of solid growth pattern
Grade III tumors comprise of poorly differentiated lesions growing predominantly in solid sheets of cells.

Carcinoma in situ is used to denote a small focus of tumor in an otherwise benign curetting. There is tufting & infolding into glands lumen. The cells in each instance are tall and pale and even with hyperplasia. There may be some evidence of secretory activity.

CERVICAL BIOPSY

On cervical biopsy following findings are present.

(a) Chronic Cervicitis

Characterized by an extensive subepithelial inflammatory infiltrate of plasma cells with scattered polymorphs nuclear leucocytes and large mononuclear cells. The epithelium may be quite normal, flattened to appear cubical or squamoid or desquamated.

(ii) Mild Dysplasia -

The neoplastic cells extend one quarter to one third of the way from the basal layer to the surface.

(iii) Moderate Dysplasia :

The cellular aberrations extend through one half of two third of the thickness of the epithelial layer. The atypia includes the small cell non keratinizing, the large cell non kerathizing

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and the large cell keratinize varieties. The loss of polarity and the general disarray of the growth patterns are prominent.

(iv) **Severe Dysplasia**

The anaplastic cells penetrate through 75 - 90% of the epithelium loss of polarity & disarray of cells in the deeper zone may be associated with early stromal invasion or microinvasion even through complete loss of stratification is not present.

(v) **Carcinoma-in-situ**

Lesion in which the entire thickness of the squamous epithelial layers is replaced by cells microscopically indistinguishable from those of frank cervical cancer with complete loss of stratification but with no evidence of stromal invasion.

(vi) **Microinvasive Carcinoma**

A lesion is properly considered invasive, if there is breakthrough of so called basal lamina with cancer cells spreading into the stromal tissue, there is a tendency towards an abundant cosinophilic cytoplasm.