Material & Methods
MATERIAL AND METHOD

The present study was carried out in the department of Obstetrics & gynaecology, M.L.B. Medical College, Jhansi (U.P.) The patients were selected from gynaecology OPD and ward.

Total 50 postmenopausal females with amenorrhoea of more than 6 months duration, were selected for the study.

1. SOURCE:

Patients are selected from - OPD
- Gynaecology ward

Natural menopause - Age above 40 years with more than 6 months amenorrhoea

Artificial menopause - Hysterectomy with Bilateral oopherectomy.

2. CRITERIA:

(a) Presenting Complaints

Hot flushes, night sweats, insomnia, dry vagina, dyspareunia, palpitations, burning micturition, vaginal discharge, frequency and urgency of urine, bone pains, spontaneous fracture.

(b) History of the patient

# Name, age, parity, caste, socio-economic status, place of residence, occupation.

[50]
# Age of menopause
# Duration of menopause
# Type of menopause whether natural or artificial
# Menstrual history.
# Obstetrical history.
# Personal History. - H/o smoking, Alcohol, H/o past surgery.
# Past history - Tuberculosis, Hypertension, Ischemic heart disease diabetes mellitus, malignancy.
# Drug History.

(c) Clinical Examination

General Examination - General condition, weight, blood pressure, pulse rate, respiratory rate, body temperature, pallor, cyanosis, jaundice, oedema, lymphadenopathy

Chest Examination

Cardiovascular System -

Any evidence of coronary artery disease, hypertension and thromboembolic phenomenon.

Central Nervous System -

Irritability, loss of memory, any psychological symptom.

Per Abdomen Examination

Breast Examination

Any lump, discharge, fixity, tenderness
**Per Speculum and per Vaginum Examination**

Condition of vulva
Condition of vagina and any vaginal secretions
Any pathology of cervix.
Size and position of uterus and adnexa.

(C) Investigations

Hb%, TLC, DLC, ESR
Urine, Routine & Microscopic
Blood Sugar,
Blood Urea
Electrocardiogram

**Lipid Profile**

Serum total cholesterol (STC)
Serum Triglycerides (STG)
High Density Lipoproteins (HDL)
Low Density Lipoproteins (LDL)

**Method of Collection of Blood sample for Lipid profile Estimations**

5 ml of blood was with drawn from antecubital vein of the female in recumbent posture with all aseptic precautions

- After 12 - 14 hours fasting.
- Without venous stasis
After that withdrawn blood was allowed to settle down for half an hour, then centrifuged and serum was preserved with standard precaution.

**Period of Collection of Sample**

1. Basal Sample i.e. before starting the therapy.
2. After 3 months of Therapy
3. After 6 months of therapy.

**Estimation of Lipid Factors**

Various lipid factors, serum total cholesterol (STC), serum Triglycerides (STG) High density lipoprotein (HDL) were estimated by diagnostic kits, while low density lipoproteins (LDL) and very low density Lipoproteins were derived from the values of above mentioned lipids by standard formulae.

1. **Estimation of Serum Total Cholesterol**

   Serum total cholesterol (STC) was estimated by a commercial kit supplied by Ethnor. The basic principle is that cholesterol reacts with kit solution of Ferric perchlorate, Ethyl Acetate and sulphuric Acid and gives lavender colour complex which is measured calorimetrically.

2. **Estimation of serum Triglycerides (STG)**

   Serum Triglycerides were estimated by acetyl acetone method. The principle behind this is that triglycerides are determined by measured glycerol. After its liberation from fatty acids by saponification glycerol is oxidized by sodium
metaperiodate to formaldehyde which is directly proportional to the amount of triglycerides.

3. **Estimation of High Density Lipoproteins (HDL)**

HDL was estimated by utilizing commercial kit supplied by Ethnor. The basic principle is that HDL cholesterol fraction is separated by using a precipitating reagent. The precipitants contain chylomicrons, VLDL, LDL which are removed by centrifugation. The supernatants contain HDL-C which is estimated by HDL-C colour reagent which gives purple colour complex. Intensity of colour developed is proportional to the concentration of HDL cholesterol in the specimen under test.

4. **Estimation of Very low density lipoproteins (VLDL)**

VLDL was estimated by formula given by *Friedwald et al (1972)* this formula is valid upto STG values to less than 400 mg%.

\[
\text{VLDL (mg/dl)} = \frac{\text{STG}}{5}.
\]

5. **Estimation of low density lipoproteins (LDL)**

LDL was estimated by using *Fredrickson DA (1972)* formula

\[
\text{LDL (mg/dl)} = \text{STC} - \left(\frac{\text{STG}}{5} + \text{HDL}\right)
\]

\[
= \text{STC} - \left(\text{VLDL} + \text{HDL}\right)
\]

**ENDOMETRIAL BIOPSY**

*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 bladder, were catheterized by plain catheter. Sedation was given to the patient in the form of Inj. Pentazocine 30 mg. with Inj. Promethazine 20 mg. For taking endometrial biopsy, patient was made to lie down in lithotomy position.

**Technique** -

The vulva was painted with antiseptic solution followed by cleaning of vagina with antiseptic solution. The part was draped by a sterile cut sheet. The vaginal examination was done to know the size of the uterus, whether anteverted or retroverted position of the cervix. The posterior vaginal wall was retracted by sim’s speculum. The anterior vaginal wall was retracted by anterior vaginal wall retractor. The volsellum was used to hold the anterior lip of cervix. A uterine sound was passed to know the length of the uterine cavity and patency of cervical os determined. If dilatation of cervical os was required, it was done with gradually increasing size of Hegar’s dilator, so that endometrial curette can be introduced. An endometrial biopsy curette was introduced and curettage of endometrial cavity done. The curettings obtained were placed in a bottle containing formalin.

*Interpretation of Histology Slides (Anderson' pathology volume 2, Tenth edition)*

a) *Early Proliferative* - During early proliferative phase (days 5-7), endometrial glands are sparse, straight, narrow
(tubular). They are lined by columnar epithelium with few mitoses and little or none pseudostratification. The stroma is dense.

b) **Mid proliferative phase** - During mid proliferative phase (days 8-10), the glandular epithelium shows increasing pseudostratification and more mitoses as compared to early proliferative phase. The stroma is loosened by oedema.

c) **Late proliferative** - There is spiralling of glands and vessels. Glands are cystic, tortuous. The stroma is dense, diffuse stratification and mitoses are abundant.

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

e) **Late Secretory Phase** - The gands are filled with secretion. The luminal borders are frayed. The stroma is loose and shows decidual reaction. There is infiltration of leucocytes.

f) **Endometrial Hyperplasias**

Silverberg classified the hyperplasias into four categories which are in the increasing order of severity - 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 morphology of proliferative phase is universally encountered.

ii) **Cystic Hyperplasia**

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

iii) **Adenomatous Hyperplasia**

Above findings plus irregularity and abnormal shape of 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, then in the less severe forms.

iv) **Endometrial Carcinoma**

Endometrial adenocarcinoma are usually glandular in pattern with a smaller percentage being papillary. Increasing anaplasia of tumour 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 tumour growing in glandular or papillary. Pattern. Grade II tumors have an intermediate pattern of growth, there is moderate differentiation of tumour cells manifesting predominantly glandular elements but with a mixture of solid growth pattern. Grade III tumours comprise of poorly differentiated lesions growing predominantly in solid sheet of cells.

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

**Estrogenicity Scoring system - Step 1 : Score Individual biopsy specimens according to eight morphologic features**

<table>
<thead>
<tr>
<th>Morphologic feature</th>
<th>None (0)</th>
<th>Limited (1)</th>
<th>Higher (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glandular effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td>Small, tubular, straight</td>
<td>Open, straight</td>
<td>Open/cystic, tortuous</td>
</tr>
<tr>
<td>Nuclear/cytoplasmic ratio</td>
<td>Very high (&gt;75%)</td>
<td>Moderate (75%-50%)</td>
<td>Low (&lt;50%)</td>
</tr>
<tr>
<td>Pseudostratification</td>
<td>None</td>
<td>Limited</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Mitoses</td>
<td>None</td>
<td>Rare</td>
<td>Scattered to many</td>
</tr>
<tr>
<td>Stromal effects</td>
<td>Compact or fibrous</td>
<td>Loosely cellular</td>
<td>Edematous</td>
</tr>
<tr>
<td>Density</td>
<td>None</td>
<td>Rare</td>
<td>Few to many</td>
</tr>
<tr>
<td>Mitoses</td>
<td></td>
<td>Rare</td>
<td>Few to many</td>
</tr>
<tr>
<td>Other effects</td>
<td>None</td>
<td>Moderate- Much being intact</td>
<td>Abundant Intact</td>
</tr>
<tr>
<td>Metaplasias</td>
<td>Disrupted or scant intact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue volume</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Estrogenicity scoring system - Step 2: Total morphologic feature scores and assign estrogen effect grade

<table>
<thead>
<tr>
<th>Total score</th>
<th>Estrogen effect grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>0</td>
<td>Typical post menopausal endometrium with little or no estrogenic effect</td>
</tr>
<tr>
<td>4-6</td>
<td>1</td>
<td>Definite but limited estrogenic effect</td>
</tr>
<tr>
<td>7-10</td>
<td>2</td>
<td>Significant estrogenic effect</td>
</tr>
<tr>
<td>&gt;10</td>
<td>3</td>
<td>Marked estrogenic effect</td>
</tr>
</tbody>
</table>

**BONE MINERAL DENSITY (BMD)**

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with consequent increase in fragility and susceptibility to fracture.

Bone mass is measured by bone densitometry. In this study, it was measured by peripheral dual energy X-ray absorbimetry (P-D\(\text{DEXA}\)) and region involved was wrist of left hand. It was done before starting the therapy and after 6 months of treatment.

**Interpretation of BMD Results**

WHO has established general diagnostic categories of bone loss based on the degree of deviations from mean bone mass density.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Within 1SD below normal (T Score equal to or above -1)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>1-2.5 standard deviation below normal adults (T score between -1 and -2.5)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>&gt; 2.5 SD below normal, No history of fracture (T score at or below -2.5)</td>
</tr>
<tr>
<td>Severe Osteoporosis</td>
<td>&gt; 2.5SD below normal, History of non-violent fractures</td>
</tr>
</tbody>
</table>

'T Score' - is generally used to relate the results to normal values found in healthy young population, that is matched for race & gender.

'Z Score' - compared the individual results to those of an age matched population that is also matched for race & sex.

**Exclusion of Patients:**

Following patients were excluded from study such as -

- Patients with undiagnosed vaginal bleeding, genital neoplasm, carcinoma breast or history of carcinoma breast in family.

- Patients who had any major complications in postoperative period.

- Patients with cardiovascular disease, hypertension, Diabetes mellitus. History of jaundice and thromboembolic phenomenon were not included in this study.

**Selection of Patients:**

Selected from the patients attending the OPD of department of obstetrics & gynaecology, M.L.B. Medical College, Jhansi.
A separate record of such patients was kept in register. This procedure was adopted till the number of post menopausal patients attained the number fifty. Datewise serial no was given to the cases. These fifty patients were divided into two groups of twenty five each.

The selection of cases for the various drugs was made on the basis of systemic random sampling.

**Mode of Administration of Drugs:**

**Group 1** - Prescribed Tab, Premarin .625 mg/day.

**Group 2** - Prescribed Tab. Ralista 60mg/d OD Both the groups were given calcium tablets along with the other drug.

Clinical examination was done at 0,3 and 6 months lipid profile was done at 0, 3, 6 months whereas bone mineral density at 0 and 6 months. Endometrial biopsy was done in those with intact uterus at 0 and 6 months.

**Enquiry was made upon -**

a) Control of symptoms

b) Any complaints like hot flushes, vaginal bleeding, breast pain or leg cramps.

c) weight gain

d) Blood pressure

e) Breast Examination.
BMD reference graph: of post menopausal woman showing normal BMD

BMD reference graph: of post menopausal woman showing osteopenia
Post Menopausal Atrophy of Endometrium

Simple Endometrial Hyperplasia