CHAPTER 2

REVIEW OF LITERATURE
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2.1: HISTORICAL ASPECT OF CANCER CERVIX

Carcinoma of uterine cervix has been known since Vedic times in India & is described in Sushruta Sanhita.

It is also well known disease in India & Egypt years before both of Christ (1500 B.C.).

Due to availability of various facilities & Methods for its diagnosis (Jeffcoate’s 7th Ed 2010). It has gained great importance in last half of century.

In 1884 Domenico Antonio Rigoni & Stern first one to show association between sexual activity & carcinoma of uterine cervix risk.

1886-Sir John Williams described the different histological types of carcinoma cervix.

1898-Werthiens of Vienna applied the operation in women with carcinoma cervix.

1900-Cullen published his classic book on Cancer uterus having an excellent picture of what today is recognized as carcinoma in situ.

1903 Margaret Cleaves used radium for 1st time for treatment carcinoma cervix.

Doctor R. Abbe in USA was the 1st who cured a patient with cancer of cervix by radium.

1910-Rubin made historical description for 1st time.

1914-Latzco described radical Wertheim’s operation with resection of parametria & regional lymph nodes.

1925-Hinselmann invented colposcope.
1928-George Papanicolau observed the cells shed from uterine cervix & recognized carcinoma cells in vaginal smears (D.C Dutta, 2010). This method termed "Cytology" still forms the mainstay of modern diagnosis and management.

1930-Schiller described a test whereby cells devoid of glycogen i.e. atypical or abnormal cells fail to stain with Lugol's iodine.

1947-J. Earnest Ayres designed the wooden spatula obtains the cells for study.

1948-Brunschwig performed pelvic exenteration as palliative procedure for various pelvic malignancies and was associated with significant morbidity & mortality.

1950-Lombard & Potter stated that multiple variables which may be etiological importance in carcinoma of uterine cervix.

1954-Winder Retkin include age at first coitus as case of control variable to be followed by the identification of this specific and also multiple sexual partners.

1967-Hill & Adelstein proved infectious agents as an important etiological factor in cervical carcinoma causation.

1968-Rawls et al., found that herpes simplex virus type 2 as a candidate for cervical cancer causation.

1972-Ratkin stated that women at higher risk for cervical cancer from lower socioeconomic classes with concomitant early onset of sexually and many sexual partners.

1973-Ratkin concluded that noncircumcision was the direct cause of cervical carcinoma.

1981-Dr. Adolf Staf introduced "cervicography" which is photographic screening technique by using acetic acid.

2.2: ANATOMICAL ASPECT OF CERVIX

The cervix is a barrel shaped lower part of the uterus. Measuring 2.5 - 3.5 cm from above downwards. Half of it projects into the vagina (portio vaginalis) while half is above the vaginal attachment (supravaginal cervix). The cervix has a central orifice, external os which gives access to the cervical canal and the internal os communicates the lumen of uterine cervix with that of the body of the uterus. The spindle shaped canal between internal os and external os is the cervical canal (Dawn, 2010).

The uterine cervix is supplied by the uterine arteries, the branches of the anterior division on internal iliac artery on both sides anastomosing with each other and forming circular artery of the cervix in the supravaginal part of the cervix. Venous drainage corresponds to that of the artery. They drain into the internal iliac vein through the vaginal plexus of vein. Veins from the cervix has also communication with middle and inferior haemorrhoidal vein and ultimately to the superior haemorrhoidal vein of portal system. The cervical vein has another communication to the presacral and lumbor channels of the vertebral plexus of vein through pelvic plexus of vein.

The lymphatic vessels of the cervix passes in three directions- laterally in the parametrium to the external iliac nodes; posterolaterally to the internal iliac nodes and backward in the sacrogenital fold to the rectal and sacral nodes. Some efferent vessels may also be reach the obturator or gluteal nodes. The secondary group of lymphnode comprises of the common iliac nodes, inguinal group & aortic (paraortic) group of nodes.

Both sympathetic & parasympathetic nerve supply is derived through pelvic nerve plexus. Para sympathetic nerve, the motor nerve arises
from T5 & T6 spinal segment, while sensory nerve arises from T10 to L5 segment. Per parasympathetic nerve both motor & sensory nerves are derived from S2, S3, S4 spinal segment (Shakuntala Baliga, 2010).

Histological aspect:

The wall of the cervix, in its upper part, is composed mainly of involuntary muscle, many of the fibres being continuous with those in the corpus. The linear half has a thin peripheral layer of muscle but is otherwise entirely composed of fibrous & collagenous tissue. Mucous membrane lining the canal varies in different parts (Jeffcoate's, 7th Ed 2010). Endocervix is lined by columnar epithelium, and ectocervix along with vagina is lined by stratified squamous epithelium. In between them is the transitional zone. There is no sub mucous layer.

Stratified squamous epithelium of the cervix:

In sexually mature women, it is possible to recognise four distinct zones in the epithelium. Each zone is composed of cells in one of the four different stages of development.

(a) Basal layer (K1 or G): This layer is also known as germinative layer. It is composed of one row of small cuboidal or columnar cells measuring about 12mm in diameter. It has a relatively higher nuclear cytoplasmic ratio 1:1.5 (Dawn, 2010). The nuclei commonly display evidence of active cellular growth, such as prominent nucleoli. Numerous chromocentres & occasional mitosis. Under normal circumstances epithelial regeneration is confined to the basal layers, the remaining zones serve as stages of cell maturation. These cells are
firmly attached to basement membrane & normally do not exfoliate. Therefore, they are not found in normal vaginal smears.

(b) **Parabasal layer (K2 or C2)**: Superficial to the basal layer lies a layer composed of several layers of round, oval or polyhedral cells with comparatively large nucleous and cellular process as reaching from one cell to the other (intracellular bridges or desmosomes). The number of the layers & the size of the cells depend upon the stage of the menstrual cycle & the extent of hormonal stimuli. The cells towards superficial layer are longer than the cells nearer to basal layer. Parabasal cells may be detached and so may be seen in vaginal smears (*Shakuntala Baliga, 2010*). Parabasal cells found in smears may be sub-divided into 2 types:

- Those poor in glycogen (aglycogenic) corresponds to atrophic epithelium of menopause and of primary or low level of oestrogen.
- Those rich in glycogen (glycogenic) characteristics of hyperplastic epithelium & usually in pregnancy of after high oestrogenic stimulation.

(c) **Intermediate cell layer (K3 or C3)**: There cells are also known as navicular cells due to appearance and lie superficial to the parabasal layer (*Dutta, 2010*). The nucleus is relatively large and flattened and usually eccentrically placed. The layers or this zone depend upon the extent of epithelial growth. During the peak of follicular activity and in pregnancy the navicular cells are distinctly outlined. Navicular cells of pregnancy differ from those of the normal menstrual cycle in that they have a longer nucleus and heavier cellular membrane.
The basal, parabasal & navicular layers constitute layers constitute the stratum basalis. Glycogen is present in all the layers of the basalis with increase in quantity from the basal to navicular cells.

(d) Independent layer (K4 or C4): This layer is well defined as an independent layer when the outer layers of the epithelium undergoes complete keratinization (K4). It corresponds to the stratum granulosum of the epidermis. The cells contain Keratohyaline granules, but complete keratinization does not occur in cervix. It is usually associated with pathologic conditions such as uterine prolapse, keratosis or leukoplakia.

In normal cornified epithelium the stratum granulosum is absent, but its place is taken by a narrow dense zone (C4) consisting of flattered and densely packed eosoniphillic cells with small pyknotic cells. Sometimes this layer is considered as homologus to the intraepithelial zone found in rodent. In routine procedure the cells of the fourth zone cannot be distinguished from that of more superficial cells (Jeffcoate's, 7th Ed 2010).

(e) Superficial layer (K5 or C5): This layer consists of several layers of flattered cells which on cross-section appears elongated. Their nuclei are small and pyknotic. The thickness of this layer depends largely on the functional state of ovaries & the phase of the menstrual cycle.

In vaginal smears these cells are large, flat, sometimes folded & have an irregular polygonal form & a small pyknotic nuclei. Some of the cells are basophilic (precornified) & those undergoing cornification are acidophilic. The ratio of the superficial acidophilic cells to the basophilic cells depend upon the oestrogenic hormonal level (Shakuntala Baliga, 2010).
Normal:
- Normal cells multiply all the time.
- Old cells die and fall off
- New, completely formed, mature cells replace them
- They take over the regular function.

Cancer.....normal gone berserk:
- Normal mechanism can be disturbed by many factors (ex: virus, chemicals in Tobacco, repeated damage).
- Cancer cells are abnormal cells which multiply too fast
- Old cells refuse to die
- New cells don't mature but keep growing – become a bulk called “tumour”
- Cannot function normally & start competing with the normal cells.
- Can spread and grow anywhere else in the body and choke off the normal cells (ex: brain, lungs, liver).
Transmission electron microscopy shows a multi layered epithelium with cells bound to each other by numerous desmosomes. The cytoplasm is rich in glycogen & tonofibrilies. But most of the superficial cells which are about to be cast off are not bound to each other by desmosomes.

Scanning electron microscopy of the surface of the normal squamous epithelium discloses plate-like arrangement of larger squamous cells closely fitting with each other. Ferenczy and Richard, (2007). The surface of the cells is provided with a network of short uniform microbridges.

**Endo cervical epithelium:**

The epithelial liming of the endocervical canal and of the endocervical glands is formed by a single layer of mucus producing epithelium composed of tall clear cells with oval nuclei, usually basal in location some variability of the nuclear form may occur due to hormonal effect. Ciliated cells may be noted in the upper part of the cervical canal.

Ultra structural studies reveal mucus cells with secreting granules in the cytoplasm. On the luminal surface the cells are bound to each other by junctional complexes & in other parts by desmosomes. Ciliated endocervical cells are found in increased number when seen under scanning microscope.

In between the columner cells there are some small cells lying adjacent to the basement membrane. These cells can be demonstrated only under electron microscope. Regeneration of the epithelium takes place from these small cells.
Endocervical glands are simple, tubular & branching type. The cells of the endocervix take in the cyclic hormonal changes. The endocervical mucous is thick in much of the menstrual cycle while it becomes liquid for 3 or 4 days just prior to, during and immediately after ovulation.

**Transformation zone:**

The cervix is composed of columnar epithelium, which lines the endocervical canal & squamous epithelium, which covers the exocervix. The point at which they meet is called the squamocolumnar junction.

The squamo columnar junction rarely remains restricted to the external os. Instead, it is a dynamic point that changes in response to puberty, pregnancy, menopause & hormonal stimulation. In neonates the squamo columnar junction is located on the exocervix. At the time of menarche, the production of oestrogen causes the vaginal epithelium to fill with glycogen. Lactobacilli act on the glycogen change the pH, stimulating the subcolumnar reserve cells to undergo metaplasia. Subcolumnar reserve cells of the tips of the columnar villi, which are exposed first to the acidic vaginal environment, are stimulated first. As the metaplasia replaces the columnar epithelium, the central capillary of the villus regresses & the epithelium flattens out, leaving the epithelium with its typical network of vasculature. As metaplasia proceeds in to the cervical clefts, it replaces columnar epithelium and similarly flattens the epithelium. The deeper clefts may not be completely by the metaplastic epithelium, leaving mucous – secreting columnar epithelium trapped under the squamous epithelium. Some of these glands open into the
surface; other are completely encased, with mucus collecting in Nabothian cysts.

The metaplasia advances from the original squamo columnar junction inward, toward the external os, and over the columnar villi. This process establishes an area called the transformation zone. The transformation zone extends from the original squamo columnar junction to the physiologically squamo columnar junction. As the metaplastic in the transformation zone matures. It begins to produce glycogen and eventually resembles the original squamous epithelium colposcopically and histologically (Shakuntala, 2010).

The only way to determine where the original squamo columnar junction was located is to take for Nabothian cysts or cervical cleft openings, which indicate the presence of columnar epithelium. Once the metaplastic epithelium matures and forms glycogen it is called ‘Healed Transformation Zone’ and is relatively resistant to oncogenic stimuli. However, the entire squamo columnar junction within early metaplastic cells are more susceptible to oncogenic factors which may transform these cells into cervical intraepithelial neoplasia. (CIN). Therefore, CIN is most likely to begin either during menarche or following pregnancy, when metaplasia is most active. Conversely, a woman who has reached menopause without developing CIN has little metaplasia and is at a lower risk.

Cytology of the cervical epithelium:
When the vaginal smear is taken, it may contain columnar cells which line the fallopian tubes, endometrium & endocervix. But the greatest number of epithelial cells present in the vaginal smear are shed
from the noncornified, stratified squamous epithelium covering the vaginal vault and the portio vaginalis of the cervix.

**Squamous epithelium:**

Cells exfoliated from the squamous epithelium generally have oval nuclei with a particular chromatin pattern. In each cell the nucleus is centrally placed. As the cell matures the nucleus changes its reticular pattern gradually becomes coarse and granular and then suddenly becomes pyknotic (Keryopyknosis) and eventually breaking up and disappearing (Karyorrhexis & Karyolysis).

With maturation the cytoplasm first loses its green colouration, then blue and ultimately becomes yellow or red, at the same time it loses the elasticity and with maturity acquires the hard qualities of keratin. Thus the immature cells are found round, small and thick when exfoliated and the older cells are found as flattened, extremely thin cells and defined as wafer-thin polygon shaped cells.

According to Papanicolaou, four main types of cells can be distinguished which corresponds to the different layers of the vaginal epithelium. They are:

(i) Superficial cells
(ii) Intermediate cells
(iii) Parabasal cells
(iv) Basal cells

(i) **Superficial cells:**

During child bearing ages in women the largest populations of cells in cervical or vaginal smear are the superficial cells derived from the loosely bound superficial zone of squamous epithelium. The cells are large, delicate, polyhedral with sharply defined cell borders which
may be irregular or indented. The diameter of superficial cells is usually 35-45 micron, nuclear cytoplasmic ration being 1 : 7. The shape of the cell reflects considerable rigidly of the cytoplasm associated with the presence of numerous bundles of tonifibrils in transmission electron microscopy. Their cytoplasm is light & transparent. Occasionally a small perinuclear halo can be present. Cytoplasm is either eosinophilic or cyanophilic. Cytoplasm may sometimes contain small dark brown granules which contain lipid & the presence of which is oestrogen dependent (Masin & Masia, 1969). The nuclear diameter is rarely more than 5 micron. Karyorrhexis is frequent. Nuclear pyknosis can be easily identified when examined under phase contrast microscopy. The pyknotic nuclei display a characteristics reddish hue. Nuclear pyknosis represent the last state in maturation processes of the squamous epithelium.

The presence of large eosinophilic superficial cells with pyknotic nuclei, exfoliated from superficial layer is a sign of maximal epithelial maturation which is dependent on oestrogen stimulation and thus provides an excellent morphologic evidence of peak oestrogenic activity.

(ii) Intermediate cells:

These cells are of same size with that of superficial cells may show extreme variation. The cytoplasm is usually cyanophillic but may also be eosinophillic. The chief difference between the superficial and intermediate cells lies in the structure of the nucleus. The nuclei of intermediate cells are vesicular, round or oval, measures about 8 micron in diameter. Chromocentres and sex chromatin may be observed within such nuclei (Koss, 1979). The nuclei are either centrally or eccentrically
placed. The cytoplasm presents slight wrinkles or small folds at the sides of the cells. This appearance is generally seen in first half of the menstrual cycle. Vacuolation in cytoplasm may create curvature in the edge of the cell which gives it a special configuration known a navicular or oyster from cells of Papanicolaou (1925).

Navicular cells are the special form of intermediate cells. They are oval in shape with slightly thickened border. Cytoplasm is clear and basophilic with eccentric vesicular nucleus. They have a tendency to cluster formation. The presence of navicular cells does not indicate a particular hormonal state because they may be present in the smear when the vaginal mucosa is in a state of intermediate proliferation. However, they are present in moderate oestrogen deficiency and in condition with high progesterone activity. e.g., secretory phase of menstrual cycle, pregnancy, progesterone therapy, in the presence of functional luteal cyst and also possibly with increased androgenic activity. The importance lies in the fact that the number & size of the individual navicular cell cluster gives a reliable information or progesterone activity. Numerous large clusters consisting of at least 10 individual navicular cells indicate a good progesterone effect smaller cluster less than 10 cells denote moderate progesterone activity and single or no navicular cell denotes sprogesterone deficiency. In normal pregnancy navicular cells increase up to 75% in pregnancy at term, number and cluster of the navicular cells diminishes though the smear consists largely of navicular cells.

(iii) Parabasal cells:

These cells are less commonly found in normal smears during child bearing age and do not constitute more than 5% of cells. But they
are predominantly found in atrophic smears in childhood or after the menopause. In the presence of pathologic process within cervix the proportion of parabasal cells in smears may increase significantly.

The parabasal cells vary in size & measures from 12-30 micron in diameter. Cytoplasm is usually basophilic and contains small vacuoles. They often assume a round or oval shape with smooth cytoplasmic borders. This appearance of parabasal cells result from contraction of the cytoplasm following cell death (Dawn, 2010). Nuclei of parabasal cells are oval and round and are approximately of the same size as the vesicular nuclei of intermediate cells and contain chromatin granules or chromocentres. The nuclei occupy a large portion of the total cell volume. The nuclear Cytoplasmic ratio being 1 : 3. These cell have been reported during pregnancy, in vaginal or cervical pathology, threatened abortions, foetal death in utero, prolonged pregnancy and severe oestrogen deficiencies.

(iv) Basal cells:

Because the basal cells are tightly adherent to the basement membrane, they are practically never seen in the smears. If present it surely indicates that a pathologic process has damaged the superficial cells of the squamous epithelium. They are small round or oval cells. Their cytoplasm is basophilic, evenly distributed and scantily. The nuclei are relatively large, shows chromatin granules and occasionally contains tiny round nuclei. The nuclear cytoplasmic ratio is 1:1.5.
Aneucleate superficial cell:

In normal non-stratified squamous epithelium they are not found. But when present it indicates contamination by the introitus or an epithelial abnormality with hyperkeratosis leukoplakia. The cytoplasm retains the waferthin squamous character, the nucleus is lost. Sometimes a nuclear ghost may be identified as a round clear area in the cytoplasm. The cytoplasm may retain nuclear fragment and granules of keratin.

Columnar cell or endocervical cell:

These are elongated cylindrical cells which are well preserved in the cervical scrape smear. The cytoplasm is abundant, foamy and finely vacuolated. Occasionally the endocervical cells show recognizable cilia supported by a terminal plate.

The nucleus lies on the basal side & which is characteristically vesicular and vary in size, but retains a round or oval shape, chromatin pattern is uniformly distributed, nuclei is prominent and multiple. Multi nucleation is common during irritation but the chromatin pattern is uniform in all the nuclei.

Columnar cells from endocervix frequently exfoliate in sheets or clumps when a scraping method is used. So, adequate smears should also contain cells from the columner cell of endocervix.

Cells other than epithelial in normal smears of female genital tract:

In addition to the epithelial element, smears from female genital tract contain some other element derived from blood, connective tissue mucus bacteria, protozoa and fungi etc. These are described accordingly.
Leukocytes:

A variety of leukocytes may be seen in smear even in the absence of inflammation. They are often present during the second half of menstrual cycle and also in post-menstrual smears. Their number decreases during pregnancy through the wall. Therefore this migration to some extent depends upon the thickness of the epithelium. When epithelium is thick and multilayered as during oestrogenic stimulation, they tend to decrease in number. But in the oestrogen deficient stage the reverse is the true. Lymphocytes and polymorph are the cells commonly encountered. Plasma cells which are very uncommon in smear signify chronic inflammation.

Red blood corpuscle:

They are frequently observed in smears taken by scrapping method. A great variety of pathological conditions may cause bleeding from the genital tract. These range from minor injuries and mild inflammatory processes to benign and malignant tumours.

Macrophages or histiocytes:

The histiocytes may be small or giant cells. The small mononuclear macrophages are often present in the smear taken during late part of menstrual bleeding. They show great variation in size and shape. They may round or oval amoeboid and have round, oval or uniform eccentric nuclei. The nucleus possesses one or more nucleoli. Cytoplasm is pale and vacuolated.

Multinucleated giant cells occur in pathological condition, like chronic inflammation of vagina, cervix after abortion and following
treatment with ionising radiation. The size of the large histiocytes may exceed the parabasal cells. Cytoplasm is pale blue and the nuclei are arranged in centre or occasionally in the periphery.

**Fibrin:**

Fibrin is recognized as long threads which stains orange with papanicolaou stain. They are often present in the acute inflammatory processes and after irradiation of pelvis.

**Micro-organism:**

The normal flora of the vagina varies from person to person. Bacillus vaginalis (Doderlin) is most common nonpathogenic inhabitant of the vagina. The organism belongs to the group lacto-bacilli. They are grams positive, red and stain pale blue by papanicolaou method. It produces lactic acid from glycogen for its existence. The glycogen it utilizes is present in the intermediate cells. Therefore, the organisms are found during second half of the menstrual cycle and during pregnancy and causes cytolysis of the intermediate cells producing bare nuclei in the smear. Due to the production of lactic acid on the other hand the pH of the vagina become acidic (pH 4-4.5) and this offers protection against pathogenic flora, which cannot survive in the acidic medium (Shaw, 1978).

Other organisms which are pathogenic or potential pathogens like streptococi, staphylococci, colonic bacilli, gonococci etc. may be present. These organisms cannot be identified by routine papanicolaou method.
2.3: PHYSIOLOGICAL VARIATION IN CERVICAL CYTOLOGY

Menstrual cycle: The cyclical changes that occur in the cervical smear were observed to be constant and characteristic. Dierk (1927), showed the cyclical changes that occurred in vaginal epithelium. Papanicolaou in 1933 published his work on epithelial changes during menstrual cycle. Others like Murray (1938), Pundal (1958) - confirmed papanicolaou's findings.

During ovulatory phase which occupies first half of menstrual cycle, at first characterized by the presence of many histiocytes, polymorph, and endometrial columnar cells. Intermediate and superficial cells being equal in number. Later at the time of ovulation the smear is composed of mainly superficial cells, large number of which are cornified, stain pink and have pyknotic nuclei. The cells are flat unfolded with transparent cytoplasm.

Luteal phase corresponds with later half of menstrual cycle. The intermediate cells are preponderant. They lie clumped together. The cell roll upon itself, sometimes they take a boat shape known as 'navicular cells'.

During menstruation, cell of all types are present accompanied by red cells, endometrial cells, polymorphic, histiocytes, etc.

Pregnancy: During pregnancy the cytology shows the picture of luteal phase in an exaggerated manner. The cells are intermediate & typically navicular and remain in clumps.

Menopause: Cytological findings in menopause has individual variation. During early menopause the findings are very much inconsistent.
During 'crowded' menopause there is moderate deficiency of oestrogen and there are cluster of intermediate and parabasal cells, hence the name. During advanced menopause the cells mature only up to parabasal level. In smear the cells are scanty with predominantly parabasal and occasional basal cells. This is the stage of atrophic menopause. In the carcinoma of the cervix though peak incidence is around 45 years of age, but carcinoma of the cervix also develops in menopause stage where this anatomical cytological changes may have a determining factors in etiopathogenesis of carcinoma of the cervix.

**Cellular Morphology:**

The cell changes associated with pre-malignant disease of the cervix were originally described by Babes of Bucharest in 1927, but was noticed only in 1941 when Papnicolaou published his monograph.

*Papanicolaou (1943)* — stated dyskaryotic cells as a group of cellular abnormality mainly the nucleus, which is -

(i) Large
(ii) Round or oval
(iii) Hyperchromatic
(iv) Nuclear membrane is waxy and undulated and not shrunken
(v) Chromatin is minimally or moderately coarse, granular but evenly distributed throughout the nucleus.

*Ayra (1944-46),* - described the criteria of a malignant cell as seen in cervical smear as follows:

- Disproportionate enlargement of the nucleus in relation to cytoplasm.
- Variation in size and shape of nucleus
- Irregularity of nuclear membrane
- Hyperchromasia & clumping of nuclear chromatin
- Multinucleation associated with any of the above.

The suspicious malignant cell may be seen singly or in cluster. The loss of normal cellular pattern & over crowding of cells in cluster from has importance in malignancy. Thickening of nuclear membrane is seen not only in malignancy but also in chronic inflammation or irradiation. Hence it is not specific of malignancy.

**Dysplasia:**

Dysplasia is an alteration of adult cells, characterized by variation in their size, shape and organization. Though the term can be applied both to epithelial and mesenchymal cells, it is mostly in case of epithelial cells which have lost its normal orientation and have undergone alterations in cellular size and shape, as well as nuclear size and shape & staining characteristics.

Dysplasia in uterine cervix usually occurs in the transitional zone, that is, in the squamocolumnar junction. It may completely encircle the cervical os or may be confined to one or more isolated foci. In relation to canal axis, dysplasia has a mean linear extent of 9.0 ± 4.4 mm. In two thirds of cases there is involvement of both the surface & the underlying glands, while in one- thirds of cases the lesion is confined to surface epithelium (Reagon & Wentz, 1959). The degree of epithelial involvement along with the severity of anaplasia determines the grades of dysplasia.
According to WHO classification (1973) dysplasias are graded as:

(i) Mild dysplasia
(ii) Moderate dysplasia
(iii) Severe dysplasia

(i) **Mild Dysplasia**: On cytological study, changes are seen in both ectocervical & endocervical cells. In mild dysplasia the exfoliated cells of ectocervix consists of superficial and intermediate cells. The cells are mostly single, polyhedral or oval. They show cellular enlargement with an enlarged, swollen, occasionally vesicular nucleus with normal nucleocytoplasmic ratio. Cytoplasm changes predominant. They include fine vacuolation and fine to coarse granules. Precocious cornification of cytoplasm may be seen. Spindle shaped squamoid cells are very rare. Fluctuating dysplastic changes are characteristic of mild dyplasia.

The endocervical cells exfoliate singly in small groups. There is proportional enlargement of nucleus & cytoplasm so that the ratio remains within normal limit.

Histologically, the epithelium shows slight aberration from normal. The dysplastic cells occur only in one third of the way from the basal layer to the surface. It is difficult to differentiate this stage from epithelial reactions due to infection.

(ii) **Moderate Dysplasia**: Moderate dysplasia is characterized by a wide range of abnormal cells (i.e., basal, parabasal, intermediate & superficial). Exfoliated cells either remain singly or in groups. Nuclear abnormality is more prominent than mild dysplasia. Nucleocytoplasmic ratio is on higher side of normal. Nuclear membrane is thickened.
Nuclei are hyperchromatic with regular & finely distributed chromatin. Moderate number of precociously cornified basal cell are present. Spindle cells are rare.

Endocervical cells are exfolite in groups. Cytoplasm is finely vacuolated and cyanophilic. The nuclei show similar changes as ectocervical cells. The cells maintain polarity.

Histologically, the cellular atypism extends through one half to 2/3rd of the thickness of epithelial layer. They may show rapid maturation or penetration of parabasal and basal type of cells into the upper layers. The atypism include small cell non-keratinizing, large cell non-keratinizing and keratinizing.

(iii) Severe Dysplasia: Severe dysplasia is cytologically detectable when it appears that most of the cells have rounded or oval configuration. These cells are basal & parabasal type. The cells are exfoliated in large groups and show some degree of loss of polarity. Nuclear enlargement is more than cytoplasmic enlargement, which alters the nuclear cytoplasmic ratio. The nuclei are hyperchromatic with prominent nucleoli & coarse chromatin pattern. The cytoplasm is usually dense & basophilic. Fibre cells and ‘tab pole’ cells may be seen in severe dysplasia. In some cells perinuclear halo may be seen.

Histologically, the dysplastic cells occupy 75 - 90% of the epithelium. The basal & parabasal cells extend into the superficial zone. Bizarre pattern with individual cell keratinisation, multiple nuclei and nucleoli with variable chromatin pattern are striking features. Basal layer shows loss of polarity with irregular arrangement.
Carcinoma-in-situ:

It is a lesion in which all or most of the epithelium shows the cellular features of carcinoma, without evidence of stromal invasion. That carcinoma in situ is a forerunner of invasive carcinoma was first emphasized by Schiller (1933). Later on, his view was supported by others (Peterson, 1956).

Morphologically, carcinoma in situ does not show a recognizable lesion to the naked eye. It is suspected by Schiller test, Papanicolaou’s smear or by colposcopy.

Cytologically, the picture is similar to as moderate to marked dysplasia. The cells show pronounced nuclear & cytoplasmic abnormality. In addition to the nuclear and cytoplasmic abnormality as seen in severe dysplasia, the cytoplasm of these cells may show amphophila and may contain vacuoles. Abnormal cells may occur individually or in loose aggregates. There is often a uniformity of the cell type. Dysplastic cells derived from other areas may be present.

In Schiller test when the cervix is painted with iodine and potassium iodide, normally it turns to mahogany brown due to rich content of glycogen in cervical epithelium. But a carcinomatous area fails to stain as there is depletion of glycogen. False positive results may be obtained in inflammatory areas. In colposcopy, dysplastic lesion show areas of white epithelium termed as ‘mosaic’ or punctuation pattern.

Histologically, the whole thickness of the epithelium is involved. Small hyperchromatic nuclei are surrounded by scanty cytoplasm. Orderly stratification is lacking & cellular polarity is often vertical or diagonal rather than horizontal. Both normal & abnormal mitotic figures are found scattered throughout the epithelium. Sometimes the anaplastic changes may extend along the surface epithelium into
endocervial gland, but the basement membrane of these glands are not penetrated.

During the last decades the term Dysplasia (mild, moderate, severe) & carcinoma in situ have been replaced by Cervical Intraepithelial Neoplasia (CIN) [Richard introduced the term CIN]. The World Health Organization recommendation (Poulsen & Taylor, 1975) set out the pathological features of various stages of the disease. Grade I intraepithelial neoplasia represent less than one-third involvement which corresponds to mild dysplasia. Grade-II intraepithelial neoplasia represent $1/3^{rd}$ to $2/3^{rd}$ involvement corresponding to moderate dysplasia and Grade-III represent $2/3^{rd}$ to full thickness involvement corresponding to severe dysplasia & carcinoma in situ. In the year 1988 the National Cancer Institute established a workshop which met in Bethesda. That nomenclature is currently referred to as “The Bethesda System” as compared with the original Papanicolaou and WHO classification. This system will be discussed later on.

The term cervical intraepithelial neoplasia implied that the disease is continuous, but its grading reflects the prognostic differences in the untreated lesion. If the lesion is removed the recurrence should be the same irrespective of grading.

Natural history of dysplasia and carcinoma-in-situ:

These precursor lesions of the cervical carcinoma either may remain static or regress or progress. Many experimental studies have indicate that dysplasia and carcinoma-in-situ are the different stages in the process of carcinogenesis.

In the experimental study on mouse cervix by Von Haam & Scarpalle (1955) - had found that the initial change in the process of carcinogenesis was the dysplasia followed by an intermediate stage which was described as
non invasive. Reagon and Wentz (1959) - also induced carcinoma of the
mouse cervix experimentally and found that dysplastic changes were
the earliest morphological changes. This stage was followed by
epithelial infiltration.

Luthra et al., (1969) - induced carcinogenesis on a specially breed
Swiss Albino vergin female mice by using 3 : 4 benspyrine in acetone.
They had shown the stages of evolution of cervical carcinoma during
experimental carcinogenesis.

It showed that when carcinogen was applied to the epithelium is
underwent various cytological and histological changes, e.g., acute
inflammation, mild, moderate and marked dysplasia & finally either to
intraepithelial and/ or to invasive carcinoma. The initial lesion showed
varied biological behaviour, some of these regressed, others persisted
and some progressed as shown below:

Progression of lesion

Regression of lesion

Carcinogen

Normal epithelium

Acute inflammation of epithelium

Mild dysplasia

Moderate dysplasia

Marked dysplasia

Intraepithelial carcinoma

Invasive carcinoma

Fig. 2.2: Showing progression to cancer from normal epithelium

Follow up studies in human being had shown similar findings as in experimental carcinogenesis. Greene (1955) reported 37% cases with dysplasia showed regression to normal while 10% progressed to carcinoma-in-situ. Mackay (1959) reported 20.2% dysplastic lesion reverted to normal, 32.5% progressed to carcinoma in situ. Progression of the dysplastic lesion in 8 to 12% cases to a severe form was observed by various workers (Attwood, 1961; Figgs & coworkers, 1962). Fox (1967) had found a higher rate of progression. He found that the progression of dysplastic lesion to a severe degree was 60%. Hall & Walton (1969) have found 6% of mild dysplasia, 13% of moderate dysplasia and 19% of severe dysplasias ultimately progressed to carcinoma-in-situ.

Richart & Barron (1969) - described that, few months to years elapse when dysplasia progresses to carcinoma in situ. They have shown that the median transit time to carcinoma in situ is approximately 12 months for severe dysplasia, 38 months for moderate dysplasia, & 58 to 86 months for mild & very mild dysplasia, 44 months for all dysplasias taken together.

The existence of a transition stage between carcinoma in situ and invasive carcinoma is described by Peel (1986). These are termed microinvasion. When the tumour cells do not extend beyond a depth of 5 mm from the basement membrane then it is known as early stromal invasion & when the size of the tumour mass does not exceed 500 mm³ then it is known as microcarcinoma Burghhardt believes that the vascular invasion with microcarcinoma will lead to the risks of
metastasis, while vascular invasion in early stromal invasion does not have any significant role.

A typical sequence of events in the natural history of carcinoma of the cervix.

<table>
<thead>
<tr>
<th>Curable</th>
<th>Difficult or impossible to cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild or moderate Dysplasia &amp; CIN-I &amp; CIN-II</td>
<td>Severe dysplasia or CIN asymptomatic (CIN III)</td>
</tr>
<tr>
<td>3-8 yrs</td>
<td>10-15 yrs</td>
</tr>
</tbody>
</table>

Fig. 2.3: Showing lag period of carcinoma development


History of CIN is difficult to predict because it is modified by diagnostic procedure & by treatment itself. Genuine regression to normal probably only occurs with CIN-I because in this grade true CIN will be difficult to distinguish from inflammatory or infective changes. About one quarter of cases of CIN-I & II will progress to more severe lesion without treatment and over a half of untreated cases of CIN-III to invasive carcinoma. Low grade CIN (LSIL) reverts to normal, persist or progress. But it is not clear whether all high grade CIN starts as denovo
or start as low grade CIN. However high grade CIN is undoubtedly a true cervical cancer precursor.

Women with age >25 yrs. CIN-III is found in 0.2-0.4%. But only 20-30% of cases progress to invasive cancer.

Invasive Cervical Cancer:

In its earliest stage the invasive cervical cancers are generally not recognized grossly. Whereas, suspicious lesions may appear in various forms. It usually appears as hardened granular area at the margin of external os, which bleeds on touch. It may also appear as fine papillary area of firm consistency or as a small definite ulcer with indurated base.

In the moderately advanced stage the lesion grows and takes exophytic (overting) form and becomes papillary or cauliflower like. The lesion is fragile, easily bleeds, Necrosis & ulceration commonly occurs. The other variety is endophytic (inverting) form. Here the tumour grows into the cervical tissue rendering the cervix large, nodular & stony. They invade the surrounding vaginal wall.

In the advanced stage the disease is characterized by widespread destruction and infiltration. The growth may completely fill up the vaginal canal. In invasive variety the cancer may completely destroy the cervix forming a foul smelling cavity. Adjoining vaginal wall becomes board like, parametrium is thickened most often there is nodal involvement (Jeffcoates 7th Ed 2010).
Types of cervical cancer:

Histologically the cervical carcinomas are divided into two basic types:

(i) **Epidermoid Carcinoma**:

This is the most common malignant tumour that constitutes about 75% to 95% of all carcinoma of cervix.

The commonest site is the squamo-columnar junction. According to the presence or absence of keratinization & tumour cell size, the tumour can be divided into three sub-types.

(a) **Keratinizing carcinoma**:

This sub-type is characterized by keratinization of tumour cells with pearl formation. There is preponderance of relatively large abnormal cells with high degree of pleomorphism. The chromatin pattern is coarse and irregular. Nuclear degeneration characterized by an opaque nuclear mass is distinct feature. The cytoplasm is abundant which is often eosinophilic or orangophilic (Dutta, 2010).

(b) **Non-keratinizing large cell type**:

This is the most frequent sub-type of squamous cell carcinoma of the cervix. Mitosis is common.

In cytological smear the tumour cells remain in syncitial mass or singly, which are relatively large with cyanophillic cytoplasm. Chromatin pattern is coarse and irregular. Nucleoli are prominent. The size of the cells show moderate degree of variation (Dawn, 2010).
(c) **Non-Keratinizing small cell type:**

The cells are relatively small and uniform in size. Keratinization is absent. The nuclear cytoplasmic ratio is high. Chromatin pattern is coarse and irregular. Nucleoli are prominent (Shakuntala Baliga, 2010).

**Adenocarcinoma:**

Adenocarcinoma of the uterine cervix is relatively uncommon constituting about 5% of all cancers arising in the cervix. These neoplasms are partially or completely composed of malignant glandular epithelium. Histologically they are divided into well, moderately & poorly differentiated carcinoma.

In cytological smear the cells show acidophilic cytoplasm with occasional fine vacuolation. The nuclei is hyperchromatic and appears larger than normal with a tendency to margination. Mitosis is occasional. Nuclei is prominent and usually multiple.

**Clear cell carcinoma:**

This type of adenocarcinoma may arise from columnar epithelium or from remnants of the mesonephric duct. It has received considerable attention because of its increased frequency, in young women whose mother had been treated with diethylstiboeosterol during pregnancy for a threatened abortion (Dutta, 2010).

**Adenosquamous (Mucoepidermoid carcinoma):**

This type of tumours have mixed squamous and glandular pattern and are thought to arise from the reserve cell in the basal layers of the endocervical epithelium (Jeffcoate’s 7th Ed 2010). The tumour cells
often contain mucin. They can be differentiated from squamous cell carcinoma only by histological examination & have a less favourable prognosis than squamous cell carcinoma of similar stage.

**Concept of cellular exfoliation:**

Various concepts have been postulated in order to explain cell exfoliation (Shakuntala Baliga, 2010). But no definite universally accepted theory is available in this regard.

Many epithelial tissues of the body undergo incessant renewal of their constituent cells, throughout life. The cells continuously desquamate in large numbers and are replaced by new ones originating by mitosis from basal layer.

The cell undergoing renewal, pass through four main phases as stated by Bertanaffy (1964). The tissues with such a mechanism of cell renewal offer themselves to cytodiagnosis. The 4 main phases include:

(i) Cell formation by mitotic divisions.

(ii) Cell differentiation, i.e., increase in size, formation of cilia, stratified border, capability to elaborate secretions.

(iii) Functional or metabolic phase, when the cell performs the function peculiar to the tissue, such as secretion, absorption, protection, etc.

(iv) Desquamation.

The rate at which cell renewal occurs varies from the tissue to tissue.

*Bertanaffy (1964)* - postulated the pressure theory to explain cell exfoliation. The cell formation by mitosis is a continuous process for each added cell; another has to be removed as it can accommodate only
a fixed number of cells. Since epithelial cells possess no motility, they are shed off by pressure of increased cell in epithelium.

Glucksmann (1964) criticized Bartanaffy's concept of cell exfoliation. He believed that cell migration was more likely the factor. He further stressed that desquamation persisted even following irradiation & lack of mitotic activity in basal layers.

Bartanaffy (1964) mentioned that mitosis, though diminished, persists following irradiation. He conceded that epithelial cells did show motility, but this stops as soon as cells come into contact & form continuous sheet. He further emphasized that the pressure theory may not be solely responsible factor in cell exfoliation.

Field of Cytology:

In the expanding field of cytology one may advance in the following three directions. (Wahi; Luthra and Malis, (1969).

(i) Increase the potential of methodology in the usefulness by defining the diagnostic potential and applying it to therapeutic follow up patients.

(ii) Utilize the same methodology in other areas of application, such as cytology of oral lesions, breast secretions, cell aspirates & skin lesion.

(iii) Introduce new methods which can apply to other fields of cytology such as cytogenetics, cytochemistry & the study of ultrastructure of cells.

Gates & Warren (1947) and Graham (1965) - have pointed out different applications of vaginal smear in diagnosis of uterine cancers, such as:
(a) For study of obscure conditions like borderline lesions of uncertain significance & those that can’t be diagnosed by usual method.
(b) For diagnosis of selected cases.
(c) For determination of sensibility to irradiation of cancer of uterus in individual cases.
(d) As a routine test in general physical examination
(e) As an adjunct in biopsy in diagnosis of gynaecological cases particularly.

Some interesting literature in relation to carcinoma cervix both international & National is summarized below:

Wachtel (1956-1958) - stated that cancer tissue may be capable of producing oestrogen or oestrogen like substances which give high Karyopy knotic index, specially in post menopausal women.

Cuyler (1957) expressed the view that superficial and intermediate cell dyskaryosis was less significant than parabasal cell dyskaryosis & would be followed up cytologically.


Macgregor (1971) - carried out large population study at Ortange cancer preventive clinic in New York and found that the incidence of the asymptomatic Ca in situ was 2.4%. Similar studies were carried out by Fox (1967), observed similar findings.

Andrew et al., (1971) had studied the vaginal irrigation smear for detection of cervical cancer & metastatic ovarian cancer.

The World Health Organization had declared 1970 as the year of “Pap test” - the slogan being ‘Early detection of cancer & save life.’
Oral contraceptive use (Vessey et al, 1983) or IUCD (Blenkinsopp & Chapman, 1982) is associated with increased risk of cervical malignancy.

Beral & Sjostedt (1983) - reported that women who smoke are at high risk for development of cervical cancer.

Role of high risk female in the development of cervical cancer was suggested by Beral (1974) & Singer et al, (1976). Buckley et al., (1981) reported that women whose husbands have had multiple sex partners are said to be at high risk. Singers et al., (1976) - informed that seconds wives of man whose first wives had cervical cancer are also at high risk.

Drapper & Cook (1983) reported that the incidence of preinvasive squamous cancer, cervical intraepithelial neoplasia Grade 3 (CIN-III) has risen markedly and has been referred to as an epidemic.

Study in India:

Mali et al., (1969) - carried on a long term study & examined a total of 39,587 women where they found the incidence of all grades of dysplasia was 2.3%. They also observed the relation of age, age at marriage & parity with that of dysplasia. Similar incidence was decided by Rao et al., (1975) & Chakravarty (1976).

Lulla et al (1980) - in their eight year's study have found that the incidence of dysplasia was 2.8%, while that of carcinoma in situ, microinvasive & invasive were 0.2%, 0.04% & 1.71% respectively. They also tried to find out the high risk group in the population by correlating with the epidemiological studies.

Jamila & co-workers (1980) -followed 1,000 cases with unhealthy cervical erosion or chronic vaginal discharge for six years. They found the incidence of dysplasia al all grades was 2.5%. They also found that
cervical malignancy is commoner among Hindus than Muslim community. Their findings also stated that higher incidence of abnormal smears were in clinically suspicious or unhealthy cervix & cervical erosion which bleeds on touch.

*Sarada & her co-workers (1982)* - Screened 1000 rural women & found that the incidence was 0.4%. They also found that the commonest age group was 30-40 years (70%), with high parity 3 to 6 in 65%.

*Das et al., (1984)* had situated 960 cases & found the incidence of dysplasia, carcinoma in situ & invasive carcinoma was 10.83%, 1.14% and 1.56% respectively.

*Chauhan et al., (1987)* - studied 5778 women & found that dysplasia was present in 2.2%. They found mean age group of dysplasia was 35.4 about 50% occurring in 4th decade. Majority of the patient had cervical erosion (91.6%) and commonest symptom was irregular menstrual bleeding (62.8%). Post coital bleeding was presenting symptom in 6.8% and prolapse 1.5%. They also found that dysplasia was commonest in the very low socio-economic groups.

*Mittal et al., (1988)* - studied a group of 250 patients belonging to all age groups having history of abnormal bleeding per vagina and other gynaecological complications for early diagnosis of precancerous & cancerous lesions of genital tract & found that 74.28% correlation between cytology and biopsy of inflammatory lesions, 90.9% correlation in moderate dysplasia, 80% in severe dysplasia & 80.9% correlation invasive carcinoma.

*Singh et al., (1984)* - performed a colposcopic study in 995 women with cervical dysplasia in those women who were found to have
carcinoma in situ as diagnosed cytologically. Overall agreement between cytology & colposcopy findings was 78.10%.

WHO/ICO information centre on HPV and cervical cancer in summary report 2010 available on www.who.int/hpocentre has given a beautiful picture on the age specific cervical cancer screening practice in India as below.

Fig. 2.4: Cervical cancer screening practice in India

www.who.int/hpocentre
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