16

Cytology of Cervical Intraepithelial Glandular Lesions

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1. Introduction

Cytology of the cervix has been properly recognized and accepted in the detection and follow-up of squamous intraepithelial lesions, whereas its role in endocervical cylindrical epithelium is less well defined. Glandular lesions of the cervix uteri have been showing a rising incidence for the last 20 years, especially among young women (Nieminen et al.1995). This trend could be attributed to several factors: better diagnosis with more appropriate techniques of sampling and specimen preparation for both cytological and histological analysis, better recognition of precursor lesions, changes in nomenclature, evolving methods of treatment and an improved understanding of morphological features, having all led to the development of criteria for the diagnosis of early dysplastic lesions. Another reason for the observed rise is the increased prevalence of these lesions.

As the major purpose of the Papanicolaou smear tests is the earliest possible diagnosis of cervical cancer and its precursors, both cervix and endocervix must be adequately sampled as the most common sites of these lesions. The best time for obtaining a smear is midcycle, i.e., two weeks after the first day of the last menses. Ideally, the woman should not have had intercourse, used douches, vaginal medication, or vaginal contraceptives 48 hours prior to obtaining a smear. It is vital that detailed clinical information be provided to the cytology laboratory. This information should include: date of the last menstrual period, results of previous Papanicolaou smears, history of fertility treatments or hormone therapy, history of abnormal bleeding, usage of intrauterine contraceptive devices, history of malignancy of female genitalia, of hysterectomy, radiation, and the results of any previous cervical biopsy.

The accuracy of clinical cytology relies to a large extent on sucessful sampling in obtaining the Papanicolaou smear and on its proper fixation and staining. A specimen from the cervicovaginal area that has been satisfactorily obtained and prepared for microscopic examination exhibits an abundance of well-preserved and meticulously stained diagnostic cellular material that remains preserved for indefinite slide storage and later review.

Glandular lesions are frequently detected in histology of cytologically diagnosed squamous intraepithelial lesions (SIL).

Cytological criteria for the identification of glandular intraepithelial lesions (GIL) have not yet been fully articulated, especially for the precursors of adenocarcinoma in situ (AIS), and these lesions may frequently remain unrecognized. Documenting the sequence of neoplastic events in the endocervix poses problems, except for its lowest segment, because the endocervical canal cannot be visualized by colposcopy and, therefore, cytological sampling cannot be targeted. Also, in spite of numerous efforts, morphological recognition of sequential abnormalities of endocervical cells is much more difficult than in squamous cells (Lee, 1999). Primary factors that contributed to either screening errors or diagnostic errors in AIS were insufficient quantities of material or poorly preserved abnormal material and aggregates of glandular cells. (Ruba et al., 2004).

2. Classification

By analogy to squamous cell cervical cancer precursors that demonstrate a wide spectrum of histological changes, some authors have proposed parallel classification schemas for endocervical adenocarcinoma precursors that include lesions with a lesser degree of abnormality than AIS. Such low grade putative glandular precursor lesions were termed endocervical dysplasia (Bousfield et al., 1980), cervical intraepithelial glandular neoplasia - CIGN (Gloor & Hurlimann, 1986), endocervical columnar cell intraepithelial neoplasia - ECCTN (van Aspert - van Erp et al., 1995), low grade glandular intraepithelial lesion - LGIL (DiTomasso et al., 1996), endocervical glandular dysplasia - EGD (Casper et al., 1997), endocervical glandular atypia - EGA (Goldstin et al., 1998), and cervical glandular intraepithelial neoplasia Low grade - L-CGIN (Kurian & al-Nafussi, 1999). We prefer the term glandular intraepithelial lesion (GIL) grade 1 and 2.

In contrast to squamous intraepithelial lesions with identifiable subgroups, in the case of glandular epithelium only adenocarcinoma in situ, included in the NCI Bethesda 2001 cytological classification, has been recognized. (http://bethesda 2001 .cancer.gov)

A uniform classification of cervical cytology findings known as Zagreb 1990 (Audy-Jurkovic et al., 1992) and developed by combining the original 1988 Bethesda System (TBS) classification (NCI, 1989) and our previous classification (Audy-Jurkovic et al., 1986) has been used in Croatia since 1990. As the TBS has been supplemented and/or modified on several occasions since its introduction (NCI, 1993 2001; Kurman & Solomon, 1994), we considered it plausible to revise our classification accordingly, i.e. by modifying and/or supplementing points of dispute noted over the past years, and by harmonizing it with the NCI Bethesda System 2001.

The current classification, **Zagreb 2002**, has been introduced as a new uniform classification system of cervical cytology findings used in Croatia (Fig. 1) (Ovanin-Rakic et al., 2003).

General classification consists of two groups, **"negative"**, for intraepithelial or invasive lesions, and **"abnormal cells"**, the latter referring to all cell alterations that are morphologically consistent with intraepithelial or invasive malignant lesions. The "negative" group refers to findings which are within normal limits, cell alterations associated with particular reactive and reparatory reactions, and with the presence of cells indicative of certain risks (e.g., findings of endometrial cells of benign appearance beyond the cycle or in the postmenopausal period).

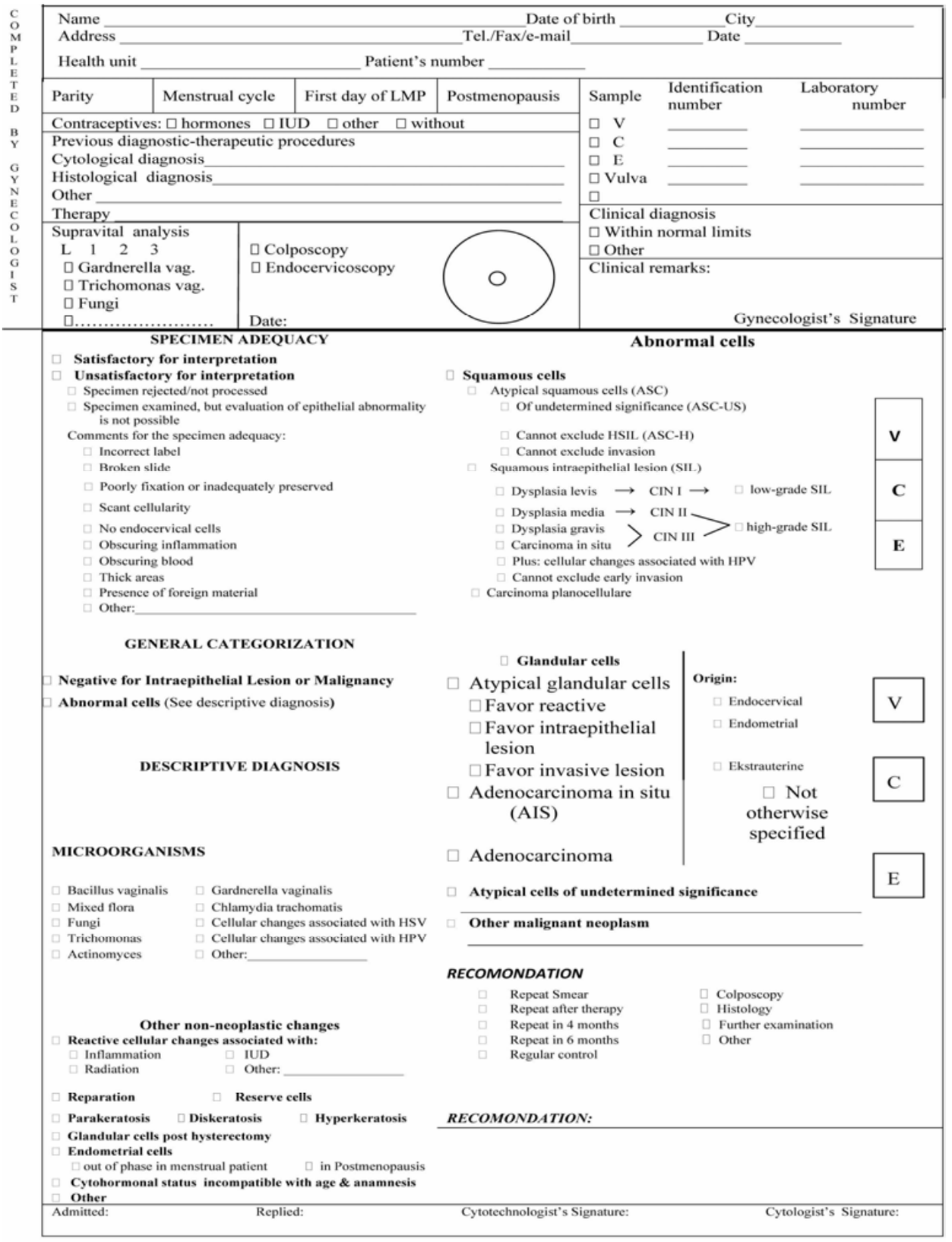

Fig. 1. Uniform classification of cytological findings of cervix uteri "Zagreb 2002", modification of the "Zagreb 1990" and "NCI Bethesda System 2001"

Unlike NCI 2001, we have kept the term **"diagnosis"** instead of "interpretation/finding result". Descriptive diagnosis contains the subgroups of **"microorganisms"** (microorganisms that can be identified directly or on the basis of a specific cytopathic effect); "**other non-neoplastic findings**" (reactive cell alterations, reparatory epithelium, reserve cells, parakeratosis, dyskeratosis, hyperkeratosis, post-hysterectomy cylindrical cells, endometrial cells beyond the cycle or in the postmenopausal period, and cytohormonal status inconsistent with age and/or history), and **"abnormal cells"** (squamous, glandular, abnormal cells of undetermined significance, and other malignant neoplasms).

In the **Zagreb 2002** classification, like in the NCI 2001, glandular lesions have been divided into three categories: **"atypical glandular cells"** (AGC), **"adenocarcinoma *in situ*"** (AIS), and **"adenocarcinoma"**. In the case of squamous epithelium, and unlike in NCI 2001, AGC have been divided into three subgroups, instead of two:

- favor reactive – cell alterations that are more pronounced than benign reactive ones but quantitatively and qualitatively less pronounced than those in intraepithelial lesions;
- favor intraepithelial (GIL1,GIL2) – cell alterations of low to moderate severity, without inflammatory cell changes, and/or suggestive of AIS, without definite criteria;
- favor invasive – cell alterations suggestive of invasive lesions, where differential cytological diagnosis cannot be made, mostly due to poor specimen preparation.

The group of adenocarcinoma in situ requires the establishment of well defined criteria.

The group of adenocarcinoma invasivum has not been modified relative to previous classifications.

For any group or subgroup of abnormal glandular cells, it is crucial to identify the origin of cylindrical epithelium whenever possible, as it is of great importance for further diagnostic and therapeutic procedures. At the end of the report, the cytologist provides the clinician with instructions on how to improve the quality of cervicovaginal smears, with guidelines on further procedures for a particular cytological finding. These instructions are in line with the current diagnostic-therapeutic protocols in use in Croatia (Ljubojevic et al., 2001).

Assessment of specimen adequacy is one of the substantial qualitative components of a finding. All criteria advocated by NCI Bethesda System 2001 (NCI, 1989,1993, 2001; Solomon et al., 2002) have been incorporated into our classification system. Information on the components of the transformation zone, i.e. finding of endocervical cylindrical epithelial cells, improves overall specimen quality thereby stimulating efforts to obtain an optimal specimen. However, the absence of such information is by no means a reason for a repeat smear (Pajtler & Audy-Jurkovic, 2002).

Cytodiagnosis of cervical cylindrical epithelial lesions lags behind the cytodiagnosis of squamous epithelial lesions both in terms of screening and differential diagnosis. The Australian (Roberts et al., 2000) modification of TBS (NCI, 1989) for glandular lesions points to the risks in the presence of high-grade abnormalities, thus resulting in more appropriate recommendations and protocols.

Cytological diagnosis of adenocarcinoma in situ of endocervical cylindrical epithelium as a separate entity was only included in the NCI Bethesda System 2001 classification, whereas dysplasia of endocervical cylindrical epithelium as an AIS precursor is still considered cytologically and histologically to be an inadequately defined entity (Zanino, 2000) and has not been included in the classification (NCI, 2001).

In most cases, morphological characteristics allow for differentiation between atypical endometrial cells and endocervical cells (Chieng & Cangiarella, 2003).

The proposed **Zagreb** classification, with amended and/or supplemented points of dispute identified in previous classifications, is uniform for Croatia. It allows for both internal and external performance quality control, along with appropriate reproducibility of cervical cytology relative to terminology adopted worldwide.

3. Epidemiology

The prevalence of AIS is not known, but is considerably lower than the prevalence of SIL. In the Surveillance Epidemiology End Results (SEER) public database, which contains data from patients entered into the database between 1973 and 1995 (Plaxe & Saltzstein, 1999), the ratio of in situ and invasive lesions is 1:3 for glandular and 5.25:1 for squamous lesions.

The rate of dysplasia of endocervical cylindrical epithelium is 16-fold that of AIS. and the mean age at diagnosis for endocervical glandular dysplasia is 37 (Brown & Wells, 1986).

The mean age at diagnosis of women with AIS in the SEER registry is 38.8, , and it is 51.7 for invasive adenocarcinoma (AI) of the cervix.

The median age of patients in our study (Ovanin-Rakic et al., 2010) was 40, which is comparable to 41, reported in the literature (Kurian & al-Nafussi, 1999), and was slightly higher than the averages from other studies (Shin et al., 2002).

Patients diagnosed with mild glandular lesions (GIL1) are on the average 10 years younger than those with the invasive disease. The mean age of AIS patients is about 13 years younger than in those with AI of the cervix. The age differnce between AIS and AI patients suggests the former to be a precursor lesion. It takes about 13 years for the AIS as an adenocarcinoma precursor to progress to AI. Such a long period of carcinogenesis recorded for lesions of endocervical cylindrical epithelium provides opportunities for their early detection and results in the reduction of incidence of AI. Additional support for implicating AIS as precursor of AI comes from several reports which had cytological or histological evidence of AIS appearing 2 - 8 years before detection of the invasive lesions (Boon et al., 1981).

Epidemiological risk factors for cervical adenocarcinoma include those that correlate with the risk of acquiring Human Papillomavirus (HPV) infections, such as multiple sexual partners and engaging in sexual intercourse at an early age. In addition, adenocarcinoma was also found to be associated with obesity and with the prolonged use of oral contraceptives.

Recent trials evaluating the efficacy of virus-like particle vaccines in the prevention of persistent infection with HPV-16 and HPV-18 in young women have been shown to be highly effective.

4. Etiology

In a series of initial cervical swabs, minimal to severe atypias of cylindrical epithelium were detected in 50% of cases with squamous epithelial lesions (Pacey & Ng, 1997), pointing to common etiological factors.

The incidence of coexisting squamous lesions was 74.8% in our study (Ovanin-Rakic, 2010), comparable to the 41 – 76.7% reported in the literature (Im et al.,1995; Shin et al., 2002).

The etiology of squamous cell carcinoma of the cervix, the most common type of cervical malignancy, is linked to infection with oncogenic types of HPV, but the pathogenesis of adenocarcinoma is less well understood. Pirog et al., 2000, detected a very high prevalence of HPV DNA in cervical adenocarcinoma relative to most previous reports. The relative difficulty in detecting HPV DNA in adenocarcinoma, in contrast to squamous cell carcinomas, may be attributed to lower viral load in glandular lesions as compared to squamous lesions. Premalignant and malignant squamous lesions, in particular those associated with HPV 16, contain a large number of episomal viral particles, in addition to integrated HPV sequences (Stoler et al., 1992). Glandular epithelium does not support productive viral infection, and HPV DNA in endocervical neoplasms (notably HPV 18) is usually present in integrated form (Park et al., 1997).

Associations between endocervical glandular atypia (dysplasia) and HPV are more contraversial. In the original study by Tase et al., 1989, only 2 of 36 cases of endocervical dysplasia contained HPV DNA. However, another study (Higgins et al., 1992) reported that 94% were associated with HPV DNA and 75% were associated with HPV 18.

5. Clinical features and management

Most patients diagnosed with GIL are free from clinical symptoms, thus a lesion is detected by cytology on routine swab sampling ("PAP" smears), or by histology (endocervical curettage - ECC, biopsy specimen, conization specimen, loop excision, hysterectomy material) on examination for SIL, or during operative procedure for myoma. (Ovanin-Rakic et al., 2010) In women who are symptomatic, the most common complaint is abnormal vaginal bleeding, either postcoital, postmenopausal, or out of phase. In intraepithelial glandular lesions, the portion is of normal macroscopic appearance and colposcopic images have long been considered nonspecific. However, some authors state that characteristic vascular changes are found in glandular lesions (Singer & Monaghan, 2000). Cytology has a very prominent and responsible role in detection of these lesions.

The anatomical distribution of AIS showed that AIS involved both surface and gland epithelia, a variable number of quadrants, glands beneath the transformation zone in about two thirds of cases, was multifocal only occasionally, and extend up the endocervical canal for a variable distance up to 30mm (Bertrand et al., 1987; Im et al., 1995). Several reports suggest that women of childbearing age may safely be followed after cold-knife conisation with minimal risk provided that the margins are negative. The cone should be cylindrical, encompassing the entire transformation zone if possible, and the sampling depth of endocervical glands should be 5mm from the canal.

It should extend parallel to the endocervical canal for at least 25mm before a 90-degree turn toward the endocervical canal (Bertrand et al., 1987). If the diagnosis is established with a loop excision, even with negative margins, a cold-knife conisation should be performed. After the completion of childbearing, a hysterectomy is recommended because of the paucity of data concerning the long-term history of AIS. In those women for whom childbearing is not important, simple hysterectomy in the face of negative margins is acceptable (Östör et al., 2000; Shin et al., 2002). Some reports indicated that a deep surgical excision with negative margins might be sufficient treatment for some women. (Azodi et al., 1999).

The treatment of glandular leasons is more difficult than that of their squmous counterparts because of the younger age at diagnosis. Managemant of fertility is often an issue, with strong desire for conservation of the uterus. Careful documentation of discussions regarding the risk of conservative management is important as well as documentation of the need for hysterectomy once the childbearing is completed.

6. Cytological features

The interpretation of observed cells requires meticulous scientific training, dedication and experience. Reaching a definitive diagnosis utilizing cells that have desquamated freely from epithelial surfaces or cells that have been forcibly removed from various tissues, demands detailed examination of all available evidence. One of the most important aspects of cytological interpretation is the acquisition of comprehensive knowledge of the normal environment of the tissue to be examined. This knowledge has to take into account the diverse physiological as well as pathological settings that would normally be found in that particular tissue. Without such detailed understanding, the exercise of cytological interpretation can become a trap for a novice. In order to recognize the cytological appearance of endocervical glandular neoplasia with maximal sensitivity and specificity, a solid understanding of normal and variant normal morphology of the cells is necessary.

6.1 Normal columnar cells

The columnar epithelial cells characteristically have basally placed nuclei and tall, uniform, finely granular cytoplasm filled with mucinous droplets. The cells lining the luminal surface have been termed "picket cells" because of their resemblance to a picket fence. It is not known whether regeneration occurs from the underlying subcolumnar reserve cells.

The nuclei of endocervical cells are finely granular and of approximately the **same size as the nuclei of intermediate squamous cells**. The nuclei tend to form dence, dark, nipple-like protrusion that usually appears as a homogeneous extension of the nucleus into the adjacent cytoplasm. The remainder of the nucleus is usually less dense and has a normal appearance. (Boon ME & Gray W, 2003) .

6.1.1 Endocervical reserve cells

Rarely seen, endocervical reserve cells are young, endocervical, parabasal cells in close contact with the basement membrane.

They have multipotential differentiation and may be seen in sheets or in loose clusters of single cells. (Fig.6.1.1.1.) Their cytoplasm is adequate to scanty, cyanophilic and finely

vacuolated. Their round to oval nuclei are centrally located, with fine, uniformly distributed chromatin. Small, round chromocenters are often multiple. Mitoses are occasionally seen and have no significance (Naib, 1996).

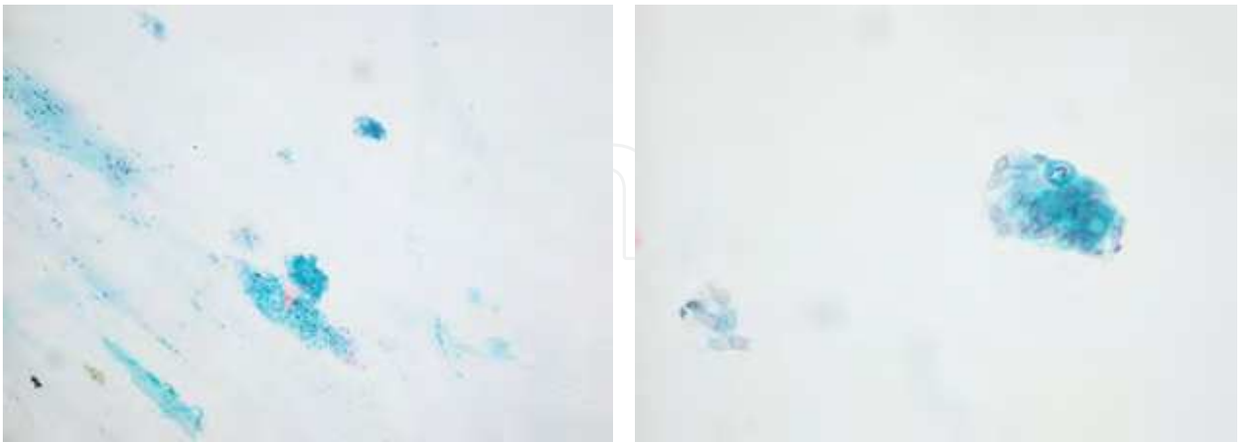

Fig. 6.1.1.1. Loose clusters of normal endocervical reserve sells. The mitotic figure has little diagnostic significance. (Papanicolaou x100, and x400).

6.1.2 Ciliated endocervical cells

Ciliated endocervical cells are the result of direct traumatic exfoliation. They can be single, in tight clusters, in small sheets, or in palisade formations when viewed from the side and honeycomb-like in appearance when their apical ends are in focus. Their size varies, but their shape is fairly constant, cylindrical or pyramidal. When a cell is well preserved, delicate pink cilia are attached to this lavender or red terminal bar or plate. (Fig.6.1.2.1.) This terminal bar can persist even after the cilia have been lost through degeneration. Their length varies according to the original position of the exfoliated cell in relation to the axes of the endocervical canal. The cytoplasm is elongated, with a semitransparent, lacy appearance and cytoplasmic borders that are thin and distinct, in contrast to those found in other types of endocervical cells. They stain darker than the pale mucus-producing endocervical cells. (Naib, 1996; Boon & Gray, 2003).

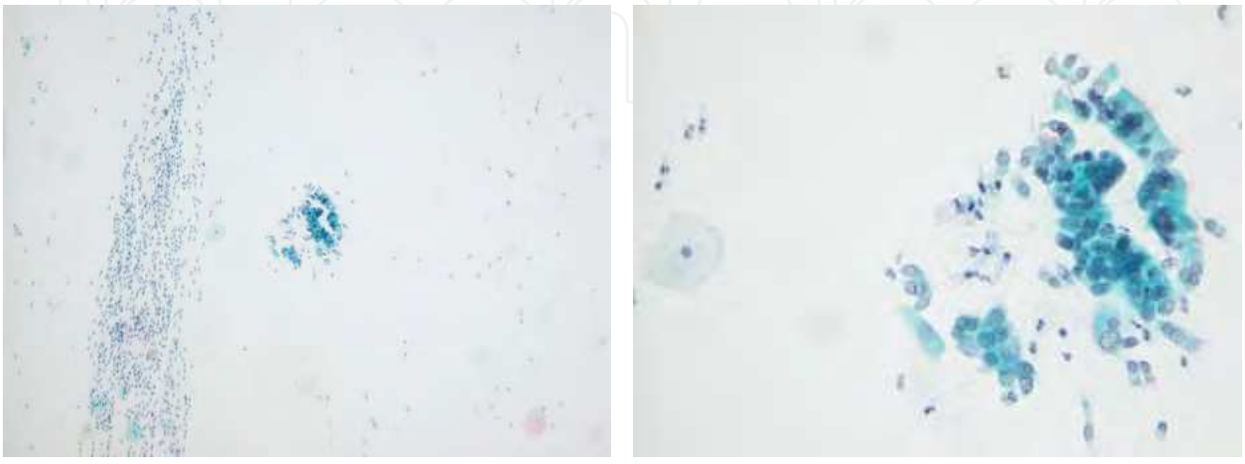

Fig. 6.1.2.1. Ciliated endocervical cells. Note the multincleation (Papanicolaou x100, x400).

Depending on the stage of maturation of the cell and its function, the nuclei are centrally placed or close to the apical cellular end, in contrast to the position of the nuclei in nonciliated cells. These nuclei are round or oval in shape and vary moderately in size. Their chromatin is finely granular and uniformly distributed. The nuclear borders are even and smooth, and they often merge with the cytoplasmic membrane on both sides. When multiple, the nuclei may overlap with little moulding.

Occasionally, nonsecretory cells with cilia are observed, the main function of which appears to relate to the distribution and mobilization of endocervical mucus. Rare, small, dark, nipple like protrusions may be seen in the nuclei of mature or reserve endocervical cells.

Detached ciliary tufts or ciliocytophthoria in cervicovaginal smears, are a very rare event and cannot be correlated with time of cycle or age of patient.(Fig.6.1.2.2.)

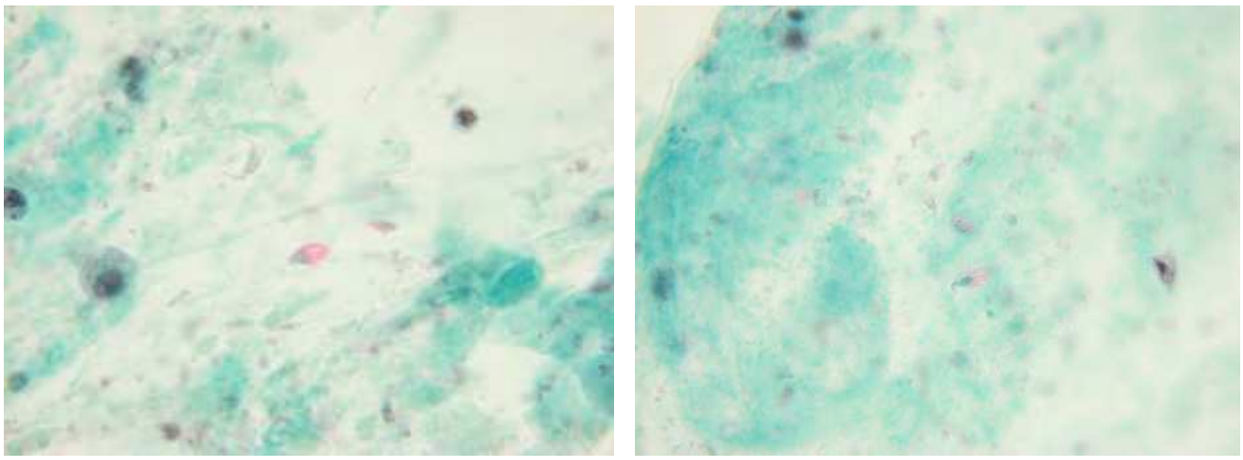

Fig. 6.1.2.2. Ciliocitophthoria. (Papanicolaou x1000).

6.1.3 Nonciliated endocervical cells

Nonciliated endocervical cells occur as single cells, in clusters, or in palisade formations and with a honeycomb-like appearance. (Fig.6.1.3.1.; Fig.6.1.3.2.)

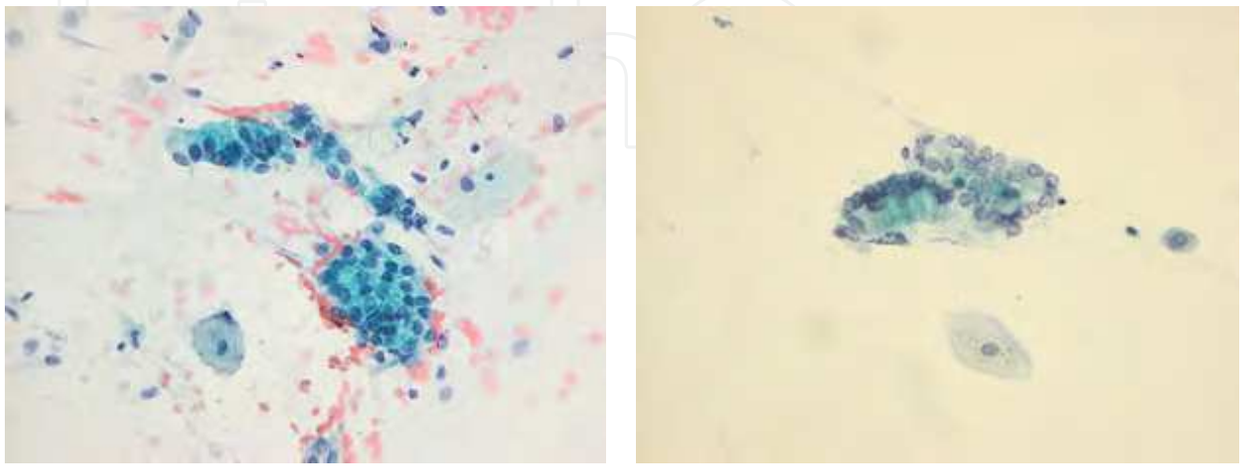

Fig. 6.1.3.1. Group of nonciciliated endocervical cells in sheet, palisade and rosettes. Note the same size nuclei of columnar and intermediate squamous cells. (Papanicolaou x400)

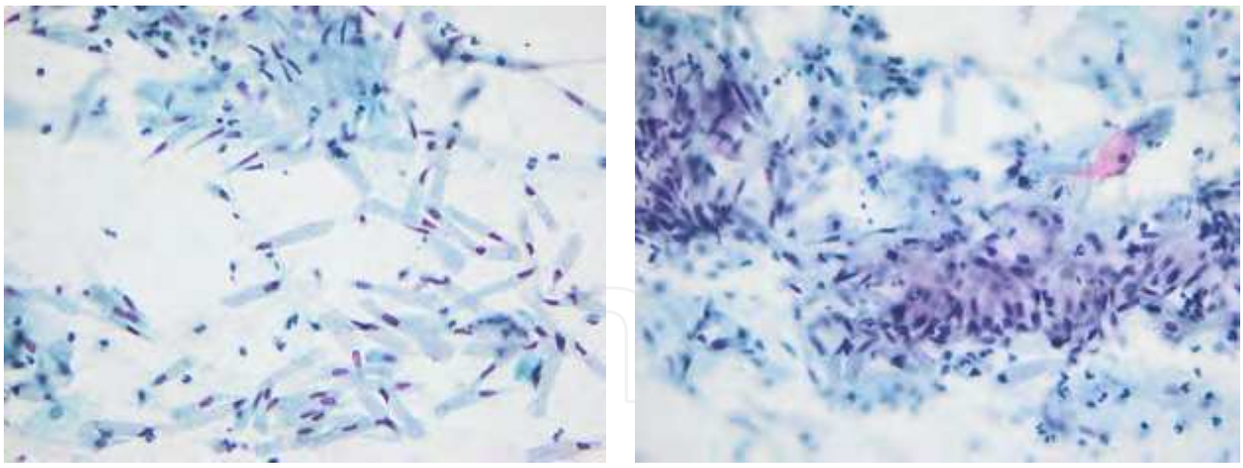

Fig. 6.1.3.2. Nonciliated singly, in cluster and palisade formation; very distended endocervical cells (Papanicolaou x400).

These long, columnar cells vary in size and are uniform in shape and elongated. Their adequate cytoplasm is narrow, and their borders are sharp, smooth, and delicate. The cytoplasm is semitransparent and finely vacuolated, and stains poorly and unevenly as pale blue. In some, fine acidophilic granules can be seen (Naib, 1996; Boon & Gray, 2003)

6.1.4 Secretory endocervical cells

Secretory endocervical cells are found in increased number with chronic irritation, pregnancy, glandular endocervical polyps, or intake of various hormones and contraceptive pills. They vary in size and exfoliate singly or in clusters. (Fig.6.1.4.1). Their shape varies from round to triangular. Their cytoplasm is usually distended by single or multiple small or large secretory vacuoles.

When degenerated, they may contain numerous, large, healthy polymorphonuclear cells. The borders of the cytoplasm are often indistinct, thin, and very delicate. Because of the fragility of the cytoplasm, it is common to find numerous stripped nuclei with only a wisp of transparent cytoplasm still attached in strands of thick cervical mucus in the smear.

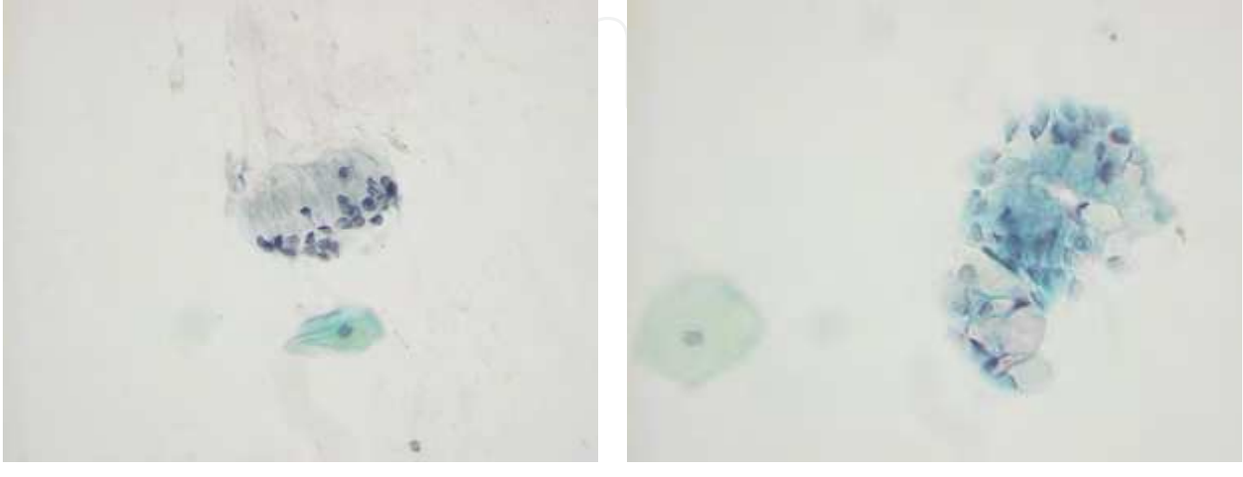

Fig. 6.1.4.1. Secretory endocervical cells in palisade and rosette formation.(Papanicolaou x400)

The nucleoli may be prominent, spherical in shape, and variable in number. Multinucleation is common, especially in cases of hormonal hyperplasia and chronic or acute cervicitis.

The nuclei are often enlarged, oval-to crescent-shaped, and eccentrically situated toward the narrow end of the cell as the result of the cell's displacement by the secretory vacuols.

The size of the nucleus may vary in diameter. The nuclear membrane is often fuzzy. The chromatin is coarsely clumped and has tendency to condense toward the nuclear membrane. Some of the nuclei may, in cases of hyper secretion, appear almost completely pyknotic with an extreme crescent-like shape. (Fig.6.1.4.2.)

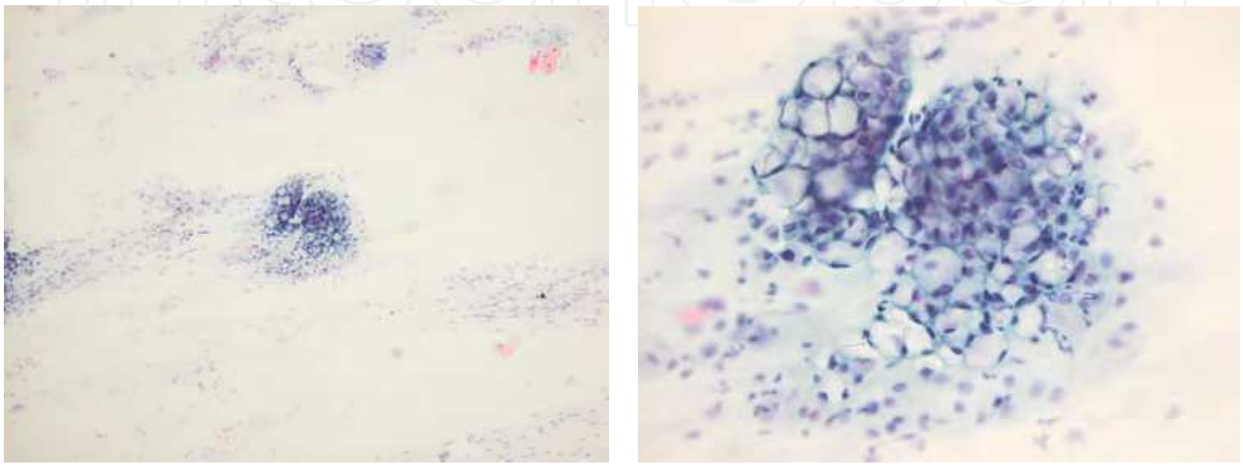

Fig. 6.1.4.2. Groups of secretory endocervical cells. Note the cytoplasmic secretory vacuoles. (Papanicolaou x100, and x400)

6.1.5 Endocervical stripped nuclei

Endocervical stripped nuclei, so-called bare, or naked, which often have a wisp of cytoplasm still attached, are commonly seen in smears from postmenopausal or pregnant women or from women with an endocervical ectropion.

These nuclei are uniformly round or oval but may vary moderately in size. Their nuclear membrane is regular and sharp with a small sign of degeneration. The chromatin pattern is uniform and finely granular with occasional clumping, similar to the normal nucleus of intact endocervical cell. (Fig.6.1.5.1.)

Condensation of the chromatin material toward the nuclear rim, pseudo hyperchromatism, or a clear, bland chromatic pattern may occur as a result of cellular degeneration. Occasional, single, small, central, reddish nucleoli can be seen in better-preserved naked nuclei.

These stripped nuclei should not be confused with poorly differentiated small-cell squamous carcinoma. Both may vary in size, but the shape of the endocervical nuclei is regular, with a smooth nuclear membrane, with chromatin finely granular, and uniformly distributed.

The cytological diagnosis of atypia should never be rendered from stripped nuclei alone.

An examination of better-preserved cells with intact cytoplasm is necessary. (Naib, 1996; Boon & Gray, 2003).

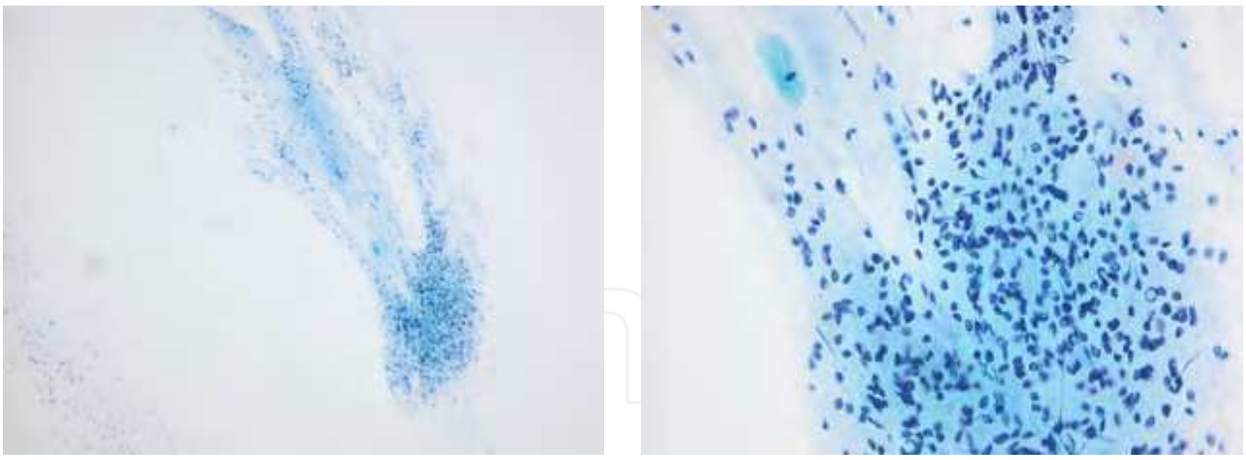

Fig. 6.1.5.1. "Stripped nuclei" of endocervical cells are uniformly round or oval. Note the chromatin pattern is uniform and finely granular similar to normal nucleus of intact endocervical cell. (Papanicolaou x 100, x 400)

6.2 Atypical Glandular Cells (AGC)

The cytologic features of atypical endocervical cells vary depending on the degree of the underlying histopathologic abnormality. The particular feature that may confound interpretation in these specimens is, again the presence of more crowded hyperchromatic groupings and the lack of spreading out that occurs in the making of conventional smears. This can lead to difficulty in identifying key nuclear and cytoplasmic features that could have otherwise made the interpretation more definitive, either toward benign/reactive or neoplastic. (Solomon, 2002; Chieng & Cangiarella, 2003; Waddell, 2003; Willson & Jones, 2004) .

6.2.1 Atypical Glandular Cells (AGC) favouring reactive process

These include endocervical cells from dense two- or three-dimensional aggregates, or sheets and palisades that have minor degrees of nucleolar overlapping. However, the changes may be reactive changes due to inflammation or trauma, as well as reflecting the earliest stages of GIL.(Fig.6.2.1.1.)

There is an increased number of intensely stained endocervical cells. Their abundant cytoplasm is dense, acidophilic, or overdistended by large secretory vacuoles, often containing well-preserved leukocytes or mucus secretions. Their nuclei are enlarged, with a smooth nuclear membrane and coarsely granular chromatin that is uniformly distributed and nucleolar feathering can be seen at the periphery of the cellular aggregates.

There is overlap between the nuclear features which may be seen in extreme inflammatory changes, and those which may be seen in some examples of glandular intraepithelial lesions.

(Fig.6.2.1.2.) They differ by regular distribution of their clumped chromatin and their smooth nuclear membrane. The nucleoli may be prominent, massive, spherical, and usually single, but they may vary in number.

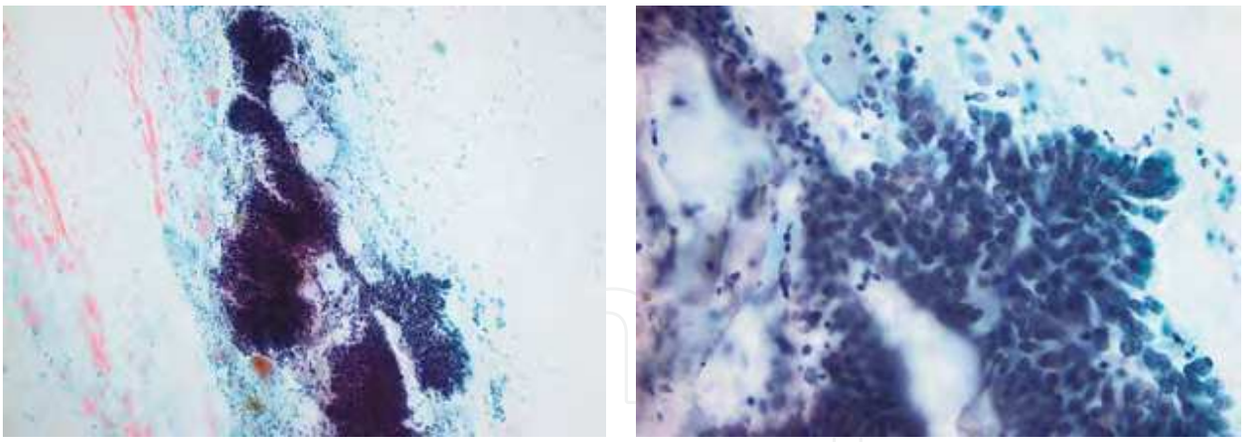

Fig. 6.2.1.1. Crowded sheets of endocervical cells. The nuclei are overlapping and hyperchromatic, but show only a mild variation in size within the sheet.

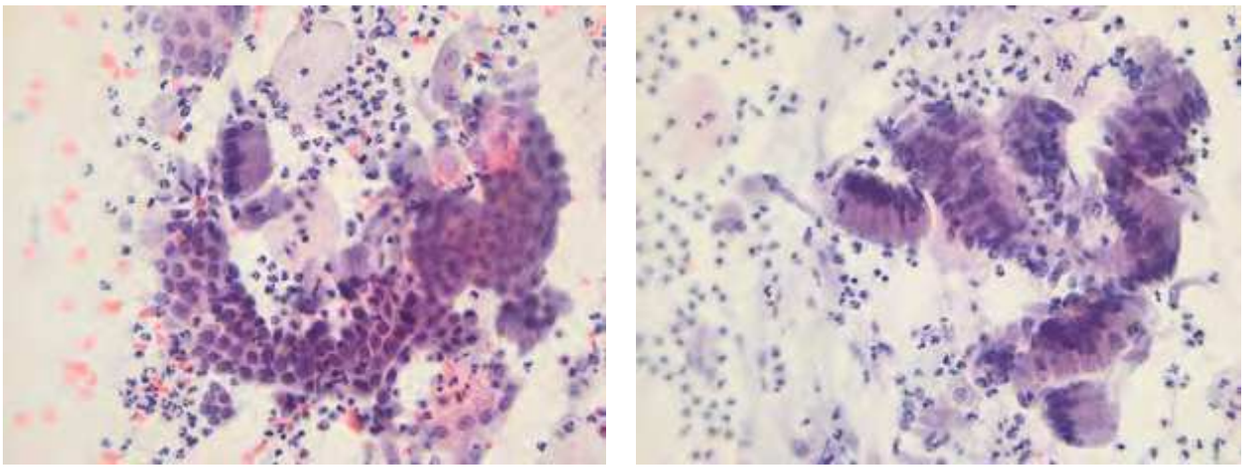

Fig. 6.2.1.2. In sheets and palisades pseudostratification of endocervical cells is present. Nuclei are slightly enlarged. (Papanicolaou x 100, x 400)

6.2.2 Atypical Glandular Cells (AGC), favouring intraepithelial lesions (glandular dysplasia)

Apearance of cytological atypias of the endocervical epithelium falls between those seen in normal glands and in AIS.

Mild (GIL1) and moderate (GIL2) glandular intraepithelial lesions have not been clearly defined, while reproducibility of the cytological and histological criteria for their identification has not been fully explored. Cellular alterations in GIL1 and GIL2 are similar to but less pronounced than those in AIS. The type of desquamation is also similar, except that the cylindrical cells are slightly packed showing a palisading pattern with mild pseudostratification, with less pronounced nuclear overlapping and observable feathering, rosettes, and glandular opening. (Fig.6.2.2.1.; Fig.6.2.2.2.; Fig.6.2.2.3.)

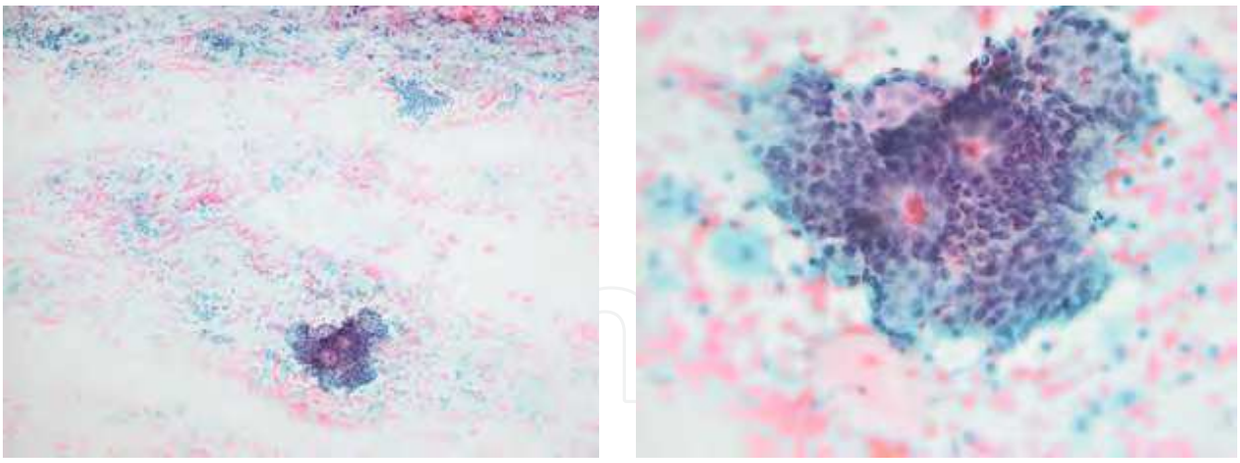

Fig. 6.2.2.1. A cluster of atypical endocervical cells (GIL 1) with glandular opening, with slight nuclear enlargement and overlapping. (Papanicolaou x100, x400)

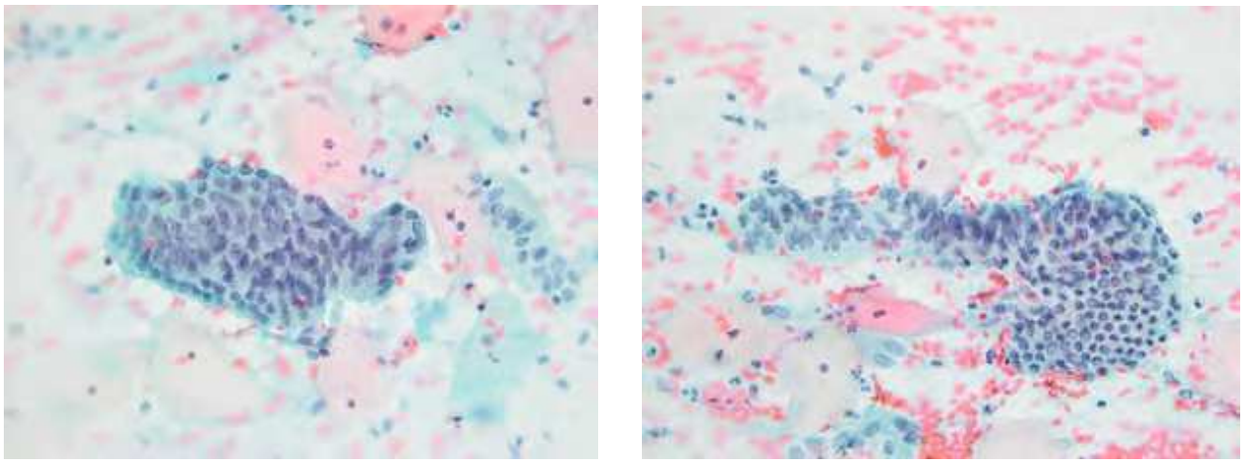

Fig. 6.2.2.2. Sheet of cells (left field) with slight nuclear enlargement and overlapping (GIL1) Note sheet of normal endocervical cells (right field) and palisade with slight nuclear enlargement and pseudostratification (GIL 1). (Papanicolaou x400)

According to the results some studies (Rabelo-Santos et al., 2008), feathering was the best criterion for predicting glandular neoplasia. Feathering was the criterion for distinguishing glandular from squamous neoplasia and also for distinguishing between glandular and . non-neoplastic diagnosis.

Rosettes and pseudostratified strips did not perfom as well. Some rosette formations can be seen in non-neoplastic cases. Squamous neoplasia, especially CIN 3 (cervical intraepithelial neoplasia), is frequently found to have rudimentary gland formation or micro-acinar structures, which can mimic AIS. These facts might help to explain the lower perfomance of the rosette when compared with feathering in the prediction of glandular neoplasia.

The cell size is like that in normal findings or slightly enlarged. **Nuclear size within a cluster varies to a greater extent than in the AIS** (Bousfield et al., 1980). The nucleus is round or oval, hyperchromasia is less pronounced, chromatin is finely granular and evenly distributed, and nucleoli are small and round. Mitoses are rare.

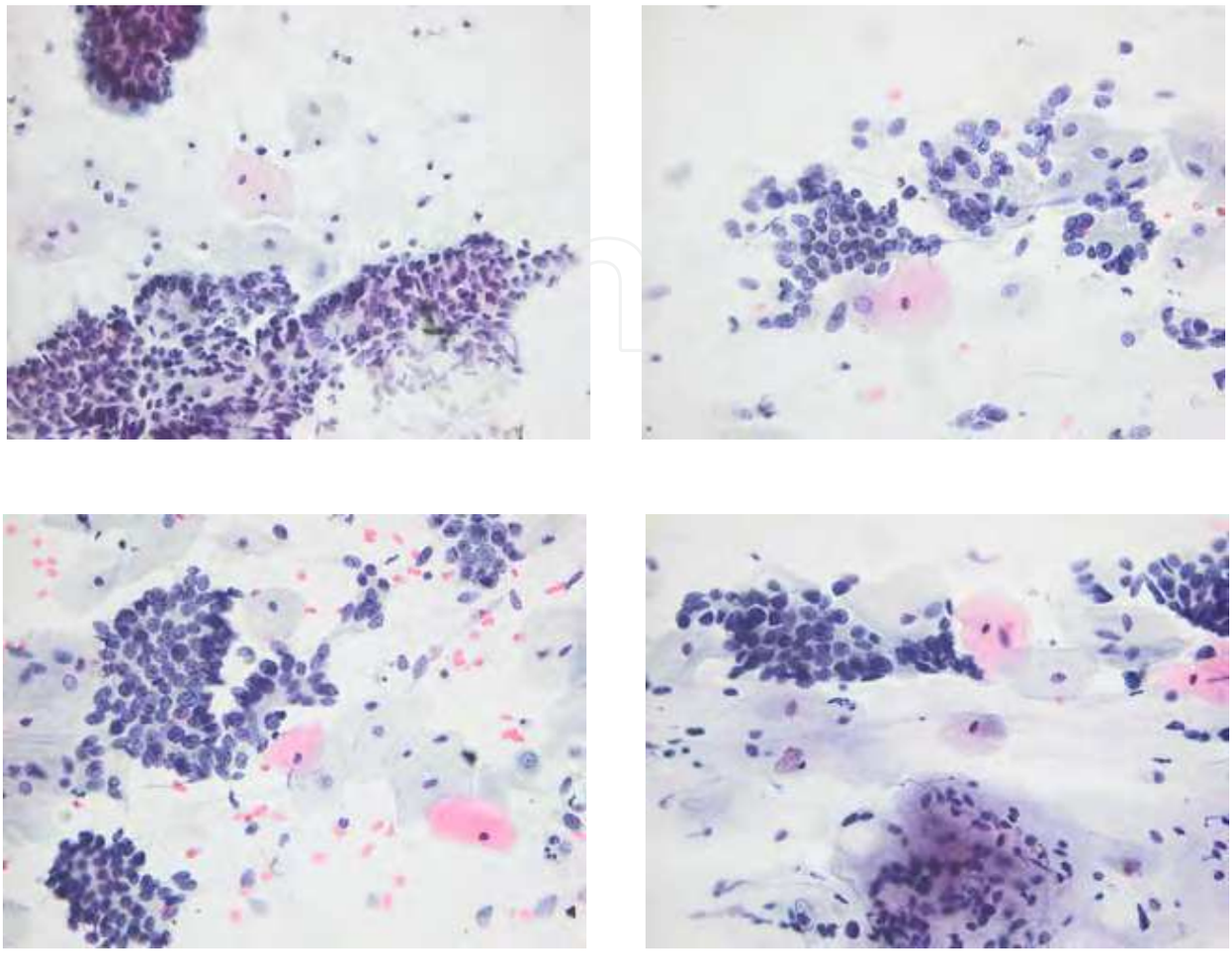

Fig. 6.2.2.3. The cervical smears contains sheets of crowded mild and moderate hyperchromatic endocervical cells with partial rosette and feathering.(GIL1, GIL2) A cluster of atypical cells (down right field) compared with normal cells in the same fields. (Papanicolaou x400).

Recognition of the characteristic architectural features in cell groups is very important in diagnosis. Without obvious and unequivocal nuclear change in endocervical cells, cytological diagnosis of GIL should not be made in the absence of these architectural features. Three-dimensional cell groups with disorderly cell arrangements, coarse grainy chromatin, and hyperchromasia with intercellular variation in nuclear staining intensity may be seen. None of the architectural abnormalities characteristic of GIL is present. . (Bousfield et al., 1980; Gloor & Hurlimann, 1986; vanAspert-van Erp et al., 1995; diTomassso et al., 1996; Golstein et al., 1998; Zaino, 2000).

Examples of abnormalities can usually be seen repeatedly in abnormal cellular material. This means that if the cellular material in question is scanty in a smear, a confident diagnosis of GIL may not be possible

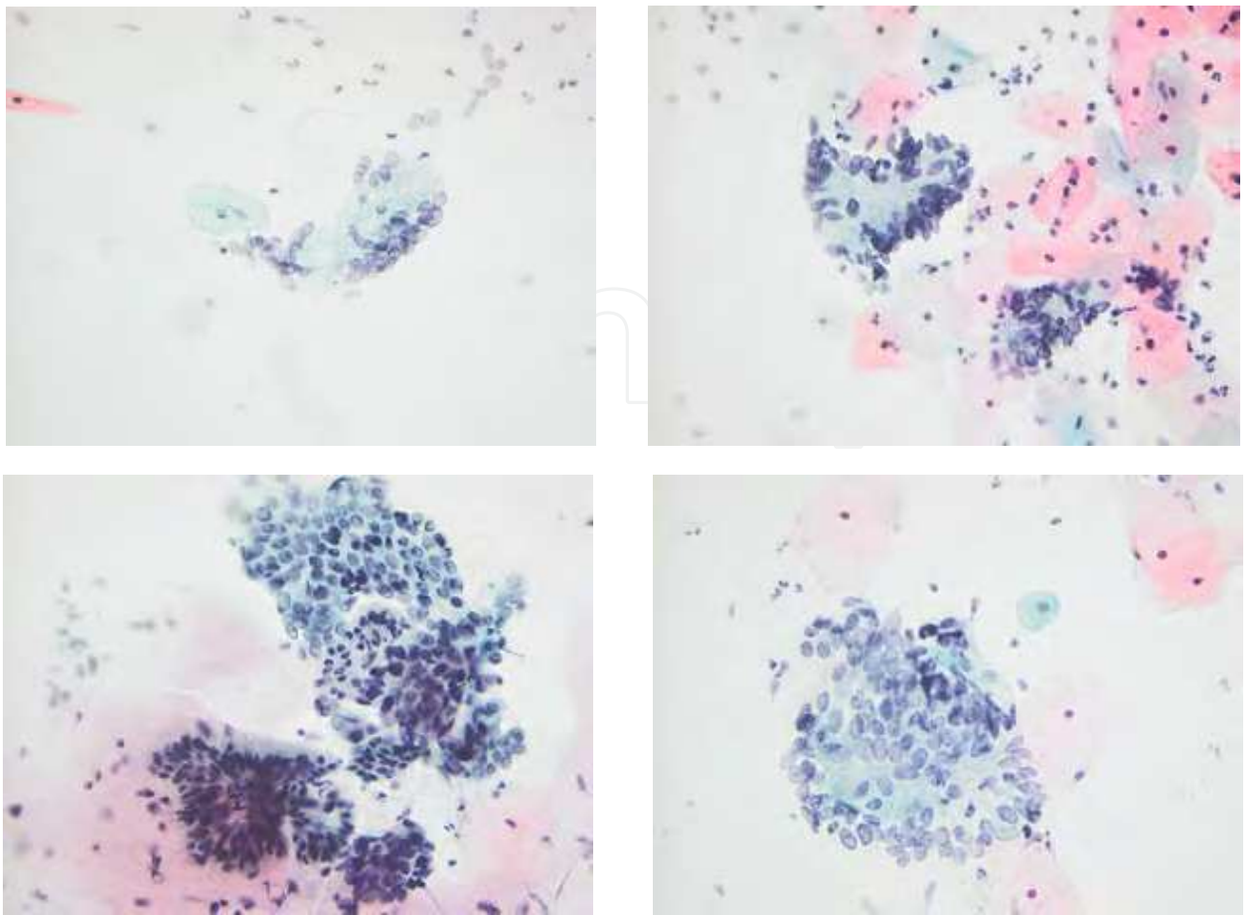

Fig. 6.2.6. The cervical smears (all four fields) contain sheets of moderate crowded endocervical cells with rosettes, partial rosettes and feathering with enlarged hyperchromatic nuclei. (GIL 2) A cluster of atypical cells compared with normal cells (pseudorosette and sheet) in the same fields (down left). Note the different nuclear size within a cluster (Papanicolaou x400).

6.2.3 Atypical Glandular Cells (AGC), favouring invasive lesions

The number of exfoliated diagnostic cells in smears varies according to the site, type, and size of the tumor and the technique used. Although generally larger than normal, the tumor cells, with few exceptions, imitate the appearance of the benign columnar cells from which they originate. In loose clusters or tight three-dimensional formations, abnormal degenerating columnar cells are identified in the "dirty" background of fresh and old degenerating blood cells, and cellular debris, consistent with the tumour diathesis.

The nuclei are enlarged, oval or round with eosinophilic granular cytoplasm. There is considerable nuclear overlapping and pleomorphism, and the chromatin pattern is coarsely granular, irregularly distributed and nucleoli are identifiable.

Unlike adenocarcinoma of the endometrium, the cells retain, especially at the periphery of clusters, their columnar configuration. A definitive cytological diagnosis cannot be made, mostly due to **poor specimen preparation.** (Fig.6.2.3.1.). Mitotic figures are occasionally seen.

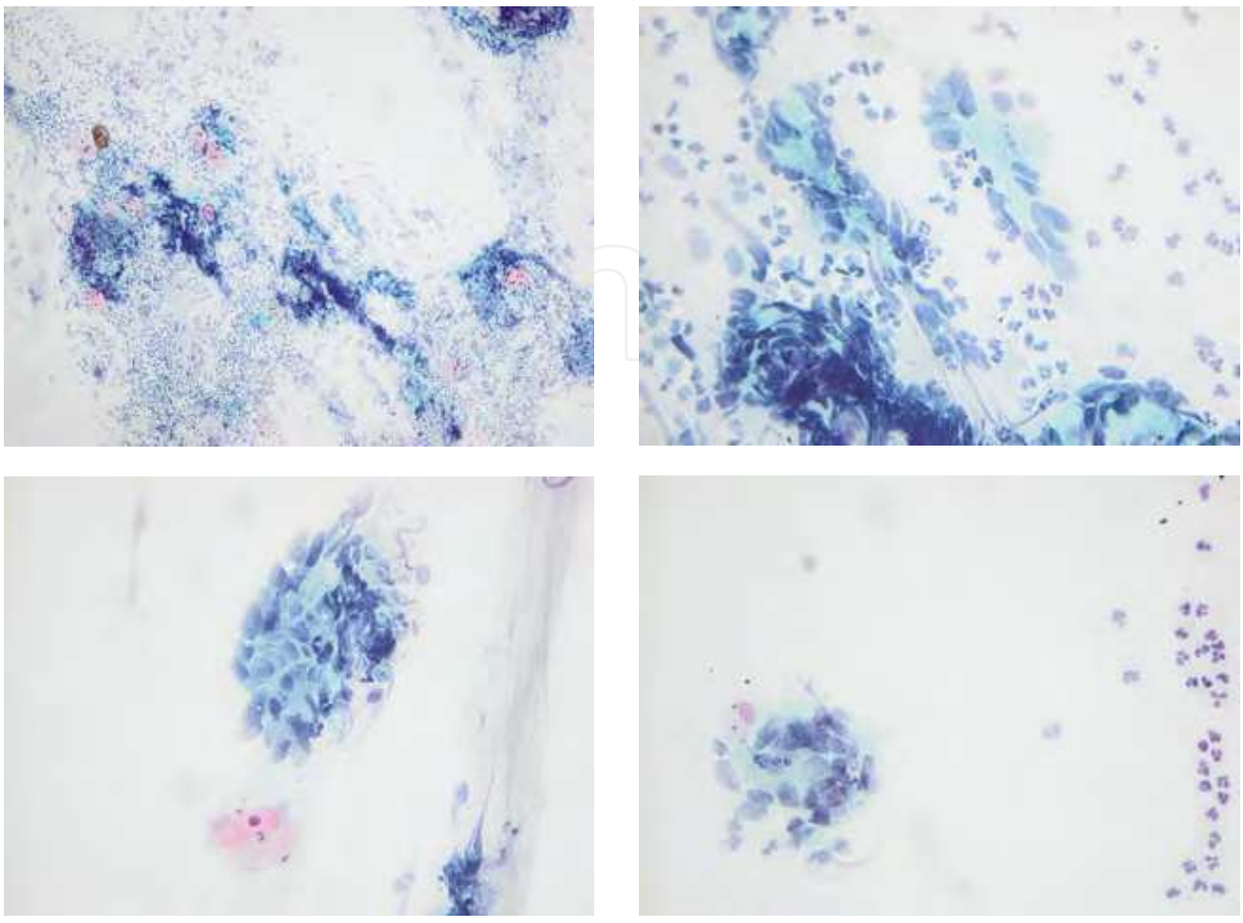

Fig. 6.2.3.1. Crowded and loose clusters, end rosettes of markedly atypical and degenerating endocervical cells. The nuclei are large, show irregular contours and coarse chromatin. Note the poor specimen preparation (Papanicolaou x100, x400 x400 x400).

6.3 Adenocarcinoma in situ

The cytomorphological criteria for diagnosis of AIS refers to changes in architectural features (sheet of cells, "strips", "rosettes", gland opening, "feathering"), and in the cells themselves. The cell size is uniform and enlarged. The cytoplasm is cyanophilic and occasionally vacuolated.

Examination of the sheets of cells does not reveal the typical honeycomb formation of normal endocervical epithelium due to crowding and overlapping of the nuclei. (Krumins et al., 1977; Bousfield et al., 1980; Gloor & Hurlimann, 1986; Betstil & Clark, 1987; Ayer et al., 1987; Pacey & Ng, 1997;Biscotti et al., 1997; Waddell,2003).

The columnar origin of cells can be recognized when lacunae, corresponding to glandular orifices, are present. At the edge of the sheets of cells, pseudo stratification of the nuclei may be observed. The glandular cells at the edge of a sheet are oriented with their long axis perpendicular to the edge. Some nuclei may have lost their surrounding cytoplasm and form irregular margins, resembling feathers at the edge of a bird's wing.

The smallest fragment is the case for 'strips' containing cells arranged in parallel with pseudo stratified nuclei and for 'rosettes', small round groups of cells with peripheral nuclei.

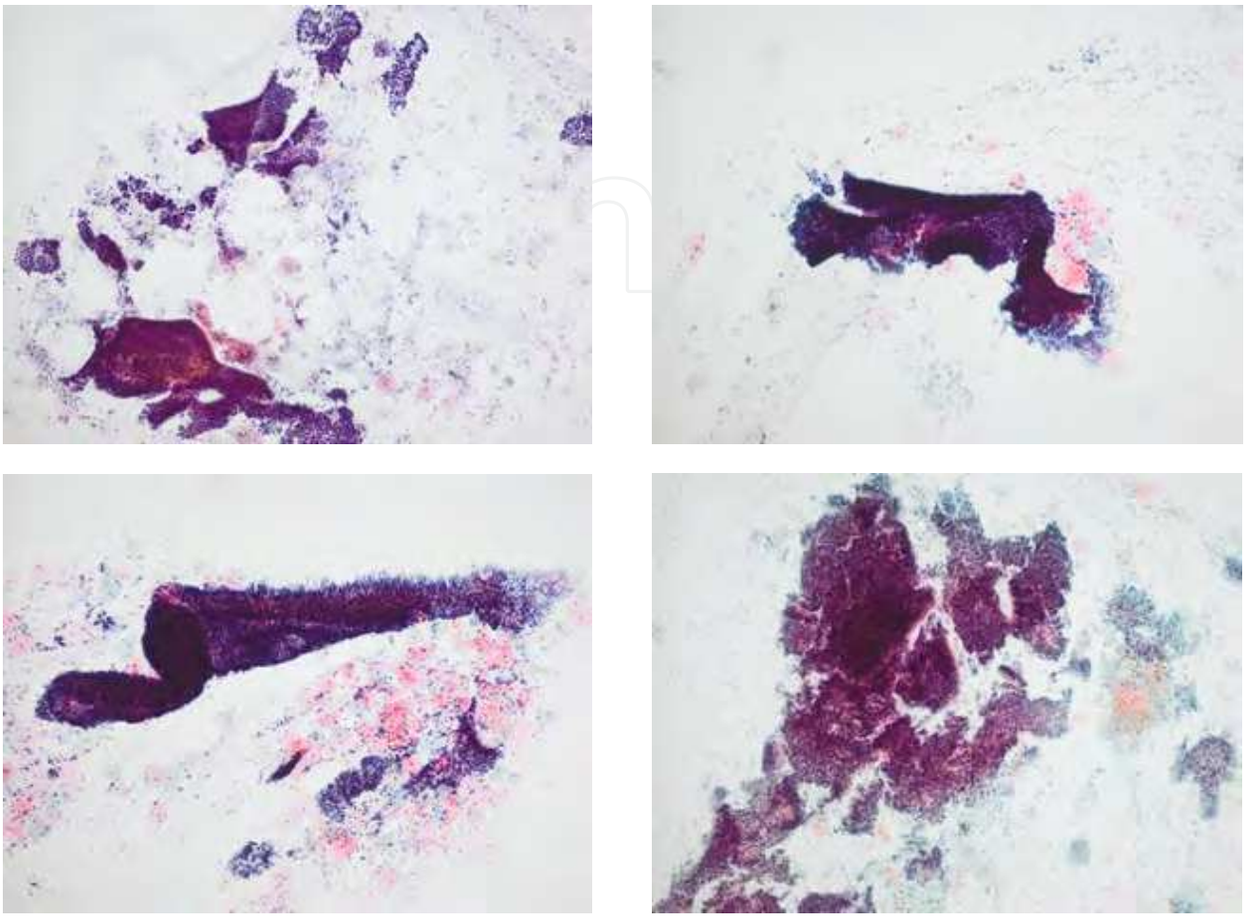

Fig. 6.3.1. AIS. Tightly crowded sheets, strips, rosettes, palisade, gland opening, feathering, of the malignant endocervical cells. The cell size is uniform and enlarged. Note crowding and overlapping of the nuclei. [Papanicolaou x 100 (all four fields)]

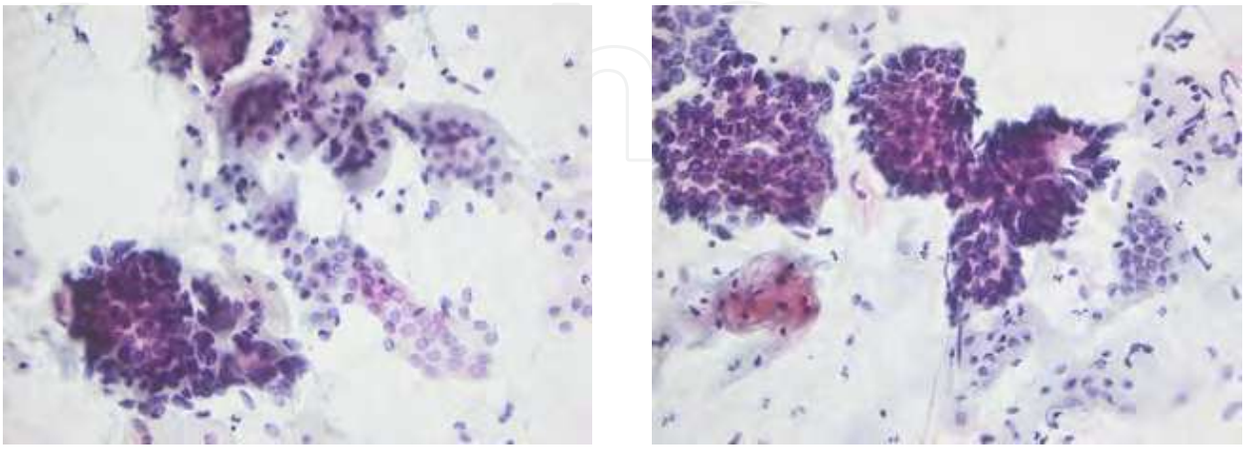

Fig. 6.3.2. AIS. Clusters of uniform small dark neoplastic cells and sheets of normal endocervical cells on the same field. (Papanicolaou x400)

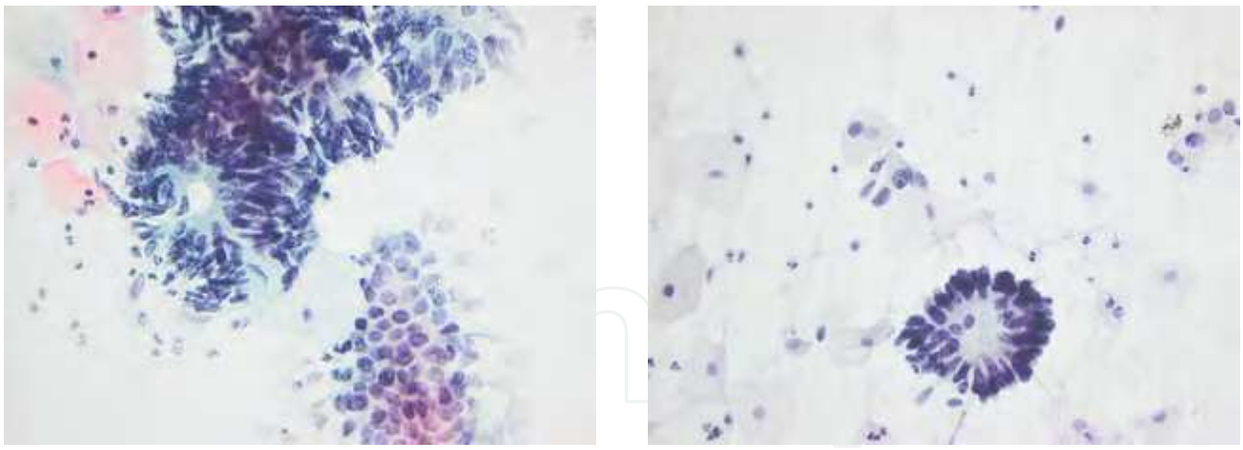

Fig. 6.3.3. AIS. Crowded sheet with "gland opening" and "rosette" of neoplastic cells. The nuclei are elongated, cigar-shaped, and hyperchromatic. Note a sheet of endocervical cells (left field) with slight nuclear enlargement and overlapping (GIL 1). (Papanicolaou x400)

The distinction between well differentiated and poorly differentiated AIS is based on nuclear features. In cell groups, the nuclei of cells of well-differentiated AIS are enlarged, oval or round, uniform, and have a regular nuclear membrane. When the cells are crowded the nuclei may be elongated, cigar-shaped, and hyper chromatic.

The chromatin is granular and evenly distributed. The nuclei in a portion of cells contain small nucleoli. Mitotic figures and apoptotic bodies are occasionally present.

Poorly differentiated AIS occurs less frequently then the well-differentiated type. (Fig.6.3.4.)

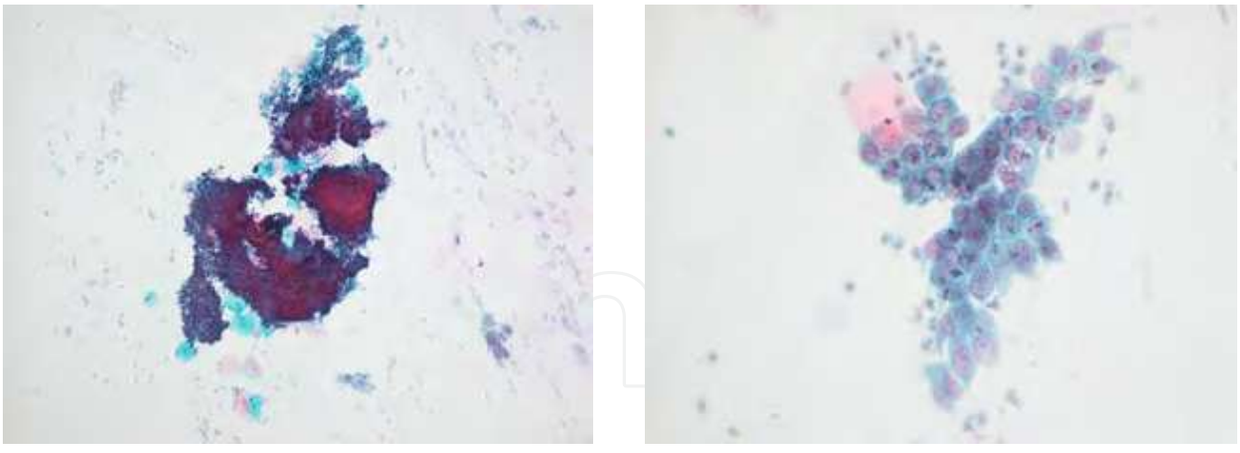

Fig. 6.3.4 Poorly differentiated AIS. The nuclei of the cells are round or irregular in shape and greatly enlarged, but less hyperchromatic and with finely granular chromatin. Nucleoli are multiple, irregular and enlarged. (Papanicolaou x100, x400).

In comparison to the cytological features of well-differentiated AIS, the nuclei of these cells are larger, but less hyperchromatic and with finely granular chromatin. The nuclei may be round and always contain nucleoli which may be multiple, irregular and/or enlarged. Mitotic figures can be seen. (Ayer et al., 1987; Pacey & Ng, 1997).

Although most endocervical adenocarcinoma in situ are of the usual 'endocervical' type, it is important to recognize that other variants sometimes occur. These include endometrioid, serous, and intestinal variants.

Of these, the most significant diagnostic variant is the endometrioid pattern (Lee, 1999). This pattern contains small cells in densely packed groups, having coarse nuclear chromatin exhibiting lack of pleomorphism (Fig.6.3.5.).

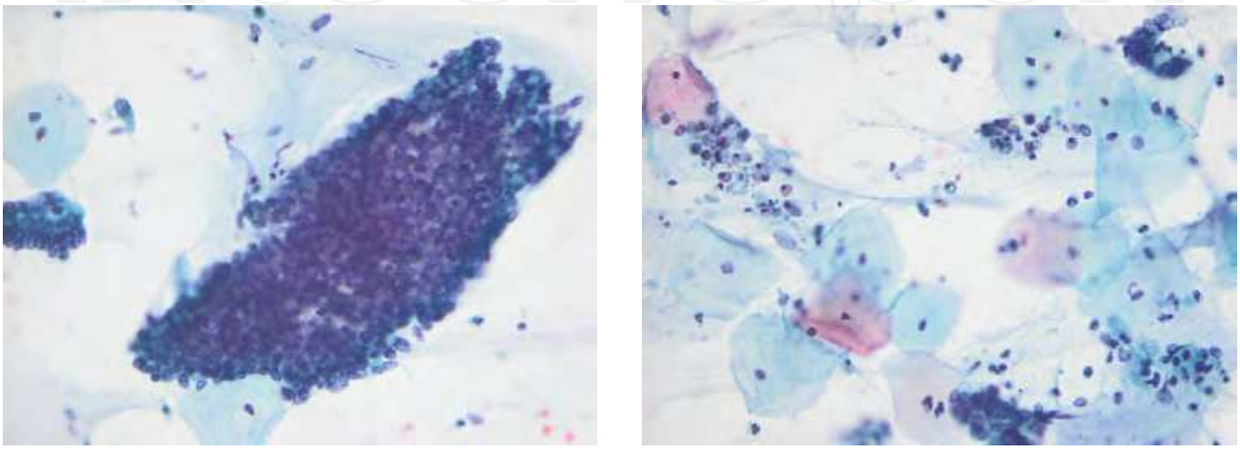

Fig. 6.3.5. AIS, endometrioid pattern, contains small cells in densely packet groups, having corse nuclear chromatin, exhibiting lack of pleomorphisam. Note apoptotic bodies on the right field (Papanicolaou x400).

These groups were more commonly misinterpreted as being of benign endometrial or endocervical origin (tubal metaplasia endometrioid variants). Criteria were developed to identify these cases as abnormal, at least to the level of atypical glandular cells: the absence of endometrial stromal cells and endometrial-like tubules, coarse chromatin patterns, extreme nuclear crowding, mitotic figures, and marginal feathering.

Key features of endocervical adenocarcinoma in situ are: hyperchromatic crowded groupings of cells, pseudostratified strips of columnar cells, epithelial rosettes, gland opening, nuclear and cytoplasmic 'feathering', twofold larger than normal nuclear size, endocervical nuclei, beyond normal increase of nucleus to cytoplasmic ratio, endocervical cells, coarsely granular and evenly distributed hyperchromatic chromatin, possible presence of small nucleoli, presence of mitotic figures and apoptotic bodies not associated with a background tumor diathesis

In a significant number of cases, abnormal squamous cells are present in association. (Fig.6.3.6) Focusing on these more commonly seen lesions can lead to a lack of identification of extant abnormal glandular processes. Careful observation and analysis of cervical cell samples should be exercised to identify these cells. Single malignant columnar cells may be mistaken as undifferentiated basal cells.

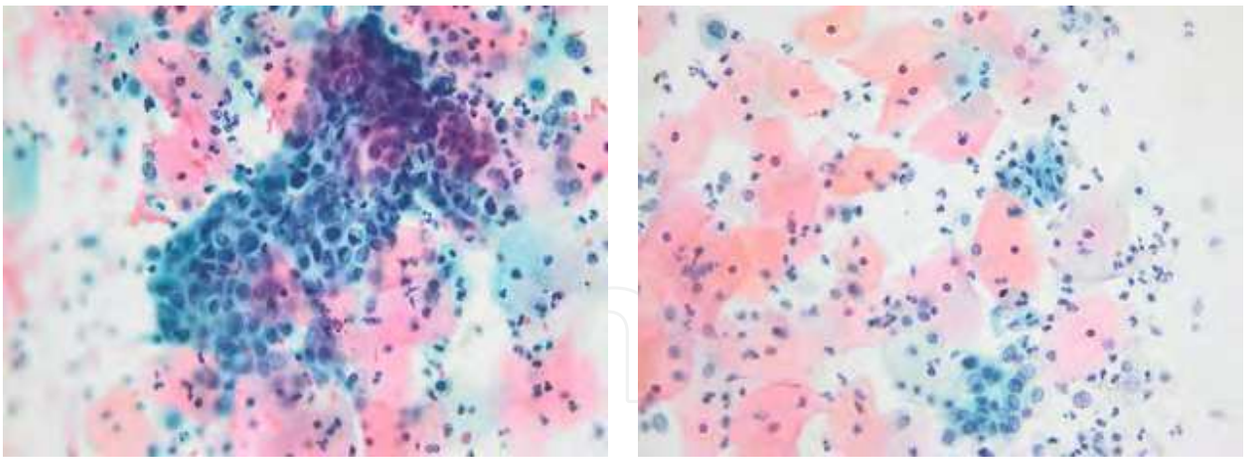

Fig. 6.3.6 CIS. A cluster of malignant squamous cells is present in association with small strip and single abnormal glandular cells (leading to lack of identification) (Papanicolaou x400).

7. Accuracy of cervical cytology

Intraepithelial lesions of the endocervical epithelium are more difficult to detect by cytology. However, cellular changes may frequently be less pronounced than those in squamous lesions and are difficult to observe unless architectural alterations call for attention. In mixed lesions, the glandular component may be eclipsed in abnormal cell count and intensity by the squamous component. (Boon et al., 1981; Di Tomasso et al., 1996).

In order to reach as accurate and precise a cytological diagnosis of intraepithelial lesions of endocervical cylindrical epithelium as possible, the cytological findings of patients with histologically verified adenocarcinoma in situ and mild to moderate glandular intraepithelial lesions were analyzed.(Ovanin-Rakic et al., 2010)

During the period 1993-2007, the value of cytology in the detection and differential diagnosis considering lesion severity and/or type of altered epithelium was assessed in 123 patients with a definite histological diagnosis of glandular lesions (AIS – n=13; GIL1 – n=11; GIL 2 – n7), glandular lesion associated with a squamous component (AIS+CIN/CI – n=58; GIL 1/GIL 2+CIN – n=28; GIL + MIC - n=6) (Table 2.).

Intraepithelial endocervical cylindrical lesions, with or without intraepithelial or invasive squamous component, were diagnosed in histological samples (78 biopsy specimens, 82 excochleation specimens, 70 conization specimens and 24 hysterectomy materials) from 123 patients aged 22-73 (mean 40).

The patients were divided into two categories : the first including 71 patients who were histologically diagnosed as AIS or AIS + CIN/CI, while the second included 52 patients who were histologically diagnosed as mild or moderate glandular intraepithelial lesions with squamous component (GIL1/ GIL2 + CIN, GIL + MIC) or without it (GIL1, GIL 2) .

In the first group, (table 2) cytological findings indicated epithelial abnormalities in 98.6% (70/71) patients. Considering lesion severity, the cytological and histological diagnoses were identical in 93% (66/71) patients.

Cytology	n	Histology			
		AIS	AIS + CIN	AIS + CI	AIS+CIN/CI
		n %	n	n	n %
AIS	9	**8 61,5**	1		1 1,7
AIS + CIN	15	1 7,7	**12**	2	14 24,2
AC/Abnormal	6	3 23,1	3		3 5,2
AI + CIN	2		2		2 3,4
AI + CI	4		3	1	4 6,9
GIL + CIN	9		8	1	9 15,6
CIN	21		20	1	21 36,2
MIC	2		1	1	2 3,4
Abnormal	2		2		2 3,4
Inflammation	1	1 7,7			
Total	71	13 (100,0)	52	6	58 100,0
%	100,0	18,3		81,7	

Table 2. Cytohistologic correlation of either pure adenocarcinoma in situ (AIS) or a mixed AIS/squamous abnormality

The accuracy of cytological diagnosis according to lesion severity and type of epithelium was 92.3% (12/13) for glandular lesions and 56.9% (33/58) for mixed lesions.

In predicting the type of epithelium involved, the agreement between cytological and histological diagnosis was recorded in 61.5% (8/13) of histologically pure (AIS) and 20.7% (12/58) of mixed lesions (AIS + CIN / CI).

The accuracy of cytological identification of abnormalities of a particular type of epithelium, histologically diagnosed as either pure or mixed lesions, was 92.3% (12/13) and 96.6% (56/58) for cylindrical and squamous epithelium.

In the second group, (table 3), cytological findings indicated epithelial abnormality in 90.4% (47/52) patients. Considering lesion severity, the cytological and histological diagnoses were identical in 80.8% (42/52) patients.

The accuracy of cytologic diagnosis according to lesion severity and type of epithelium was 61.1% (11/18) for glandular lesions and 35.3% (12/34) for mixed lesions.

In predicting the type of epithelium involved, the agreement between the cytological and histological diagnosis was recorded in 22.2% (4/18) of histologically pure (GIL I) and 20.6% (7/34) of mixed lesions (GIL1,2 + CIN / MIC).

The accuracy of cytologic diagnosis according to lesion severity and type of epithelium was 61.1% (11/18) for glandular lesions and 35.3% (12/34) for mixed lesions.

In predicting the type of epithelium involved, the agreement between the cytological and histological diagnosis was recorded in 22.2% (4/18) of histologically pure (GIL I) and 20.6% (7/34) of mixed lesions (GIL1,2 + CIN / MIC).

Cytology	n	Histology							
		GIL I	GIL II	Total		GIL I + CIN	GIL II + CIN	GIL + MIC	Total	
		n	n	n	%	n	n	n	n	%
GIL I	4	**4**		4	22,2					
GIL I + CIN	8	2	2	4	22,2	3		1	4	11,8
GIL II+ CIN	5	1		1	5,6	1	**3**		4	1,8
AIS + CIN	4		2	2	11,1	1	1		2	5,9
GIL + MIC	2					1		**1**	2	5,9
CIN	24	1	1	2	11,1	8	10	4	22	64,6
Inflammation	5	3	2	5	27,8					
Total	52	11	7	18	100,0	14	14	6	34	100,0
%	100,0	34,6				65,4				

Table 3. Cytohistologic correlation of either pure gladular ysplasia (GIL) or a mixed GIL/squamous sbnormality

The rate of cytological identification of abnormalities of a particular type of epithelium, histologically diagnosed as either pure or mixed lesions, was 61.1% (11/18) and 100% (34/34) for cylindrical and squamous epithelium, respectively.

However, the fact that AIS patients are older than women with squamous CIS (Brown & Wells, 1986) and that the reverse is true for AI and CI could imply that the progression of GIL to AIS must be slower than the progression of CIN lesions to CIS.

In contrast, AIS should progress to AI significantly more rapidly than does CIS into CI That , indeed, seems to be the case. This would leave ample time for detection of glandular dysplasia, but not necessarily AIS (Plaxe & Saltzstein, 1999).

A coexisting SIL may obscure the presence of glandular lesion because abnormalities involving exclusively squamous components were quite frequently observed in the latter, either because of more distinct criteria and easier recognition, or due to more pronounced cellular lesions, or because of the predominant population of abnormal squamous cells, especially when extensive or high grade.

Historically, only sporadic cases of AIS were reported after it was first defined in 1953 by Friedell & McKay, 1953, who described only its histological appearance.

In the 1970s and 1980s, descriptive studies detailing the cytological criteria necessary for the prospective cytological diagnosis of AIS of the cervix uteri were published, increasing awareness and the diagnostic skill of cytologists.

For the cytologist intraepithelial glandular lesions pose possibly the greatest challenge in cervical sreening.

In a number of cases published over the period of 15 years, Papanicolaou smear screening detected a glandular abnormality before confirmation of AIS on cone biopsy or

hysterectomy in 32- 79% cases. (Ayer et al., 1987; Azodi et al., 1999; Östör et al., 2000; Shin et al., 2002; Ovanin-Rakic et al., 2010).

Our observation has been that the number of AIS cases we identified has increased with time after our first identification in 1986.

The Papanicolaou smear in our patients had a sensitivity of 74.2% in detecting a glandular abnormality preoperatively. The cytological differential diagnosis of AIS showed a 61.5% and of GIL 1 22.2% accuracy. These results are similar to other reports (Ayer et al., 1987; Ioffe et al., 2003). Ioffe et al., 2003 have shown that the application of a semiquantitative system for the diagnosis of noninvasive endocervical glandular lesions results in better diagnostic reproducibility even in diagnostically problematic cases. Papanicolaou smear that includes adequate material from the transformation zone and endocervix can be a useful method for detecting precursor lesions of adenocarcinoma of the cervix. It bears remembering that cytology should not be recommended as the definitive diagnostic investigation for adenocarcinoma of the cervix uteri. If a clinician is suspicious of cancer during clinical examination, then he or she should proceed to colposcopy and biopsy regardless of the cytologic findings (Pacey & Ng, 1997).

8. Differential diagnosis

Cytological analysis of glandular lesion abnormalities in vaginal-cervical-endocervical (VCE) smears is associated with a number of diagnostic difficulties. Interpretation of the results must be based on scientific knowledge, meticulous training and experience, and demands dedication. To reach a definitive diagnosis utilizing cells that have desquamated freely from epithelial surfaces or cells that have been forcibly removed from various tissues, requires detailed examination of all available evidence. In this case, consideration must be given to both procedural aspects of the cytology laboratory and to changes that modify individual cells or cell groups.

There is overlap between the cytological criteria for various glandular lesions of the cervix, thus requiring more rigorous criteria for defining both benign and malignant cervical glandular lesions.

The emphasis is on criteria that discriminate among non-neoplastic conditions, benign neoplasms that may mimic malignancy, and malignant neoplasms that may pose as benign entities. Appropriate clinical data are certainly of great help in solving some of these diagnostic issues. Awarwness of cellular changes, together with pertinent clinical information , will prevent diagnostic errors.

8.1 Non-neoplastic lesions

8.1.1 Inflammatory changes in endocervical cells

Endocervical cells are usually present in small clusters, sheets, strips and pseudorosettes (small round groups of cells with peripheral cytoplasm), with minimal nuclear overlapping.

Hyperchromasia and mild anisonucleosis may be present in round or oval nuclei. Chromatin is finely stippled and may be smudged in the texture. Reactive/reparative atypical glandular cells are uniform in size and shape with small to medium-sized regular nucleoli and abundant cytoplasm. They are typically arranged in groups, rather than singly.

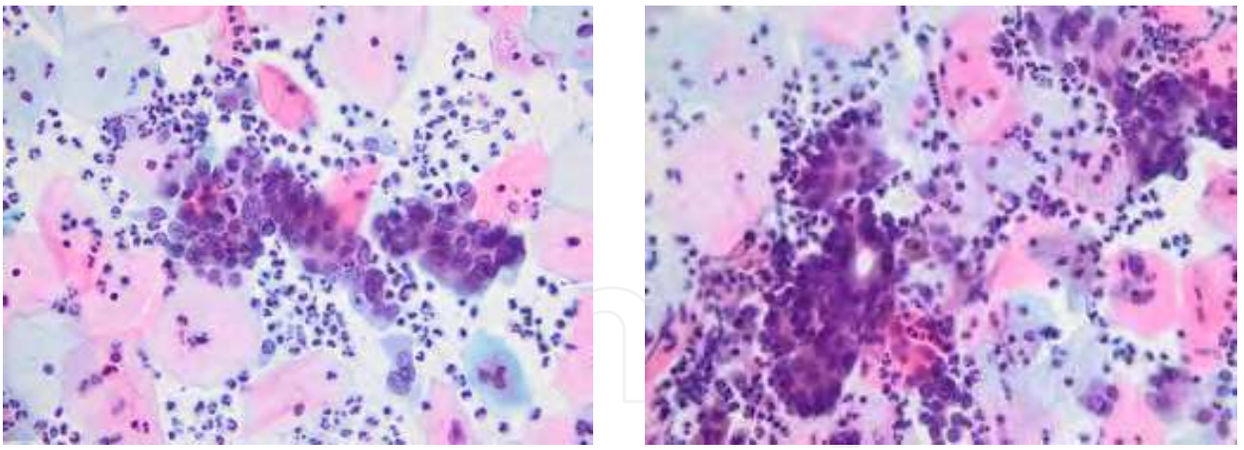

Fig. 8.1.1.1. A sheet of rective endocervical cells infiltrated by neutrophils with enlarged nuclei. Note a mitotic figure (left field) and gland opening (right field). These cells simulate glandular displasia.

The nucleoli are prominent, massive, spheroidal, and usually single, but they may vary in number. The cells can be multinucleated, variable in their size and shape, moulding, or overlapping each other, with very occasional mitoses seen in regenerating epithelial cells. There is a danger of mistaking these cells for endocervical adenocarcinoma cells. They differ by the regular distribution of their clumped chromatin and their smooth nuclear membrane. The most important feature which distinguishes sheets and clusters of endocervical cells with inflammatory changes from those of GIL is the exfoliation pattern. Nuclear stratification and feathering at the edge of sheets are features of GIL, which are rarely present in inflammatory smears. However, the cells seen in reactive conditions are usually monolayered with abundant cytoplasm. There is no stratification or 'feathering' of nuclei. Cells with marked nuclear enlargement, hyperchromasia and prominent nucleoli may be seen in polyps.

During pregnancy or the postpartum period, as a result of acute or chronic irritation, groups of endocervical cells can become considerably larger, with monstrous nuclei. They can be confused with anaplastic malignant cells, except for the persisting regularity of their smooth nuclear membrane and the abundance of their benign-appearing cytoplasm.

It is important to obtain clinical information in these situations and appraise cytological criteria for AIS with care (Naib, 1996; Pacey & Ng, 1997; Waddell, 2003).

8.1.2 Atypical repair

Reactive changes in epithelial cells are well described and generally well recognized as such by cytologists. Under some circumstances cells react to some injury of the epithelium. This condition of extreme reactivity, also known as atypical epithelial repair, can be problematic.

The cells seen in this condition may mimic a glandular abnormality, specifically invasive adenocarcinoma of the endocervix. (Fig. 8.1.2.1.) Cytoplasmic boundaries are well-defined and can clearly be seen in the overlapping or syncytial appearance of the groups noted in many neoplastic processes. Nuclei may be large with coarse chromatin and regular macro nucleoli are noted in virtually all nuclei. When repair becomes atypical, the nuclei begin to

show variable degrees of pleomorphism of size and shape within the groups, often taking on nuclear contour irregularities. Chromatin patterns can turn from uniformly distributed to irregular and show coarse granularity. (Naib, 1996; Pacey & Ng, 1997; Waddell, 2003).

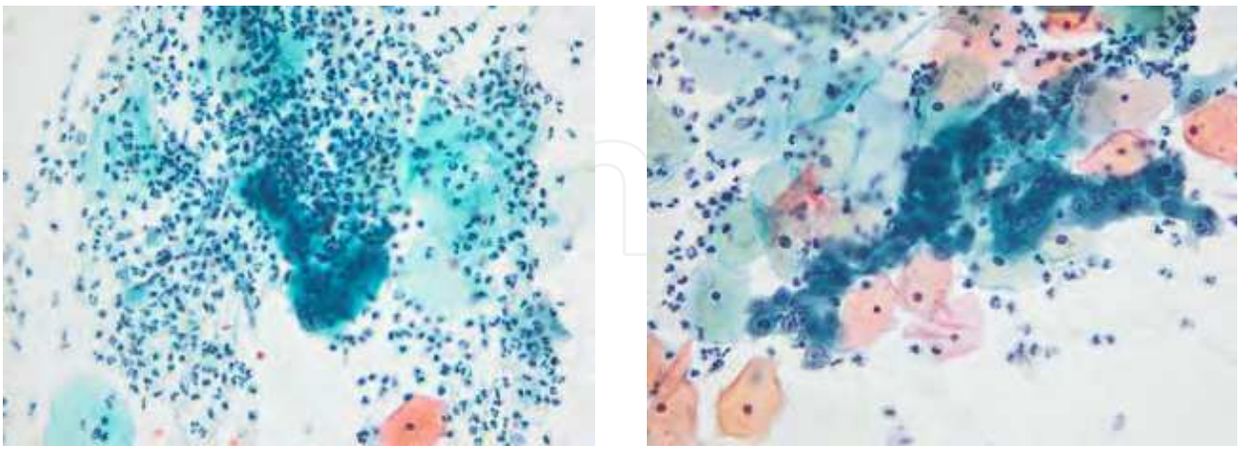

Fig. 8.1.2.1. This strips of atypical epithelial repair with enlarged nuclei. Some appear hyperchromatic and other have prominent nucleoli simulating adenocarcinoma. (Papanicolaou x400).

In determining diagnosis between atypical repair and invasive carcinoma, a designation of atypical glandular cells is warranted and an endocervical sampling procedure is required, as will be discussed below under management options.

8.1.3 Micro glandular endocervical hyperplasia

Micro glandular endocervical hyperplasia (MEH) is a localized proliferation of endocervical cells that can be mistaken for adenocarcinoma. MEH represents a non-neoplastic endocervical change usually related to progesterone effect or oral contraceptives. It is rarely seen in postmenopausal women.

The cytological manifestations of MEH falls in the spectrum of 'glandular atypia'. (Fig.8.1.3.1.)

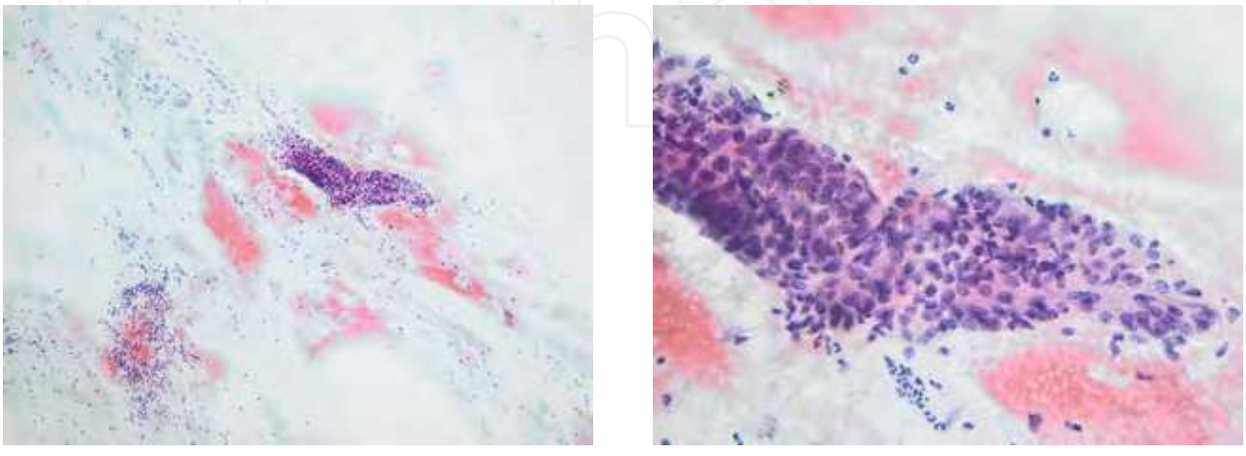

Fig. 8.1.3.1. A pseudostratified strip of endocervical cells is present. Nucleolar feathering at the periphery of the cluster, and nuclei are slightly enlarged. (Papanicolaou x 100, x 400).

The most common cytological findings are nuclear enlargement, nuclear hyperchromasia with fine nuclear chromatin, and nuclear overlap (Selvaggi and Haefner, 1997)

These are the presence of two- and three-dimensional fenestrated large sheets of cuboidal and columnar glandular cells, with finely vacuolated cytoplasm and with micro-rosette in sheets. Immature metaplastic cells, with dense basaloid cytoplasm, and reserve cells with little or no cytoplasm may also be seen. Reactive changes resulting in anisonucleosis, nuclear enlargement and prominent nuclei may lead to suspicion of either glandular or squamous neoplasia. The absence of chromatin heterogeneity, macro nucleolus formation, and tumour diathesis are the best discriminators in avoiding erroneous interpretation.

8.1.4 Sampling of the lower uterine segment, or post cone biopsy smears.

A cone biopsy shortens the endocervical canal allowing easier access to endometrial cells. Post cone biopsy smears may contain cells from this region which are referred to as lower uterine segment or LUS cells. Charateristic here is the presence of long tubular, branching glands embedded in loose monomorphic stroma. This is best observed on low power magnification.

Sampling of the lower uterine segment (LUS) following conisation is a result of endocervical brush or broom sampling of the endometrial cavity secondary to a shortened endocervical canal. This may occur following a conisation procedure or vigorous use of endocervical brushes in patients who have not undergone conisation. (Fig.8.1.4.1.)

Post cone biopsy smears are screened with a high index of suspicion, so the unwary can overreact to the presence of high endocervical cells or debrided endometrial cells in the smears.

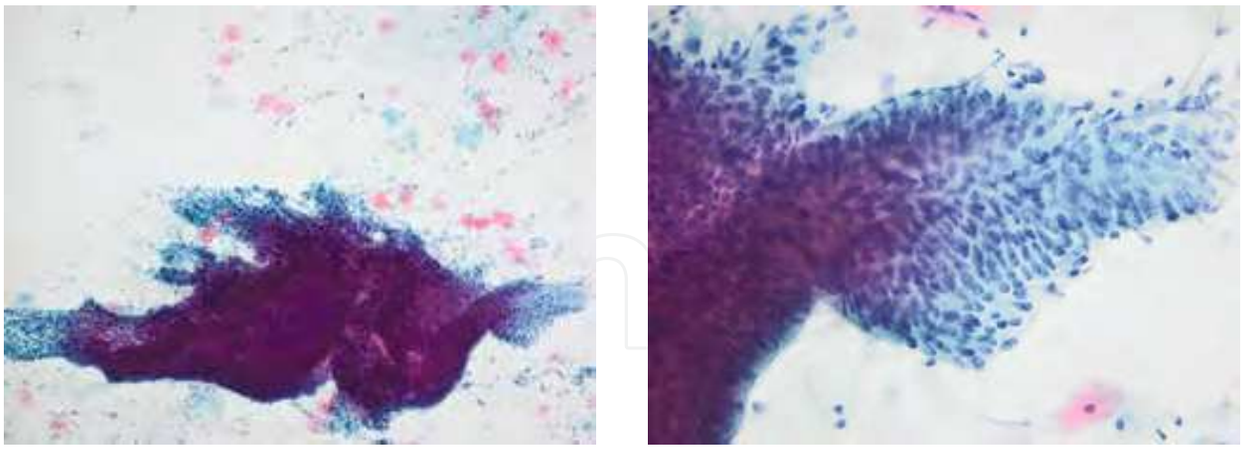

Fig. 8.1.4.1. Tightly crowded large group with pseudostratification and peripheral streaming of nuclei mimicking feathering (low uterine segment sampling) (Papanicolaou x100, x400).

Smears from the LUS show cellular two- or three-dimensional fragments with branching tubular glands that are embedded in stroma that is composed of round to spindle-shaped cells. In such circumstances, it is advisable to review the smear with the histology of the cone biopsy and with the previous abnormal Papanicolaou smear samples that led to the cone biopsy.

When compared with AIS smears, LUS sampling smears show smaller nuclei with less distinct nuclear membranes; densely dispersed, but finely granular chromatin, less frequent mitotic figures, and abundant endometrial-type stromal cells in the background (Hong et al., 2001). Presence of the endometrial-type stromal cells is significant; it is absent from the background of all cases of AIS.

Most false-positive interpretations are secondary to the presence of groups of nonciliated small glandular cells from either the upper endocervical canal or lower uterine segment of the endometrium (Lee, 1993, 1999).

These tightly crowded groups differ from AIS by having smaller, less hyperchromatic nuclei with finer chromatin, and by being intermixed with benign epithelial cells, and, occasionally, endometrial stromal cells. (Fig.8.1.4.2).

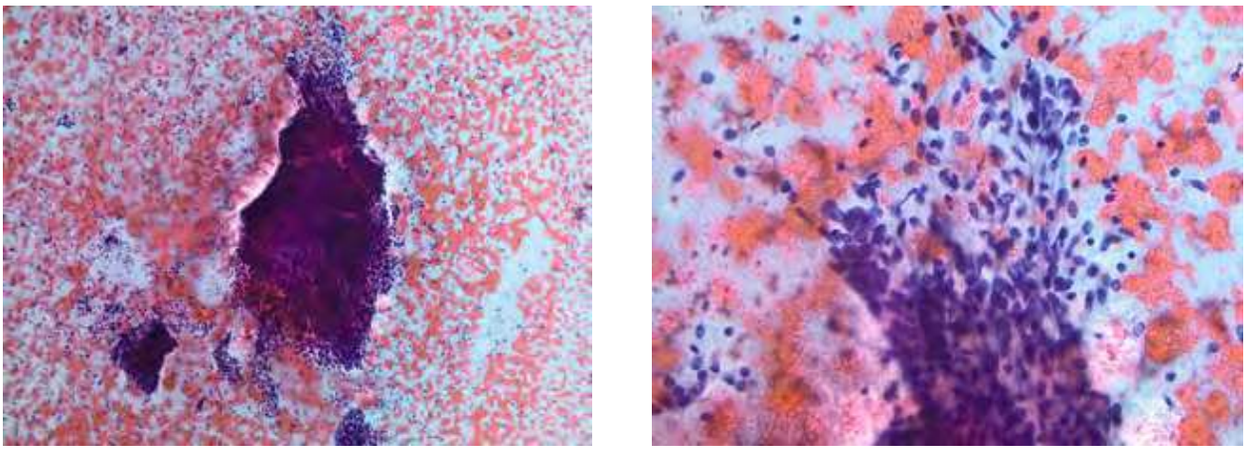

Fig. 8.1.4.2. Tightly crowded groups with benign epithelial cells, and, occasionally, endometrial stromal cells (post cone biopsy smears). (Papanicolaou x100, x400)..

More striking are the large and branching fragments of crowded glandular tissue.

Appreciation of the cytological features of LUS cells is essential to avoid misdiagnosis.

Presence of glandular cells of endometrial origin showing round nuclei, finely granular chromatin and nuclear crowding. Nucleoli are inconspicuous.

Occasional peripheral palisading of cells is noted and glandular openings are often visible. Presence of stromal cells showing uniform round to spindle-shaped nuclei, fine granular chromatin and scant cytoplasm. Peripheral cells are loosely attached and appear 'strung out'. It is important to exercise caution in examining post cone smears especially in women who have had a previous diagnosis of adenocarcinoma. Residual tumor may be present and careful scrutiny is required to differentiate abnormal from LUS cells.

8.1.5 Tubal metaplasia

Tubal metaplasia may pose a cytological problem. This refers to the replacement of normal endocervical glandular epithelium by foci of benign epithelium resembling that of normal fallopian tube epithelium. Apart from the smooth chromatin pattern of the nuclei, the most valuable feature for identification of tubal metaplasia is the density of cell cytoplasm, with blunted luminal edges bearing terminal bars and cilia. (Fig.8.1.5.1)

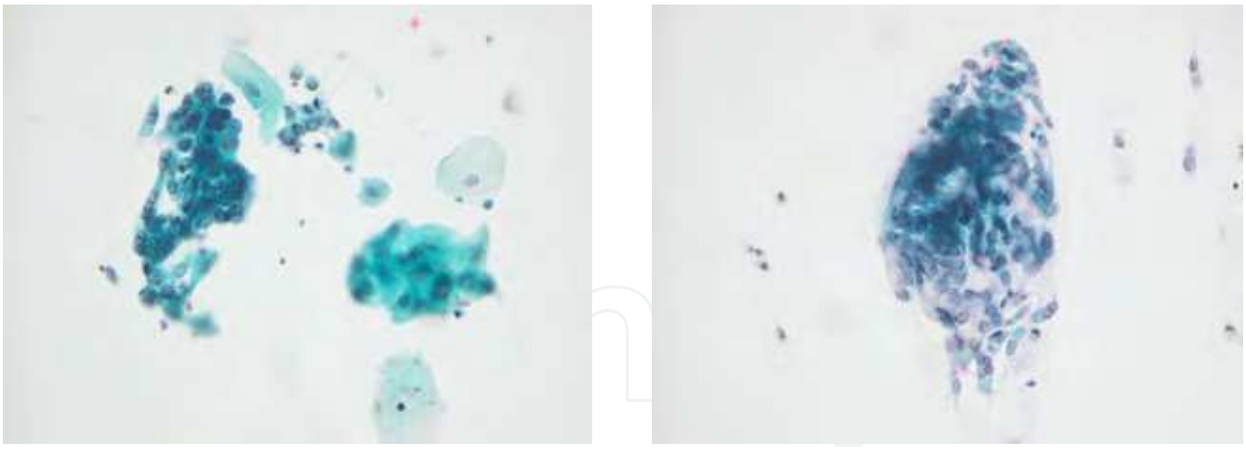

Fig. 8.1.5.1. Groups of cells with nuclear crowding, but the nuclear chromatin is finely granular. Note the terminal cilia. (Papanicolaou x400).

However, this may be a significant cause of false-positive smears for glandular neoplasia. When seen, it appears as flat sheets or cohesive clusters, and in palisade or mosaic patterns. It can mimic AIS because of nuclear crowding, nuclear overlap, nuclear feathering and nuclear palisading. Rosettes are uncommon, and, most importantly, the nuclear chromatin is finely granular and evenly distributed. Mitotic figures and apoptosis are rare. The identification of terminal bars and cilia is the most helpful cytological finding, but these features may not be present in some cases (Lee, 1993, 1999; Salvaggi & Haefner, 1997), since they may be lost during processing. Conversely, terminal bars and cilia are rarely seen in AIS.

The key to distinguishing difficult presentations of tubal metaplasia where cilia are absent is a careful review of nuclear chromatin. Most often the cells of tubal metaplasia will have normal 'endocervical' chromatin.(Fig.8.1.5.2.; Fig.8.1.5.3.; Fig 8.1.5.4.)

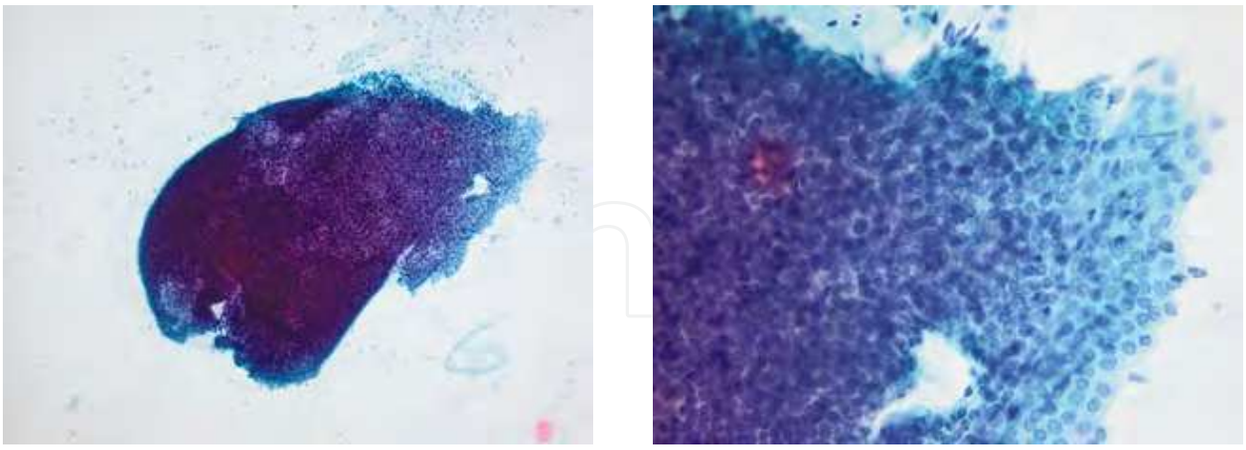

Fig. 8.1.5.2. Large three-dimensional and crowded groups of glandular cells. The cells at the edge of the fragments retain their cytoplasm and have a relatively smooth border and there is slight nuclear overlapping. (Papanicolaou x100, x400).

The typical chromatin pattern of AIS is coarse and evenly distributed. In addition, apoptotic nuclear fragments are not generally found in cases of benign tubal metaplasia, but may be commonly noted in neoplasias.

Diagnostic difficulties arise sometimes when cilia are not identified in large three-dimensional and crowded groups of glandular cells. Then, a borderline report may be justified as the possibility of coexistence of tubal metaplasia and glandular neoplasia must be borne in mind.

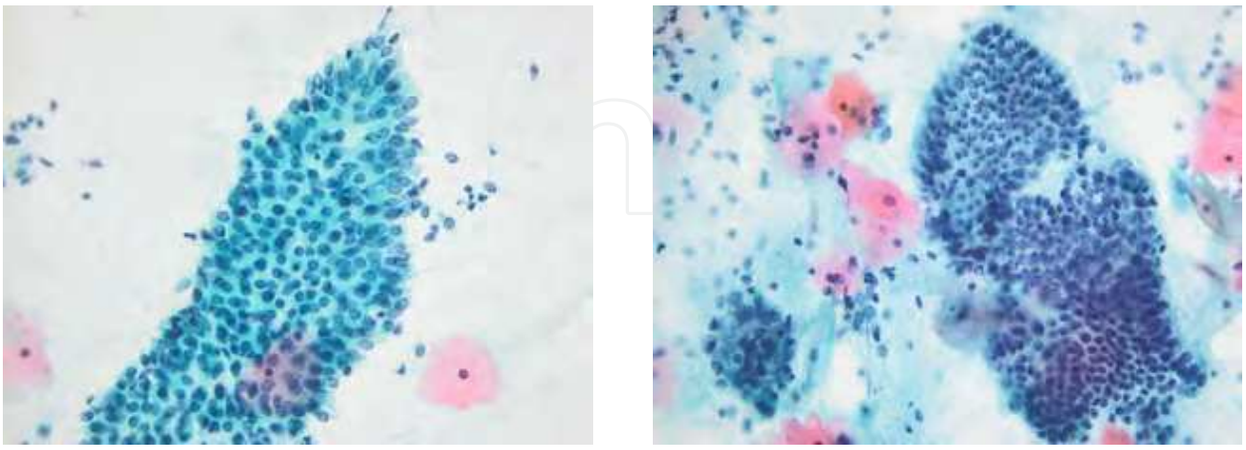

Fig. 8.1.5.3. Large groups of glandular cells with pseudo-stratification et the edge od the clusters. that mimicking feathering. The nuclear chromatin is finely granular and evenly distributed. (Papanicolaou x400).

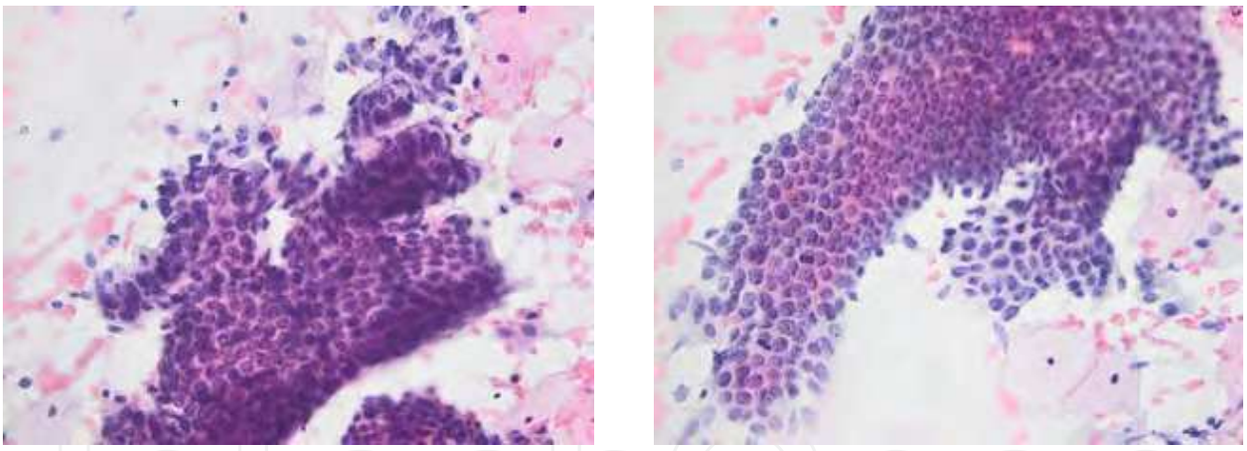

Fig. 8.1.5.4. . Large group and strips of glandular cells with palisading (left field). Large cluster that mimicking feathering (right field) . The nuclear chromatin is finely granular and evenly distributed. Note a mitotic figure on the right field. (Papanicolaou x400).

8.2 Neoplastic lesions

8.2.1 Carcinoma in situ

One of the major differential cytological diagnoses of AIS is endocervical gland involvement by CIS. In these cases, highly atypical nuclei are identified in the center of the cell aggregate, and some of the cells at the periphery of the aggregate appear to be endocervical cells. Involvement of endocervical glands by squamous CIS shows a syncytial arrangement, loss of cell polarity, and nuclear overlapping within the center of cell clusters whereas AIS cells generally maintain cell polarity. (Fig.8.2.1.1.)

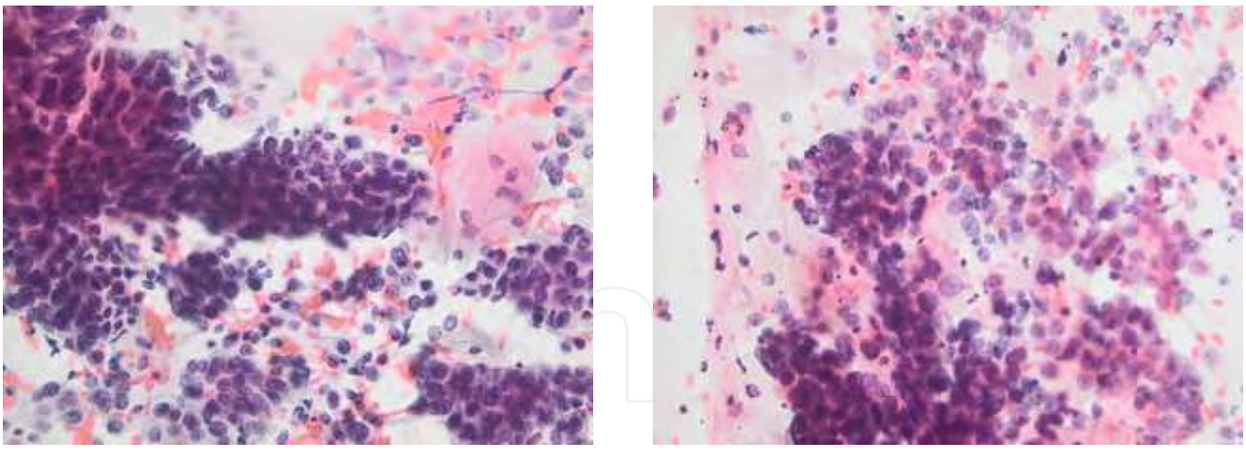

Fig. 8.2.1.1. CIS. A syncytial arrangement, loss of cell polarity, and nuclear overlapping within the center of cell clusters. Some of the cells at the periphery appear to be endocervical. (Papanicolaou x400).

The identification of occasional cells with dense eosinophilic cytoplasm and hyper chromatic cigar-shaped nuclei favors squamous CIS over AIS.

The identification of occasional cells with dense eosinophilic cytoplasm and hyper chromatic cigar-shaped nuclei favors squamous CIS over AIS.

Strips, rosettes, and gland formations, which are characteristic of AIS, are not observed in squamous CIS. In the infrequent cases that defy the above distinction, a diagnosis of atypical endocervical cells favouring AIS with a notation that squamous CIS cannot be excluded may be considered.

8.2.2 Adenocarcinoma in situ and invasive carcinoma

The most serious error is mistaking AIS for a benign process: small cell 'endometrioid' AIS, mistaken for direct sampling of the lower uterine segment endometrial cells; AIS mimicking tubal/tubo-endometrial metaplasia cells. This differential diagnosis may be extremely difficult or, in some cases, impossible in Papanicolaou smears. (Lee, 1993, 1999)

Endometrial adenocarcinoma may be mistaken for AIS if there is extension into the cervix and if the lesion is directly sampled.

Squamous carcinoma may be mistaken for adenocarcinoma if poorly differentiated.

9. New methods

A number of new technologies have been developed to improve the detection of cervical lesions, and a wide array of immuno-histochemical markers have been evaluated with respect to their specificity in staining abnormal cells in cervical cytological smears. However, there is still a significant demand for better biomarkers to identify neoplastic cervical glandular epithelial cells precisely. The most important advancement in cervical cytology has been the introduction of **liquid-based cytology (LBC)**. The advantages of LBC - compared to conventional cytology - are its increased sensitivity for detecting epithelial

cell abnormality, reduced number of specimens with obscuring blood and inflammation, and the possibility of performing **molecular assay** directly from liquid-based specimens when a diagnosis of atypical cells is made (Bishop, 2002).

Human Papillomavirus (HPV DNA) detection is a potential biomarker of a neoplastic diagnosis in women with glandular abnormalities in their cervical smears. A positive HPV test is more strongly associated with squamous neoplasia than with glandular lesions.

Studies have shown that the prevalence of HPV in adenocarcinoma may be underestimated because the glandular epithelium does not support productive viral infections. HPV DNA in endocervical neoplasia is usually present in integrated form and not in the episomal particles. This integration may result in deletion of the viral genome. Detection of HPV DNA in the assay could depend on the presence of intact episomal HPV copies (Pirog et al., 2000).

Tumor suppressor protein (p16INK4a). Some studies have shown increased high-risk viral oncogene expression in dysplastic cervical epithelia, and have demonstrated that p16INK4a protein as a specific biomarker for the identification of dysplastic cervical epithelia in sections of cervical biopsy samples or cervical smears and in thin-layer LBC specimens (Murphy et al., 2002, Juric et al., 2006, 2010). The use of p16INK4a protein as a definitive marker for cervical neoplasia would be a valuable supplementary test in gynecologic cytology. A test result is considered positive if brownish granules are found in the nuclei and/or cytoplasm of dysplastic or malignant cells. (Fig.9.1.)

Murphy et al. 2004, compared the expression patterns of p16INK4a in benign and neoplastic glandular lesions and tubo-endometrioid metaplasia. All cases in each category displayed some p16INK4a expression.

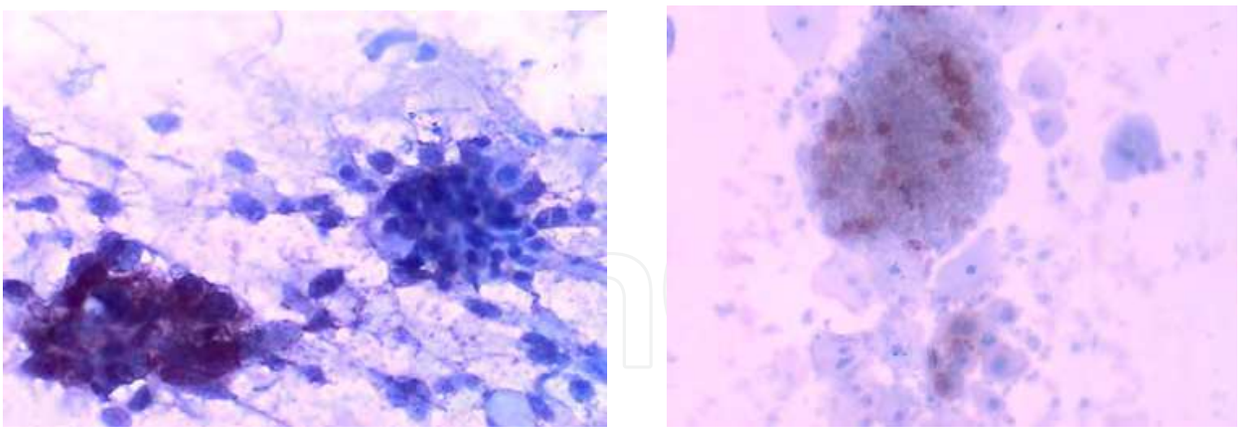

Fig. 9.1. p16INK4a postive staining of cluster malignant edocervical cells (AIS) left field, and of atypical endocervical cells (GIL2) and of high-grade squamous intraepthelial lesions (HSIL) on right field. Note a cluster of normal glandular cells p16INK4a negative staining on the left field. (x400, 100)

While p16INK4a has been demonstrated to be an excellent marker of cervical dysplasia in squamous neoplastic lesions of the cervix, it has potential pitfalls in cervical glandular lesions that may limit the utility of this biomarker in resolving the nature of suspicious glandular lesions, particularly in cytopathology.

Based on our results in detecting SIL lesions and carcinoma of the uterine cervix (Juric et al.,2010), immunocytochemical expressions of p16INK4a in ThinPrep cervical specimens correlate closely with the HPV-high-risk typed specimens through the polymerase chain reaction method (PCR) in the same samples.

We can assume that the combination of these tests can identify two groups within low-grade lesions, i.e. one with low risk for the development of premalignant cervical lesions, for which both of these tests are negative, and another group with both tests positive and with an increased risk of squamous intraepithelial lesions.

The value of immunocytochemical expressions of p16INK4a as adjunct methods for detection and differential diagnosis of glandular lesions has been investigated.

Imaging of silver-stained nucleolar organizer regions (AgNORs) is one of the more recent methods (Ploton et al. 1986). Nucleolar Organizer Regions (NORs) are structured from loops of ribosomal deoxyribonucleic acid (rDNA). Under the influence of RNA polymerase I, they are transcribed to ribosomes and proteins sited on the short arms of acrocentric chromosomes 13, 14, 15, 21, and 22. Since they have the central role in the transcription of nucleic acid into proteins, their number and size can be a reflection of cell proliferation, transformation or overt malignancy (Crocker, 1990). This method reveals AgNORs in the form of brown-black dots of different sizes within the nucleus. In numerous papers, the differential diagnostic and prognostic value of AgNOR analysis has been emphasized, on histological (Crocker, 1990; Darne et al., 1990) as well as cytological

(Fiorella et al., 1994; Audy i sur. 1995; Ovanin-Rakic & Audy-Jurkovic, 1998; Mahovlic et al., 1999) samples of benign, borderline and malignant lesions at various locations, and its significance has rarely been disputed.

Automated image analysis is applied to avoid the subjective error of an observer and to decrease the time necessary for data processing. This automated process was applied **in 1996** as a fast, reproducible method on archival cytological specimens from cervix uteri stained by the Papanicolaou method (Ovanin-Rakic & Audy-Jurkovic, 1998) from 16 patients with a histological diagnosis (4 endocervical glandular dysplasia, 5 adenocarcinoma in situ, 7 adenocarcinoma invasivum) and 10 patients with benign endocervcal cells at the Institute of Gynecological Cytology, Department of Obstetrics and Gynecology, Medical School, University of Zagreb.

AgNORs are shown in the nucleus as dark brown to black dots. The count, area and size of AgNOR per square micrometer (minute <0.24; small 0.25 - 0.74; medium 0.75 - 1.4; large 1.5 - 2.4; extra large > 2.25) were analyzed in 50 cells per smears magnified 1,000x, on the focal plane. The SFORM system was used for digital image analysis (VAMS, Zagreb, Croatia) at the Institute of Pathology and Pathological Anatomy, Medical School, University of Zagreb. The system includes a high-resolution CCD color TV camera transferring images from the microscope (Olympus BHS, Tokyo, Japan) to a PC-compatible computer via a picture digitizer, with a resolution of 512 x 512 pixels, whereby each of them can assume a value described by 24 bits.

While measuring, the results of parameters measured are automatically transferred and logged in previously defined tables. The data obtained were processed on a PC by the

SPSS/PC+ 3.0 program (Chicago, Illinois, U.S.A.). Mann-Whitney and x2 tests were applied to test the differences between the groups, while statistical significance was tested at the level P= .05.

Our results showed that the mean values of AgNOR count and area per nucleus increased from benign endocervical cells (1.9; 2.17 μ2), and dysplasia (2.11; 2.53 μ2), and AIS (3.1; 3.27 μ2) to AI (3.,7; 5.49 μ2). The differences between all groups are statistically significant (P<.05)

Regarding AgNOR size and histological diagnosis, most frequently found were minute AgNORs in AIS (7.8%) and AI (6.7%), then benign (2,1%), and dysplasia (1.9%), while extra large AgNORs most frequently found in AI (15.9%). The differences between groups are statistically significant (P<.05) except for the pairs benign endocervical glandular cells and dysplasia.

One AgNOR per nucleus was usually present in benign endocervical cells (43.6%), and four or more in adenocarcinoma, especially adenocarcinoma invasivum (37.6%; 51.7%) with the differences between all groups being statistically significant (P<.05). (Fig.9.2.)

The AgNOR technique is a simple, inexpensive and reliable method applicable to both histological and cytological samples. AgNOR number is considered to be a reflection of cell proliferation. According to the literature, digital AgNOR image analysis of endocervical benign and abnormal glandular cells has not been performed before.

Our results indicate an increase in the mean value of AgNOR count from normal to intraepithelial and invasive glandular lesions, corresponding to the results on histological samples (Allen & Galimore, 1992; Darne et al., 1990; Miller et al., 1994), and cytological smears (Fiorella et al., 1994; Audy-Jurkovic et al., 1995). A significant finding of four or more AgNORs in 51,7% indicating adenocarcinoma invasivum that correlates to the results on histological samples (Miller et al., 1994).

Digital AgNOR image analysis (count, size and area) in cytological specimens of the cervix uteri indicated that the method is helpful in differentiating benign, intraepithelial and invasive lesions of the endocervical cylindrical epithelium, because statistically significant differences were obtained among all groups except for the benign state – dysplasia pair according to AgNOR size (p=0.8946).

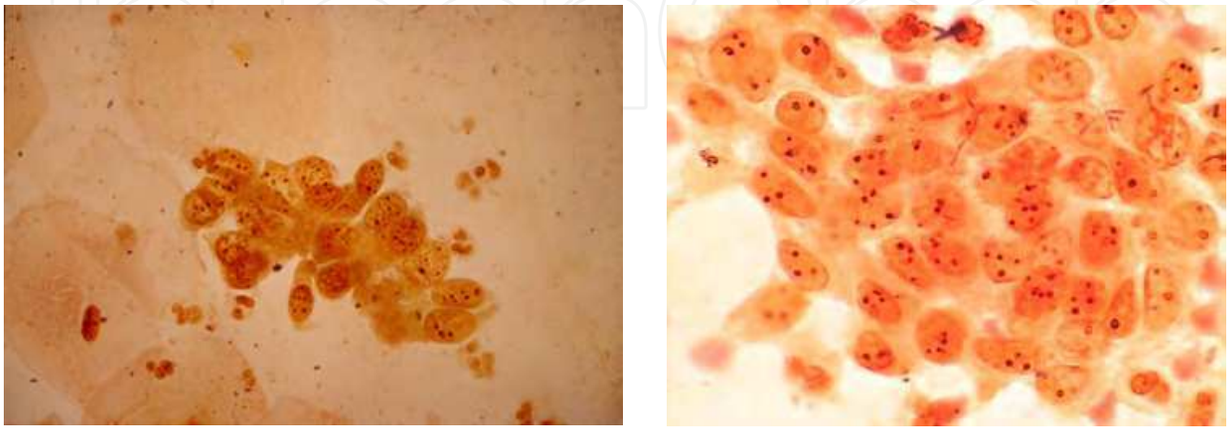

Fig. 9.2. AgNOR-stained. Cluster of adenocarcinoma in situ (left field), and adenocarcinoma invasivum (right fields). Note different types of brown-black dots within the nucleus.

10. Conclusion

Intraepithelial lesions of the endocervical epithelium are difficult to detect by cytology. However, recent studies show some favourable trends. In our study, the cytological differential diagnosis of AIS showed a 61.5% accuracy. The diagnostic accuracy of cytology is by far higher for pure (cylindrical only) than for mixed (cylindrical + squamous) lesions, because the abnormalities involving exclusively squamous component were quite frequently observed in the latter, either because of more distinct criteria and easier recognition, or due to more pronounced cellular lesions, or because of the predominant population of abnormal squamous cells.

The cytodiagnosis of cervical cylindrical epithelial lesions lags behind the cytodiagnosis of squamous epithelial lesions both in terms of screening and differential diagnosis. As data continue to accumulate, the clinical characteristics of pre-invasive glandular cervical lesions are becoming progressively better defined. Cytological screening for these lesions is imprecise. A major problem is the relative infrequency of glandular lesions and inexperience with sometimes difficult differentiation between benign glandular cells and the endocervix or lower segment of the endometrium. However, modifications to current classification systems may improve overall diagnostic accuracy. Nevertheless, all glandular abnormalities on the Papanicolaou smear require judicious evaluation and careful follow-up.

At present, the solution lies in better education. When in the hands of experienced cytologists, difficult cases of intraepithelial glandular lesions can be reliably distinguished from benign processes most of the time. The problem is in translating this experience to the entire community of cytologists, including cytotechnologists. Experience demands increased sensitivity, and cytologists and cytotechnologists both play a critical role in attempts to increase sensitivity in the face of demands for diagnostic specificity.

As our understanding of glandular lesions continues to expand and cervical sampling techniques continue to improve, we may expect continued enhancement in our ability to detect and treat intraepithelial glandular lesions, and thus help to decrease morbidity and mortality from cervical adenocarcinoma

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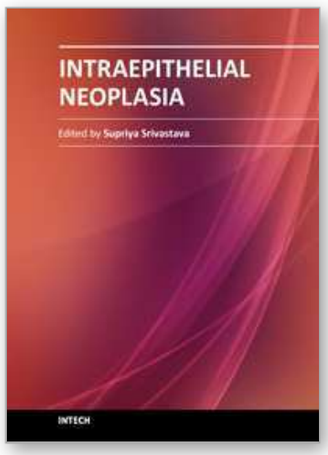

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The book "Intraepithelial neoplasia" is till date the most comprehensive book dedicated entirely to preinvasive lesions of the human body. Created and published with an aim of helping clinicians to not only diagnose but also understand the etiopathogenesis of the precursor lesions, the book also attempts to identify its molecular and genetic mechanisms. All of the chapters contain a considerable amount of new information, with an updated bibliographical list as well as the latest WHO classification of intraepithelial lesions that has been included wherever needed. The text has been updated according to the latest technical advances.This book can be described as concise, informative, logical and useful at all levels discussing thoroughly the invaluable role of molecular diagnostics and genetic mechanisms of the intraepithelial lesions. To make the materials easily digestive, the book is illustrated with colorful images.

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