

# Bizarre atypia of the cervical epithelium due to chemotherapy with busulfan and cyclophosphamide

## Servikal epitelde busulfan ve siklofosfamid kemoterapisine bağılı bizar atipi

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### ABSTRACT

We present a 20 year-old female patient with highly atypical epithelial changes in the uterine cervix discovered on cervical smear and biopsy specimens. She had recently been diagnosed with acute lymphoblastic lymphoma and received alkylating agent chemotherapy. We thought that the epithelial atypia was related to chemotherapy in the light of the reports in the literature which are discussed in the present text along with a brief review of related histopathological and cytological criteria.

**Key words:** Cervical dysplasia, chemotherapy, busulfan, cyclophosphamide

### ÖZET

Yazımızda uterus serviksinde sitoloji ve biyopsi ile ağır epitelyal atipik değişiklikler gösterilen 20 yaşındaki kadın hastayı sunmaktayız. Hasta yakın zamanda akut lenfoblastik lenfoma tanısı almış olup alkilleyici ajanlar ile kemoterapi almıştır. Hastamızda izlenen atipik değişiklikler, konuyla ilgili literatürün de ışığında kullanılan alkilleyici ajanlara bağlanmış olup, bu yazıda ilgili histopatolojik ve sitolojik kriterler tartışılmıştır.

**Anahtar sözcükler:** Servikal displazi, kemoterapi, busulfan, siklofosfamid

### INTRODUCTION

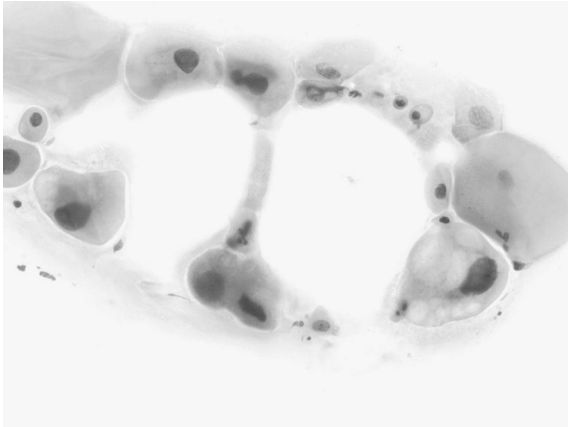
Chemotherapy with alkylating agents has been known to cause high grade dysplastic alterations in epithelial cells (1-4). These changes can lead to an erroneous diagnosis of true pre-neoplastic dysplasia in cervical smears. The present case demonstrates this issue with a smear followed by a biopsy of the uterine cervix.

### CASE REPORT

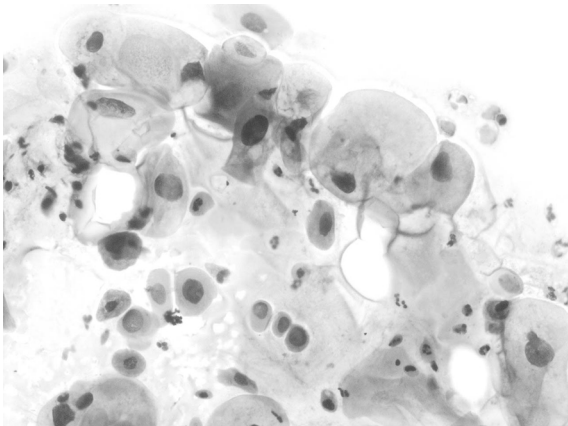
A 20 year-old female patient was diagnosed as acute lymphoblastic leukemia and she re-

ceived a regimen of busulfan and cyclophosphamide. She had a cervical smear in her follow-up. The smear slide stained with Papanicolaou stain revealed severely enlarged cells with hyperchromatic nuclei and irregular nuclear outlines while the nuclear/cytoplasmic ratio was not increased (Figures 1 and 2). The chromatin pattern showed a homogenous basophilic staining without coarse clumping. These cells had abundant cytoplasm, some with vacuolation. Mitotic figures were absent. Necrotic diathesis was not observed. The cytology report stated that high grade dysplastic changes had been observed while a possible drug effect could not have been ruled out. A cervical cone biopsy was obtained. The specimen was inked on surgical margins, fixed in buffered formalin and processed. Sections

from the paraffin blocks were first stained with hematoxylin and eosin. The slides showed diffuse atypia in the epithelium of the uterine cervix.

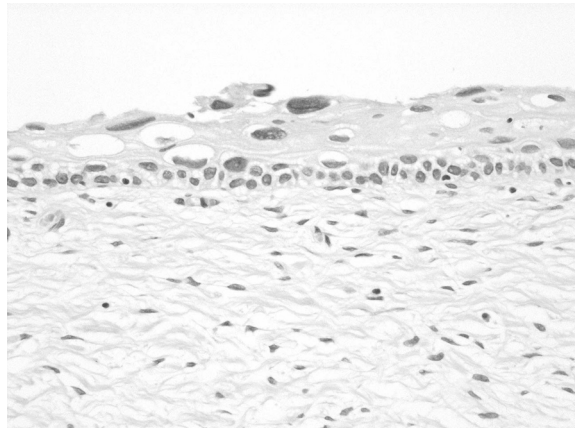


**Figure 1.** A group of cells with irregularly contoured hyperchromatic nuclei and abundant cytoplasm in the cervical smear. Cytoplasmic microvacuolation is evident in the right lower hand (Papanicolaou x400).

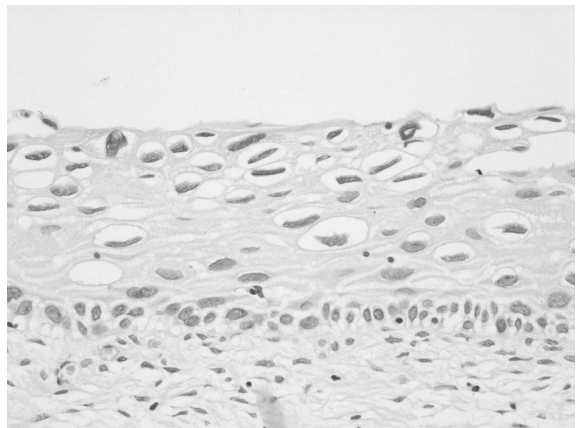


**Figure 2.** Another focus in the cervical smear shows pleomorphic, enlarged cells. Some nuclei are about five times larger than their normal counterparts. Cytoplasms are enlarged as well (Papanicolaou x400).

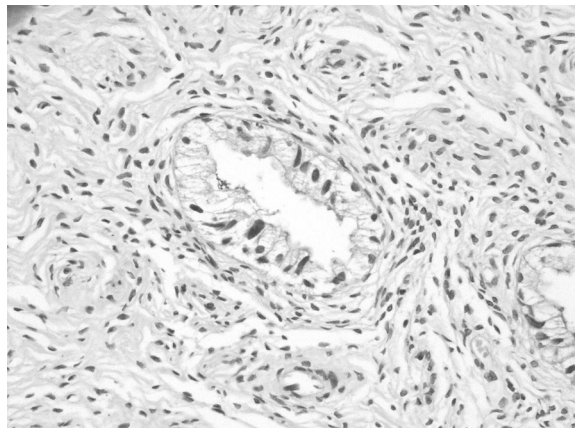
The most prominent atypical changes were found in the squamous epithelium. Especially the keratinocytes of the middle and superficial layers exhibited bizarre, enlarged and hyperchromatic nuclei with significant pleomorphism throughout (Figure 3 and 4). Oddly, the overall polarity of individual cells was mostly maintained. Hyperchromasia was homogenous; the



**Figure 3.** The cervical cone biopsy reveals highly anaplastic-looking cells in the middle and superficial layers while the basal layer is occupied by small monotonous cells (HE x400).



**Figure 4.** The squamous cells are pleomorphic but somehow maintain their polarity (HE x400).



**Figure 5.** An endocervical gland with large atypical nuclei. No mitoses were found (HE x400).



**Figure 6.** Immunohistochemistry for Ki-67. Only few basal cells show nuclear positivity (Streptavidin-peroxidase x200).

nuclear chromatin did not show clumping, vesicular appearance or conspicuous nucleoli. Cytoplasm was again abundant and contained vesicular or vacuolar areas. No mitotic activity was discovered despite a thorough search and serial sectioning. Importantly the basal cells looked uniform and normal without crowding, hyperplasia or any cytological disturbances. No evidence of an invasive neoplasia was observed. Endocervical glands focally contained cells with lost polarity and irregularly contoured, hyperchromatic nuclei (Figure 5). There was not atypia in the stromal or endothelial cells.

Immunohistochemistry for human papilloma virus (HPV), human herpes virus types I and II (HSV), cytomegalovirus (CMV), p53, p16, and Ki-67 were performed with streptavidin and peroxidase technique. Tests were negative for HPV, HSV and CMV. There was focal positive nuclear staining with p53. No positivity was found with p16. Ki-67 only stained the nuclei of the basal cells (Figure 6). Preservation of the nuclear/cytoplasmic ratio, detection of abundant vacuolized cytoplasm, degenerative-looking chromatin pattern, absence of mitoses, accompanying focal endocervical glandular dysplasia, negativity for p16 and p53 with a very low proliferative index with Ki-67, led us to make the diagnosis of epithelial atypia secondary to alkylating agent administration. Two months after,

a cervical smear result was in the normal range.

## DISCUSSION

Alkylating agents are recognized causes of non-neoplastic, reactive, high grade cytological atypia in many organ systems such as the upper aerodigestive tract, lower respiratory tract, esophagus, stomach, uterine cervix, urothelium and skin (1-9). There is agreement in the literature that cyclophosphamide is the drug most strongly associated with epithelial dysplasia (2,4) administered either alone (6,7) or with busulfan (1,3). Moreover, these two drugs together were responsible for most of the cases reported. Atypia of uterine cervix associated with cyclophosphamide was shown before (2,6,7) but opposing suggest that this drug does not cause abnormal cervical smears (10). The former studies proposed that such chemotherapy increased the risk of cervical intraepithelial neoplasia (CIN) (2,6,7).

The pathologist has to be aware of chemotherapy-related alterations in order not to make an erroneous diagnosis of malignancy. The criteria put forth for gastric chemotherapy-related atypia have great help in the diagnosis of CIN in other organs as well (11). Those features in favor of chemotherapy-related alterations were bizarre atypia with marked cellular enlargement exceeding that seen in cancer, lower nuclear to cytoplasmic ratio, cytoplasmic eosinophilia and vacuolation, lower mitoses, atypia also involving fibroblasts and endothelium and changes resembling radiation effect (11,12). In stratified epithelium, atypia in the superficial cells without accompanying hyperplasia of basal cells is a clue against malignancy (4,13). Prominent, multiple or eosinophilic nucleoli (4,14) and smudging of the chromatin (13) are common in chemotherapy-related atypia. As can be expected, Ki-67 proliferation index is low in these lesions (4).

These changes are probably related to an arrest in nuclear division due to a metabolic ef-

fect of the drug (3). The benign nature of these cells was supported by the lack of an increase in their nuclear DNA content (15).

The present case demonstrates a recognized relation of alkylating agents with bizarre atypia of epithelial linings. The pleomorphic cells observed in the cervical cytology and the biopsy specimens had anaplastic changes exceeding those of a carcinoma, and most importantly the nuclear/cytoplasmic ratio was preserved or increased while there was microvacuolar and vesicular appearance of the abundant cytoplasm. Mitoses were not found. Such alterations should have a higher index of suspicion and lead to an investigation for drug or radiation effects. Our patient had not received radiotherapy. Other possible causes of enlarged or atypical nuclei in cervical epithelium such as viral cytopathic effect or atrophy were also considered. As nuclear ground-glass appearance, multinucleation, Cowdry type A or cytoplasmic basophilic inclusions and immunohistochemical evidence for viral involvement were lacking, viral infection was ruled out. Atrophy of the cervical epithelium may exhibit high nuclear/cytoplasmic ratio and may be confused with dysplasia. The bizarre cellular atypia and pleomorphism in our case which surpass that expected in atrophy as well as the young age of the patient helped to rule out atrophy. The patient had a normal smear in the subsequent second month. We could not perform a polymerase chain reaction test or other more definitive assays to rule out HPV, which forms a gap in our report. This case shows that chemotherapy with busulfan and cyclophosphamide may cause bizarre reactive atypia of the cervical epithelium, which should be investigated with further studies. We conclude that in cytological and biopsy materials from patients with a history of chemotherapy, highly atypical epithelial changes are expected and should not be overdiagnosed.

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