OLGU SUNUMU CASE REPORT

A Retrospective Immunocytochemical Analysis of p16 and p53 Protein Expressions in Cervicovaginal Smears of Four Women with *Trichomonas vaginalis* Infection

Trichomonas vaginalis Enfeksiyonu Olan Dört Kadının Servikovaginal Yaymalarında p16 ve p53 Protein Ekspresyonuna Dair Retrospektif Bir İmmunositokimyasal Analiz

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ABSTRACT The clinical significance of positive immunostaining for p16 and p53 in cervicovaginal smears of women with *Trichomonas vaginalis* infection was investigated. We applied p16 and p53 immunostaining to cervicovaginal smears of women with *Trichomonas vaginalis* infection (the prior antibody was applied to four cases while the latter was used in two patients). Immunoreactivity, intensity of immunostaining (weak or strong) and immunostaining patterns of p16 and p53 were recorded. All cases revealed a granular, diffuse intracytoplasmic p16 immunoreactivity in varying intensities in different microorganisms. In two cases, a more intense immunostaining for p53 was demonstrated which was similar to p16 immunoreactivity regarding the immunostaining pattern. Positive p16 and p53 immunostainings demonstrated in cervicovaginal smears of subjects with *Trichomonas vaginalis* infection may lead to misinterpretation of p16 and p53 immunoreactive *Trichomonas vaginalis* microorganisms as dysplastic epithelial cells. This may subsequently result in diagnostic pitfalls hindering conventional use of those antibodies as adjunctive biomarkers of high-risk HPV infection in cervical screeening.

Key Words: *Trichomonas vaginalis*; p53 protein (325-355), human; Immunohistochemistry

ÖZET *Trichomonas vaginalis* enfeksiyonu olan kadınların servikovaginal yaymalarındaki pozitif p16 ve p53 boyanmalarının klinik anlamlılığı araştırıldı. *Trichomonas vaginalis* enfeksiyonu olan dört olgunun servikovaginal yaymalarına p16 ve p53 immunboyaması uygulandı (p16 antikoru 4 olgunun tümünde, p53 antikoru 2 olguda kullanıldı). p16 ve p53 immunoreaktivitesi, immunboyanma yoğunluğu (zayıf veya kuvvetli) ve immunboyanma paternleri incelendi. Olguların tümünde farklı mikroorganizmalarda değişen yoğunluklarda olmak üzere granüler, diffüz intrasitoplazmik p16 immunreaktivitesi tespit edildi. Olguların ikisinde ise immunboyanma paterni açısından p16 immunreaktivitesine benzer daha yoğun bir p53 immunboyanması olduğu gösterildi. *Trichomonas vaginalis* enfeksiyonu olan kadınların servikovaginal yaymalarında pozitif p16 ve p53 immunboyanması olması, p16 ve p53 immunoreaktif *Trichomonas vaginalis* mikroorganizmalarının displastik epitelyal hücreler olarak yorumlanmasına yol açabilir. Bu yüzden söz konusu antikorların serviks kanseri taramasında yüksek riskli insan papilloma virüs (HPV) enfeksiyonunun biyolojik belirteçleri olarak kullanılması tanısal tuzaklara neden olabilir.

Anahtar Kelimeler: *Trichomonas vaginalis*; p53 protein (325-355); immunohistokimya

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P16 protein (also known as INK4A) is a cyclin-dependent kinase-inhibitor that regulates G1-S phase of cell cycle and functions as a tumor supressor.[1,2] It specifically binds to cyclin-dependent kinase and inhibits the catalytic activity of the CDK4-cyclin D complex leading to an action of negative cell cycle regulation.[3] It seems to have a probable association with angiogenesis, cell senescence and spreading. p16 levels are fo-

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und to be diminished in a variety of malignant lesions involving pancreatic and lung carcinomas, melanomas, glioma and leukemia while overexpressed in cervical carcinoma and dysplastic cervical lesions associated with high-risk HPV (HR-HPV) infection.[4-7] Several investigations concluded that p16 appeared to be a surrogate marker of HPV infection and a valuable biomarker of dysplastic and malignant lesions originating from squamous and endocervical mucosa.[8-14] This is considered to occur subsequently to interaction and degradation of retinoblastoma protein by E7 which is an early viral oncoprotein synthesized by HPV. Early viral proteins, E6 and E7 of only high-risk HPV subtypes express the potential of malignant transformation.[1,15] Recent investigations reported that immunohistochemical detection of p16 is found to be even more sensitive and specific than HPV status of cervical lesions using liquid-based as well as tissue samples.[16] Overexpression of those proteins functioning in cell cycle progression of HPV-infected cells may be demonstrated by immunohistochemical analysis in all conventional Pap smears, liquid based specimens, cell blocks and may serve as satisfactory biomarkers for HR-HPV induced cervical disease.[17-19]

p53 tumor supressor protein is a DNA sequence-specific transcriptional regulator that controls expression of numerous genes in cellular outcomes regarding the cell cycle arrest and cell death in response to various forms of stress. In cervical squamous carcinoma and dysplastic lesions, mutation of p53 supressor protein has been suggested to be associated with HPV infection.[20] E6 which is the oncoprotein indicated to be overexpressed in cervix cancer binds host p53 supressor gene protein product and causes degradation and disruption of its cell regulatory role.[5,11,21]

Pantanowitz and colleagues suggested that *Trichomonas vaginalis* was stained positively in cervicovaginal smears and urine while evaluating for p16 immunocytochemistry.[8,23] They concluded that positive *Trichomonas vaginalis* immunoreactivity for p16 in smears may result in misinterpretation of this reaction as malignancy or dysplasia and may hinder the use of p16 in cervical cytology.

In this study, we aimed to investigate about any specific and clinically significant positive immunostaining for p16 and p53 in cervicovaginal smears of four women with *Trichomonas vaginalis* infection.

CASE REPORTS

The study was conducted on conventional PAP smears of 4 patients examined between 1996-2005 at Kocaeli University, School of Medicine, Department of Obstetrics and Gynecology (mean age: 34 years ranging between 21-44 years of age). Study subjects were determined to be negative regarding precancerous intraepithelial lesion or malignancy and all cervicovaginal smears were demonstrated to have *Trichomonas vaginalis*. All the microscopic slides included in this study were reassessed and the diagnosis of *Trichomonas vaginalis* infection was confirmed by two independent pathologists (YG, DT). Quantity of *Trichomonas vaginalis* protozoa was described as abundant, moderate or few in case of >20 microorganisms per high power field (hpf), 10-20/hpf and <10/hpf respectively. Endocervical cells and endometrial cells, inflammation and genital infections were recorded if present. The microscopic slides were divided into two parts by a hydrophobic pen in order to apply immunostaining by both p16 and p53 antibodies (the prior antibody was applied to all four cases while the latter was used in two subjects). Prior to immunostaining procedure, each specimen underwent ethylenediaminetetraacetic acid (EDTA) antigen retrieval twice (5 minutes) and microwave treatment, followed by cooling at room temperature for 20 minutes. Immunostaining was performed by biotin-streptavidin-peroxidase method using mouse monoclonal antibodies against activated p16 (16p07 Neomarkers, Fremont, CA, USA) 1:20 and p53 (DO-1 Neomarkers, Fremont, CA, USA) 1:200 diluted in primary antibody diluents. Antibody detection was done by adding biotinylated secondary antibodies, streptavidin peroxidase and AEC chromogen. The slides were counterstained by hematoxylin. Non-immune serum and appropriate tissues were used as negative and positive controls respectively. Immunoreactivity, intensity of immunostaining

(weak or strong) and immunostaining patterns of p16 and p53 were examined by the aforementioned pathologists by means of light microscopy (YG, DT).

The mean age of the patients was determined to be 34 ranging between 21-44 years of age. Two of the cases were suffering from foul smelling vaginal discharge while the other two subjects were diagnosed to have *Trichomonas vaginalis* infection following gynecologic examination for another complaint. *Trichomonas vaginalis* infection was confirmed by pathological and microbiological evaluation. *Trichomonas vaginalis* quantity was found to be abundant in one of the cases, moderate in two of the cases and just few in the other one. Endometrial cells were shown to be present in none of the cases while acute inflammatory cells were apparently demonstrated in all of the subjects. All four cases revealed a granular, diffuse intracytoplasmic p16 immunoreactivity in varying intensities in different microorganisms. A pale, weak immunostaining was observed in nuclear localization of the protozoa (Figure 1, Figure 2). In two cases, a more intense immunostaining for p53 was demonstrated which was similar to p16 immunoreactivity regarding the immunostaining pattern (Figure 3, Figure 4).

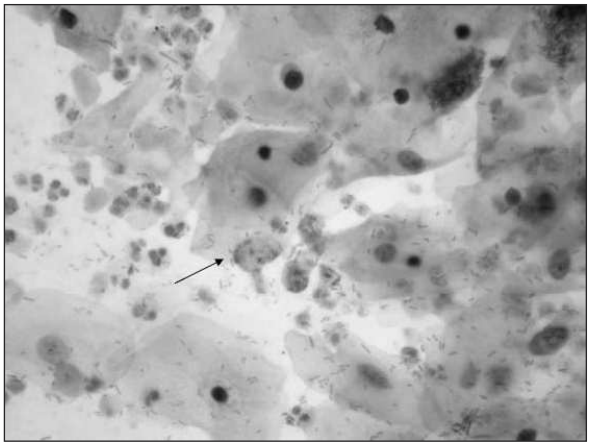

FIGURE 1: p16 immunoreactivity in *Trichomonas vaginalis* colony is demonstrated (immunohistochemistry p16 X200).

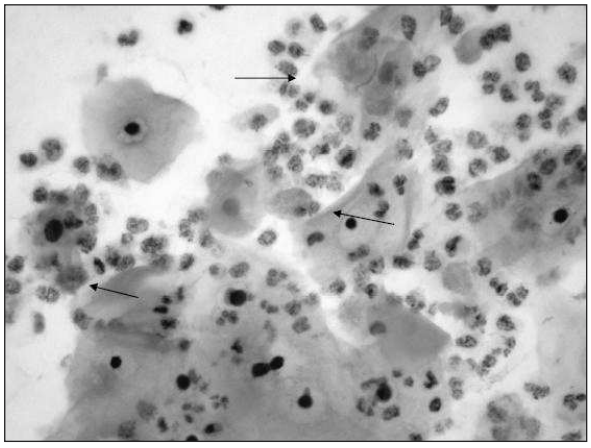

FIGURE 2: Pale intracytoplasmic p16 immunoreactivity in *Trichomonas vaginalis* microorganisms is demonstrated (immunohistochemistry p16 X400).

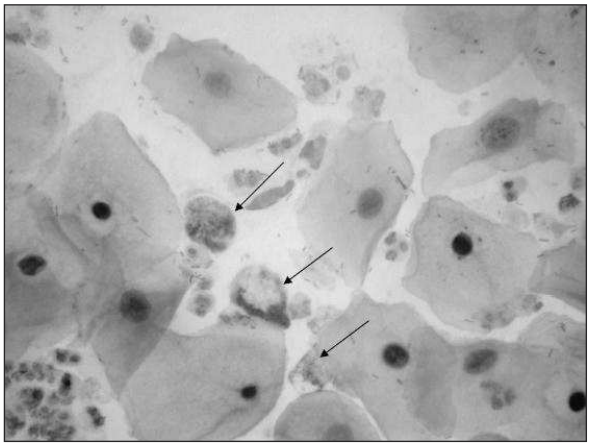

FIGURE 3: Strong and granular intracytoplasmic p53 immunoreactivity in three *Trichomonas vaginalis* microorganisms is demonstrated (immunohistochemistry p53 X400).

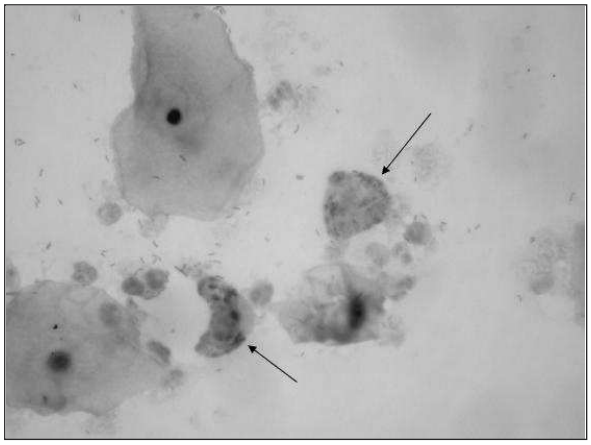

FIGURE 4: p53 immunoreactivity in cytoplasm of two *Trichomonas vaginalis* microorganisms with a pale area in the nuclear region is demonstrated (immunohistochemistry p53 X400).

DISCUSSION

Several investigators reported a well-recognized association between HR-HPV and p16 which is a tumor supressor protein that decelerates the cell cycle by inactivating cyclin dependent kinases resulting from phosphorylation of retinoblastoma protein.[15] Since p16 is shown to be overexpressed in HR-HPV induced premalignant and malignant lesions, p16 immunocytochemical examination successfully applied to conventional Pap smears, liquid-based specimens and cell blocks may be used as a satisfactory biomarker in order to clarify this controversial issue of gynecologic cytopathology.[7,9,11-14,17]

However a variety of studies suggested that p16 immunoreactivity may lead to a diagnostic pitfall in case of cytopathologic evaluation in specimens confounded by a background of inflammation, atrophy and microorganisms. Inflammatory cells, bacteria and mucus have been reported to cause potential false positive p16 immunostaining due to background staining.[14] p16 has been demonstrated to stain endocervical glandular cells, bacilli, benign endometrial cells, tubal metaplasia, microglandular hyperplasia, multinucleated giant cells and occasional metaplastic cells of various stages of differentiation.[11-14,23,24] Pantanowitz and colleagues have demonstrated that *Trichomonas vaginalis* protozoa in cervicovaginal smears and urine were positively stained with p16 immunocytochemistry.[8,22]

p53 is a well-known tumor supressor protein which is a DNA sequence specific transcription regulator functioning in cell cycle arrest and cell death. Ability to recognize and bind specific DNA sequences and recruitment of both general and specialized transcription regulators are required. Multiple interactions with co-activators and co-repressors allow p53 to either promote or inhibit transcription of different target genes.[20]

In this study, we confirmed the findings of Pantanowitz and colleagues regarding positive p16 immunostaining for *Trichomonas vaginalis* in cervicovaginal smears. p53 immunoreactivity for *Trichomonas vaginalis* has been shown for the first time in literature by the present study. We detected a more intense p53 staining for trichomonas vaginalis with a similar pattern of p16 immunostaining in our subjects.

Since *Trichomonas vaginalis* is known to be the most prevalent non-viral sexually transmitted disease worldwide, positive p16 immunostaining may not be an infrequent finding of cervicovaginal smears of women with *Trichomonas vaginalis*. It is not absolutely known why those protozoa are stained positively with p16. Pantanowitz and colleagues concluded that this phenomenon may be due to a non-specific binding or less likely due to a specific labelling by p16 monoclonal antibody and may be related with the fact that cyclin dependent kinases and and cell division control sequences are strongly conserved among eukaryotes involving *Trichomonas vaginalis*. However, concurrent p53 immunoreactivity (even more intense but with a similar pattern) demonstrated in our study points out that this is a non-specific staining independent of cyclin dependent kinase activity of the microorganism. It possibly seems to be a non-specific immunostaining independent from the primary antibody used in laboratory protocol. This possibility may be investigated by using different primary antibodies in immunostaining of Pap smears with *Trichomonas vaginalis*.

As a conclusion, the investigators should be aware of potential misinterpretation of p16 immunoreactive *Trichomonas vaginalis* microorganisms as small dysplastic or malignant epithelial cells in cervicovaginal smears. The presence of this protozoan may prevent conventional use of p16 as an adjunctive marker of HR-HPV infection in cervical screeening.

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