T.C.

YEDİTEPE UNIVERSITY

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THE EVALUATION OF CERVICOVAGINAL FLUID ALPHA-ACTININ-4 (ACTN4) LEVELS IN PATIENTS WITH HIGH RISK GENITAL HPV INFECTIONS

DOCTOR OF PHILOSOPHY THESIS

NİLÜFER ÇETİNKAYA KOCADAL, MD

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

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DECLARATION

I hereby declare that this thesis is my own work and does not contain material previously published or written by anyone else. If needed references are given in the text.

Nilüfer ÇETİNKAYA KOCADAL

DEDICATION

Onuruma… Nazıma… Yarınlarımıza… Mutluluğumuza…

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LIST OF SYMBOLS AND ABBREVIATIONS

ACTN4: Alpha actinin 4

ACS: American Cancer Society

AGC: Atypical glandular cells

ASCCP: American Society for Colposcopy and Cervical Pathology

ASC-H: Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion

ASC-US: Atypical squamous cells of undetermined significance

CAP: The College of American Pathologists

CIN: Cervical intraepithelial neoplasia

HPV: Human papilloma virus

HSIL: High-grade squamous intraepithelial lesion

LSIL: Low-grade squamous intraepithelial lesion

IFCPC: International Federation of Cervical Pathology and Colposcopy

TZ: Transformation zone

Hr-HPV: High risk HPV

P-HPV: Persistant HPV

SM: Squamous metaplasia

ABSTRACT

Cetinkaya Kocadal, N. (2024). The Evaluation of Cervicovaginal Fluid Alpha-actinin-4 (ACTN4) Levels in Patients With High Risk Genital HPV Infections. Yeditepe University, Institute of Health Science, Department of Molecular Medicine, PhD thesis, İstanbul.

Cervical squamous cell lesions are commonly seen in reproductive aged women. HPV type 16 and 18 are responsible for more than two-thirds of lesions. The rate of spontaneous regression within two-three years is nearly 90%. However permanent and/or latent HPV infection in the form of re-infection or re-activation are responsible for dysplasia progression. Therefore close follow-up visits are needed in women with genital HPV infection and new alternative diagnostic methods are under development with the aim of early diagnosis of persistent HPV. Alpha-actinin 4 (ACTN4) is a protein with an actin-binding properties and has a role in remodelling of actin cytoskeleton. It is demonstrated that elevated vaginal ACTN4 levels might have role in cervical dysplasia progression. For this reason we evaluated the relation between vaginal fluid ACTN4 levels and squamous cell lesions in patients with high risk genital HPV infection. A total of 104 patients were grouped according to high risk HPV type and cervical cytological results as follows: Group1: HPV type 16 and/or 18 (+) and cytology ≥ ASCUS, Group 2: HPV type 16 and/or 18 (+) and benign cytology, Group 3: HPV type non-16/18 (+) and cytology ≥ ASCUS, Group 4: Two years of persistent HPV type non-16/18 (+) and benign cytology, Group 5: Histologically diagnosed HSIL regardless of HPV and cytology type and Group 6: Negative HPV and benign cytology. 44.2% (n: 46) of patients were HPV type 16-18 positive and the remaining 46.2% (n:48) were HPV non-16-18 positive. Cervical cytology were benign in 39.4% (n: 41), ASCUS in 29.8% (n: 31), LSIL in 24% (n: 25), ASC-H in 3.8% (n: 4), HSIL in 1.9% (n: 2) and AGC in 1% (n: 1) of patients. Cervical biopsy results were benign in 25% (n: 24), CIN I in 42.7% (n: 41), CIN II in 19.8% (n: 19) and CIN III in 12.5% (n: 12) of patients. The mean vaginal ACTN4 values were measured as follows; 1625.06±67.19 pg/ml, 1687.93±96.22 pg/ml, 1587.87±60.41 pg/ml, 2067.56±70.48 pg/ml, 2850.12±262.19 pg/ml and 1680.37±71.9 pg/ml (Mean±SEM) in Group I to VI. Statistically significant difference was detected among vaginal ACTN4 levels between Group I, II, III, VI and Group V ($p<0.001$). Furthermore there was statistically significant difference among vaginal ACTN4 levels between Group III vs Group IV ($p=0.002$).

In the current case-control study, we found that ACTN4 levels in vaginal fluid were significantly elevated in patients with HSIL independent from type of hr-HPV and/or cervical cytology. As a conclusion we declare to the presence of elevated vaginal ACTN4 levels in

patients with HSIL and ACTN4 has potential diagnostic role for the early diagnosis of HSIL in women with high risk genital HPV infection. Furthermore elevated levels of vaginal ACTN4 in women with normal cytology, hr-HPV type non-16/-18 positivity and benign cervical histology or low grade cervical dysplasia (CIN I) might be related with HPV persistence.

Key words: HPV Infection, Cervical Dysplasia, Cervical Carcinoma

The study was supported by a grant from doctoral thesis student.

ÖZET

Cetinkaya Kocadal, N. (2024). Yüksek Riskli Genital HPV Enfeksiyonu Olan Hastalarda Servikovajinal Sıvı Alfa-aktinin-4 (ACTN4) Düzeylerinin Değerlendirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı, Doktora tezi, İstanbul.

Servikal skuamöz hücre lezyonları sıklıkla üreme çağındaki kadınlarda görülür. HPV tip 16 ve 18 displazilerin çoğundan sorumludur. Servikal displazilerin %90 kadarı kendiliğinden geriler. Bununla birlikte, re-enfeksiyon veya re-aktivasyon nedeniyle persistan HPV enfeksiyonu oluşabilir ve bu durum displaziye ilerlemeden sorumludur. Bu nedenle genital HPV enfeksiyonu olan kadınlarda yakın takiplere ihtiyaç duyulmakta ve persistan HPV'nin erken tanısı amacıyla yeni alternatif tanı yöntemleri geliştirilmektedir. Alfa-aktinin 4 (ACTN4), aktin bağlama özelliğine sahip bir proteindir ve aktin hücre iskeletinin yeniden şekillenmesinde rol oynar. Yüksek vajinal ACTN4 düzeylerinin servikal displaziye ilerlemede rol oynayabileceği gösterilmiştir. Bu çalışmada yüksek riskli genital HPV enfeksiyonu olan hastalarda vajinal sıvı ACTN4 düzeyleri ile skuamöz hücre lezyonları arasındaki ilişkinin değerlendirilmesi amaçlandı. Toplam 104 hasta, yüksek riskli HPV tipi ve servikal sitolojik sonuçlarına göre şu şekilde gruplandırıldı: Grup1: HPV tip 16 ve/veya 18 (+) ve sitoloji ≥ ASCUS olgular, Grup 2: HPV tip 16 ve/veya 18 (+) ve benign sitolojili olgular, Grup 3: HPV tipi non-16/18 (+) ve sitoloji ≥ ASCUS olgular, Grup 4: İki yıllık persistan HPV tip non-16/18 (+) ve benign sitolojili olgular, Grup 5: HPV tipi ve sitolojiden bağımsız histolojik olarak teşhis edilmiş HSIL olgular, Grup 6: HPV testi negatif ve benign sitolojili olgular. Hastaların %44.2'si (n:46) HPV tip 16-18 pozitif, geri kalan %46,2'si (n:48) HPV tip non-16-18 pozitif idi. Servikal sitolojinin %39.4'ü (n:41) benign, %29,8'i (n:31) ASCUS, %24'ü (n:25) LSIL, %3,8'i (n:4) ASC-H, %1,9'u HSIL (n:2) ve AGC hastaların %1'inde (n:1) görüldü. Servikal biyopsi sonuçları hastaların %25'inde (n:24) benign, %42.7'sinde (n:41) CIN I, %19.8'inde (n:19) CIN II ve %12.5'inde (n:12) CIN III olarak izlendi. Ortalama vajinal ACTN4 değerleri gruplarda sırasıyla 1625.06±67.19 pg/ml, 1687.93±96.22 pg/ml, 1587.87±60.41 pg/ml, 2067.56±70.48 pg/ml, 2850.12±262.19 pg/ml ve 1680.37±71.9 pg/ml ml (Ortalama±SEM) olarak saptandı. Grup I, II, III, VI ve Grup V arasında vajinal ACTN4 düzeyleri arasında istatistiksel olarak anlamlı fark olduğu tespit edildi ($p<0.001$). Ayrıca Grup III ile Grup IV arasında vajinal ACTN4 düzeyleri arasında da benzer şekilde istatistiksel olarak anlamlı farklılık saptandı ($p=0.002$). Bu çalışmada yüksek risk HPV tipinden ve/veya servikal sitoloji sonucundan bağımsız olarak HSIL olgularında vajinal sıvıda ACTN4 seviyelerinin anlamlı derecede yüksek olduğu izlendi.

Sonuç olarak, HSIL hastalarında artmış vajinal ACTN4 düzeylerinin yüksek riskli genital HPV enfeksiyonu olan kadınlarda HSIL tansında erken dönemde potansiyel role sahip olduğu görülmüştür. HPV tip non-16/-18 (+), benign sitolojili ve servikal biyopsi sonucu benign veya düşük dereceli servikal displazi (CIN I) olarak saptanan kadınlarda vajinal sıvıda görece artmış ACTN4 seviyeleri persistan HPV tip non-16/-18 enfeksiyonu ile ilişkili olabilir.

Anahtar Kelimeler: HPV İnfeksiyonu, Servikal Displazi, Servikal Kanser

Bu çalışma maddi açıdan tarafımca desteklenmiştir.

1. INTRODUCTION and PURPOSE

Cervical dysplasia and cervical squamous cell cancer are commonly seen in women in their reproductive ages. Human Papilloma Virus (HPV) infection with the prevalence of approximately 10% is known to be the most important factor for developing cervical preinvasive disease and cervical cancer when it resides in female genital tractus and results in persistent HPV infection (pHPV) (1). The precursor lesions of cervical cancer are commonly known as cervical dysplasia and are grouped as cervical intraepithelial neoplasia CIN 1, II and III. CIN II and CIN III are high-grade lesions and if not treated they results in invasive cervical cancer.

Nowadays it is known that there are more than 200 subtypes of HPV, but 40 types have been shown to be related to gynecologic cancers. HPV-16 and -18, which are in the high-risk HPV group, are responsible for more than 90% of cervical cancers seen worldwide. HPV-31, -33, -45, -52 and -58 are also considered as high-risk HPV (hr-HPV) type and they can be detected alone or as a member of co-infection in women with cervical precancerous and cancerous pathologies (2, 3).

HPV infections are usually resolve spontaneously within a few months after viral infection. The rate of spontaneous regression within two-three years in previously infected people is nearly 90% (4). But permanent and/or latent infection are responsible for cancer progression (5). Because of this knowledge evaluation of cervical HPV positivity and cervical cytological examination which means cervical co-test examination is the courner stone of cervical cancer screening algorithyms which emphasize that cervical cancer can be prevented via effective national screening programs in women through early diagnosis and treatment of cervical cancer precursors (6).

Cervical conization or LEEP electrosurgical excision techniques are used for diagnosis and treatment of cervical precursor lesions diagnosed by colposcopic evaluation and cervical biopsy in patients with abnormal co-test results. By this method transformation zone of cervix, which is the most important area of formation of cervical dysplasia and/or cervical cancer, can be excised with its dedifferentiated tissue with changed in structural proteins and the cell cycle properties due to altered gene expression profile because of HPV persistence. However HPV re-infection or re-activation of latent HPV infection can lead to recurrent cervical dysplasia formation with or without formation of cervical cancer (7).

Therefore proper follow-up visits are also needed in treated women. In addition to nowadays screening and diagnostic methods researches are still working on alternative methods to lower the high burden of HPV infections.

Alpha-actinin 4 (ACTN4) is an actin-binding protein in the spectrin superfamily. It is located in the cytoplasm and commonly participates in the cytoskeleton structure, cytokinesis and cell adhesion function. ACTN4 has a role in the formation of cellular protrusions, which results of cellular migration (8-10). For these reasons we hyposized that ACTN4 might have a role in cervical dysplasia progression in hr-HPV infected women. The current study is planned to find any association between degree of cervical dysplasia and vaginal fluid ACTN4 levels with respect to different type of hr-HPV infection.

2. LITERATURE REVIEW

2.1. Structure Of Uterine Cervix

2.1.1. Physiological Histology And Anatomy Of The Uterine Cervix

The uterine cervix is a cylindrical structure located at the bottom of the uterus in the pelvic cavity, with an average length and diameter of 2-3 cm. It opens to the vagina with the external orifice and to the uterine cavity with the internal orifice through endocervical canal.

Mullerian duct and urogenital sinus are two different embryological structures which provide the formation of genital system in women. The uterus, fallopian tubes and the proximal third of the vagina develop from Mullerian system where as distal third of vagina develops from urogenital sinüs (11). During this development process the columnar epithelium of the Mullerian system transforms into squamous epithelium and forms the original squamocolumnar junction (SCJ) in female genital tract. Transformation zone, in which most of the cervical dysplastic changes occurs, is defined as the region between the original-SCJ and the actual SCJ, which is formd under stressful circumstances due to altered vaginal acidity and extrusion of endocervical glandular structure in responce to hormonal alterations (12).

Adult cervical tissue is covered with two different epithelial structures that are in contact with each other at the squamocolumnar junction. Squamous epithelium is located entirely in the ectocervix and it has a multilayered, non-keratinized flat and glycogen-containing cells that results in staining with Lugol solution. Histologically ectocervix composes of basal, parabasal, middle and superficial layes without glandular structures (12).

Most of the cellular activity takes place within the basal layer and as newly formed epithelium organise and matures it reaches the superficial layer from where after a while it detaches and sheds in vaginal fluid. Due to this developmental process vaginal cytological examination can be performed to evaluate cellular dysmorphological changes secondary to HPV related changes via Pap-tests (13). Although endocervical canal is lined by a single layer of columnar epithelium there are numerous endocervical gland structure which secrete mucus that forms a layer that prevents pathogens from passing into the uterine cavity.

The histological and physical properties of the uterine cervix may vary depending on age, hormonal status and parity of women (14). The displacement of single layered columnar

epithelium into stratified squamous epithelium is called squamous metaplasia (SM). This is a normal differentiational process that occurs in response to chronic irritation due to various factors such as inflammation or changes in hormonal status due to cyclic reproductive physiology. SM is irreversible and as it occurs the border of the newly formed actual SCJ gradually shifts upward resulting in the occurence of a transformation zone (TZ) in between them. High mitotic activity and cellular turnover rates results in increased vulnerability of this immature metaplastic cells to toxic or oncogenic factors such as HPV infection. Uncontrolled cellular growth and proliferation of these HPV infected cells can result in abnormal dysplastic epithelium which may lead to invasive cancer with its atypical cells in terms of nuclear abnormality and chromosome content.

The majority of cervical dysplasia and cancer formation is seen in the transformation zone. TZ is classified as theree types according to the visibility of borders of the actual squamocolumnar junction.

• Type I TZ: Entire borders of actual-SCJ can be visualized through the ectocervix.

• Type 2 TZ: Borders of actual-SCJ is partially visible from the ectocervix elong the endocervical canal.

• Type 3 TZ: Borders of actual-SCJ is situated deeply in the cervical canal and it is invisible through the ectocervix (15).

2.2. Risk Factors Of Cervical Dysplasia And Cervical Cancer

2.2.1 Risk Factors Of Cervical Carcinoma

Cervical cancer is the most common type of gynecologic cancer in younged aged women and it is also related with the huge amount of gynecologic in origin cancer related deahths in women (16). Preinvasive lesions and their carcinogenesis mechanisms are well known in nowadays practice and women can be screened throughout worldwide well designed elimination programs to decrease disease burden. Therefore it is important to screen women for preventable well defined risk factors to give women opportunity for the appropriate precautions and treatments choices.

Demonstration of persistent-HPV (p-HPV) infection is the most important predictive factor that indicates the presence of high probablity of cervical dysplasia occurence due to the studies presenting about 99.7% of cervical cancers are linked to cervical HPV positivity (17). Although there are different subtypes of cervical cancer similar risk factors have been identified for squamous as well as cervical adenocarcinoma and they can be listed as follows; presence of

genital HPV infection, multiparity, sexual intercourse in the early reproductive ages and history of multiple sex partners, cigaret consuming, history of past and/or recently diagnosed sexually transmitted disease, oral contraceptive usage and immunosuppressive conditions such as HIV infection and/or history of organ transplantation (18).

Condom usage during sexual intercourse can reduce but not neglect the risk of HPV contamination. It is known that long-term usage of oral contraceptive drugs can be related to increased risk of cervical cancer throug altered degree of cellular susseptibility to HPV infection by altered subcellular metabolism. Furthermore hormonal changes through oral contraceptives increases the squamous metaplastic changes in the transformation zone which is thought to be a cause of increased vulnerability to cervical infection and progression of dysplasia to cervical carcinoma (19).

2.2.2. Human Papilloma Virus (HPV) Infection And Cervical Carcinogenesis

Papillomaviruses; are small, nonenveloped, double-stranded deoxyribonucleic acid (DNA) viruses in the Papillomaviridae family. They have propencity to invade squamous cells and if permanent infection occurs they causes the formation of proliferative lesions throughout the skin, oral cavity, throat, esophagus, nasopharynnx, uterin cervix, vagina, vulva and anogenital region (20).

Infectious process can be visualised as active-transient or latent-persistent types. Spontaneous regression is the most commonly encountered situation after HPV infection. Latent or persistent infection occurs if spontaneous HPV regression dosen't take place. Reactivation of dormant HPV might be presented with recurrent lesions in women with silent or latent HPV infection. However persistent infection increases the likelyhood of dysplastic changes which if not treated for at least a decate leads to cancer formation. Besides of this women can also be re-infected by another type of HPV secondary to new onset sexual partner (7, 21).

HPV have unique and complex molecular structures despite their small size. HPV have specialized DNA structure that includes especially three different functional regions: early region abreveted as *E*, late region abreveted as *L* and long control region abreveted as *LCR*. HPV starts to proliferate in actively proliferating cells when it enters into the cell structure and transcription of early and late regional genes take place for virus particles live cirkle to accomplish. E and L regions code for early and late structural proteins that have role in viral life cycle. HPV genom also had a capability to insert itself into the human DNA if host immunity cannot provide enough resistence to abondate cellular HPV infection. Especially

latent or persistent HPV infection predispose to increased interaction time between host DNA and HPV DNA structures. E6 and E7 proteins are viral transcriptional oncoproteins that can be synthesized abundantly when HPV genom incorporates in to the host DNA and translational products of these genes lead to fragmentation and suppression of cellular retinoblastoma (Rb) and p53 tumor suppressor proteins. E6 protein inhibits cellular p53 and E7 protein inhibits cellular Rb proteinal functions which lead to force carcinogenesis processes (20).

HPV only infects humans. More than 200 types of HPV have been isolated. Some HPV types are accepted as carcinogenic by the International Agency for Research on Cancer (IARC) because they have found to be related with increased cancer rates in women. According to carcinogenetic risk factors HPV types can be classified as high, medium and low risk groups. HPV type-16, -18, -31, -33, -45, -52, -58, -35, -56, -59, -51, -39, -68, -73, -82 are known to be major carcinogenic types whereas type-26, -53 and -66 has medium risks and type-6, -11, -40, -42, -43, -44, -54, -61, -70, -72 and -81 are charecterized as low risk group. Low risk HPV types rarely cause malignancy and they are usually related with papillary lesions called as condyloma. On the contrary high risk HPV types are associated with epithelial cancers mostly gynecologic in origin as cervical squamous cell carcinoma in women (22).

Approximately more than 70% of women with coital activity are known to be infected by HPV throughout their lives (23). Infection is usually not permanent and resolve spontaneously within a few months after viral infection. The rate of spontaneous regression within two years is around 90% (4, 5). If hr-HPV persists for longer than two year the likelihood of spontaneous clearence decreases and the likelihood of occurence of precancerous lesions increases (6).

The most frequently diagnosed hr-HPV infections are HPV type-16 and HPV type-18. Furthermore they are related with increased probability of presence of precancerous lesions and/or advanced stages cervical carcinoma in women when they are detected. HPV type-18 is much more related with cervical adenocarcinoma than type-16. On the contrary HPV type-16 is commonly related with squamous cell pathologies. All of the mentioned dysplastic changes and carcinogenetic processes occurs in the presence of chronic, latent or persistent HPV infection (6).

2.3. Cervical Cancer Screening Strategies

Cervical cancer rates and cancer related mortality ratios can be reduced by effective wordwide accepted national based cervical cancer screening protocols. Since the introduction of effective strategies numbers of newly diagnosed cervical carcinoma patients and cervical

carcinoma mortality have significantly reduced in developed countries. Nowadays early detection of genital HPV infection with PCR tecnology, documentation of abnormal cytology, appropriate cervical visualisation through colposcopy and treating preinvasive cervical dysplastic lesions are important measural factors in national screening and management programs to prevent cervical cancer. The American Society of Colposcopy and Cervical Pathology (ASCCP) recommends cervical screening in women over age 21 (6).

2.3.1. Pap Smear Tests And Bethesda Classification Of Cervical Cytology

Pap smear test is the method of cytological evaluation of the shed cervical cells to diagnose any pathological alterations. Ectocervical surface epithelium around the squamocolumnar junction and transformation zone can be sampled with the help of a brush. Although it has a low sensitivity around 60% in detecting high-grade cervical lesions, effective cytological screening algorithyms are known to increase detection of cervical dysplasia (24). The false negativity rate of Pap Test depends on many factors from false sampling methods to false negative interpretation. Cellular evaluation is performed according to Bethesda Classification system.

Transformation zone is area of interest when performing the Pap Smear test to increase the reliability of pathologic evaluation and obtain accurate results. It is desired that women should'nt have intravaginal medication or tampons or have sexual intercourse recently. If there is a vaginal infection, testing should be postponed after effective vaginal treatment. Conventional smearing ideally should give opportunity see transformational zone components with appropriate amount of endocervical cells in each slides to make assuarence evaluation.

In recent years liquid-based cytology methods known as sure-path or thin-prep have been preferred instead of conventional smearing of cervical smear samples. In liquid-based methods, cervical epithelial cells separated from blood, mucus and secretions in the laboratory environment. Cells are than brought together in a special filter and applied to the slide in a thin layer. Many studies have shown a decrease in inadequate smear results whereas documenting similar sensitivity with conventional method in the diagnosis of pathologic lesions (25).

Nowadays Pap test is used with a combination with cervical HPV PCR tests. According to worldwide accepted general screening algorithym of ASCCP presence of HPV infection influences the management of screening results and screening intervals of surgically managed women based on the probability of having CIN+3 lesion. It is generally accepted that Pap Test is not sensitive enough to make reassurent comments and it is not accepted as a single screening method in women (6, 26).

According to Bethesda Classification System abnormal cellular categorisations are reported as atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells high-grade lesion cannot be excluded (ASC-H); low-grade squamous intraepithelial lesion (LSIL) indicating HPV infection or mild dysplasia (CIN 1); high-grade squamous intraepithelial lesion (HSIL) indicating moderate (CIN 2) and severe dysplasia (CIN 3/CIS); features suggesting invasion, squamous cell carcinoma; atypical endocervical, endometrial or glandular cells; atypical endocervical or glandular cells not otherwise specified (NOS); adenocarcinoma in situ; endocervical adenocarcinoma; atypic glandular cells favor neoplasia, respectively (27, 28).

2.3.2. HPV PCR Tests

Genital HPV is common in women and due to its transient nature screening programs target the women under risk of permanent HPV infection. Primary HPV based secreening test is adviced for women over 25 years old age (29, 30). On the contrary screening with simultaneous cervical cytology and HPV tests which is known to be called as co-test is suggested to women elder than 30 years. Age cuttoffs are determined to risk of HPV persistence. ASCCP recommends The FDA-approved tests for primary HPV screening (6). Qualified HPV screening tests use cervical sampling material for isolisation of HPV DNA and/or its protein metabolites. It is important to choose assays with reliable diagnostic success. The sensitivity and specificity rates for the HR-HPV tests as a primary screening test are reported to be 98.66% and 87.15% (31).

There are specified FDA approved assays for HPV based secreening. Roche Cobas, Onclarity, Digene Hybrid Capture 2, Cervista, Cobas 4800 and Aptima HPV assays are in practical use worldwide (29). HPV DNA tests are usually performed 1, 3 and 5 yearly as a component of co-test for the screening or management purposes. More frequent HPV controls as a repead tests are not effective to conduct alternative therapies.

2.3.3. Histology Of Cervical Intraepithelial Neoplasia

The demonstration of the occurence of invasive cervical carcinoma secondary to cervical precancerous lesions that diagnosed due to cervical screening programs has led to the awarenes of cervical carcinogenesis. The currently used cytopathologic evaluation system of Bethesda Classification is revised in 2014 in the light of the current studies. While squamous intraepithelial lesion (SIL) is used in the terminology of cytological evaluations, cervical

intraepithelial neoplasia (CIN) is used in the terminology of histological alterations within cervical epithelial tissue.

Histologically ectocervical epitelium consists of stratified multilayered squamous epithelim. Mitotically active cells recides in the basalis layer of epithelium and cellular differentiation and maturation leads to upward movement of newly formed cells towards superficial epithelial layer. During this process cellular morphology changes cells become larger, fills with abundant glycogen and cell nucleus decrease in size.

HPV inoculation occurs through the destructed cellular barier secondary to micro trauma and basalis cellular layer becomes invaded. HPV infection of mitotically active cells in basal layer stimulates cellular proliferation as previously described and infected cells move upwards (20). Increased nuclear activity and viral particles forms a perinuclear halo (coilocytic alterations) in virus infected cells and these alterations can be detected via cytological evaluation and reported as low grade squamous intraepithelial lesion. Therefore dysplasia is atypical non-invasive epithelial proliferation and differentiation that affect the layers of the cervical epithelium. Mild dysplasia (cervical intraepithelial neoplasia I, CINI) has been defined as the presence of nuclear atypia, increased mitosis and cellular irregularities in the lower 1/3 of the squamous epithelium. Moderate dysplasia (CIN2) is charecterized by the involvement of distal 2/3 of epithelial layer. Severe dysplasia (CIN3) has involvement more than distal 2/3 of epithelial layer and when the full thickness epithelial layer efected with abnormal cells it is defined as carcinoma in situ (CIS) (32).

Even though it seems easy there are intra- and interobservational diagnostic difficulties-especially with moderate dysplasia-with the histologic CIN terminology. According to these difficulties classification of HPV infection related anogenital squamous cell lesions is changed by LAST (Lower Anogenital Squamous Terminology) criteria in 2012 (32, 33). In the light of these changes two tier classification system LSIL (low grade squamous intraepithelial lesion) and HSIL (high grade squamous intraepithelial lesion) is accepted instead of three tier classification system as CIN1, CIN2 and CIN3. In the light of these alterations CIN1 lesions and coilocytic alterations are classified as LSIL, CIN II lesions interpretation needs special conciderations especially with p16 immunohystochemical staining because dense expression indicates more severe disease such as HSIL whereas low p16 immunhystochemical staining gives reassuring results which means LSIL. CIN III lesions are classified as HSIL (32).

Most of the CIN1 lesions (%60) spontaneously regress within two years of diagnosis. Whereas 1% of CIN1 lesions progress to invasive cancer on the contrary 5% of CIN2 lesions and 12% of CIN3 lesions progress to invasive cancer (34).

2.3.4. Worlwide Accepted ASCCP Guideline For Cervical Cancer Screening And Management Of Abnormal Results

Immediate CIN 3+ risk of women based on current HPV DNA testing and cervical cytology result is important when dealing with efferctive screening. Based on test results we can create there different patient groups with different cancer risk. High risk test are defined as cytological interpretations such as ASC-H, AGC, HSIL, alterations favor invasive carcinoma. These women have >25% risk of cervix cancer at the time of diagnosis. Low risk tests includes hr-HPV-positive ASC-US or hr-HPV-positive LSIL cytology with unknown previous screening history and HPV-positive NILM cytology (negative for intraepithelial lesion or malignancy) occurring at 2 consecutive annual visits. These women have 4.0% risk of cervical cancer at the time of diagnosis. Third group consists of women with test results having risks below 4%. These women generally have hr-HPV-positive NILM cytology, hr-HPV-negative LSIL cytology and hr-HPV negative ASC-US cytology with unknown previous screening. The Clinical Action Threshold theory supports to evaluate women with colposcopy if 4% immediate-CIN 3+ risk is considered (6).

ASCCP recommends routine screening at 5-year intervals using HPV based testing when women have an estimated 5-year CIN 3+ risk of less than 0.15% based on past history and current test results. Repeat testing in 3 years is recommended if women have an estimated 5-year CIN 3+ risk of 0.15% or greater but less than 0.55%. Repeat testing in 1 year is recommended if women have 5-year CIN 3+ risk of 0.55% or greater but less than the threshold for immediate colposcopy (4.0%). When women have an estimated immediate risk of diagnosis of CIN 3+ of 4.0% or greater referral to colposcopy is highly recommended (6, 26).

2.4. Management Of Abnormal Screening Results

2.4.1 Colposcopy

Colposcope is a highly developed optical instrument that allows magnification about 2-40 times of area under evaluated and permits detailed examination of the cervical surface. It can be applied in the outpatient clinic to evaluate abnormal cervical cytology and/or HPV test results and ensures target directed biopsy under naked eye investigation (35).

Administration of 3-5% acetic acid solution to cervical surface facilitates to diagnose any abnormal findings during examination. Green light filter can also demonstrate abnormal vascularity of cervical lesions. Squamous cells of the ectocervix normally seen as smooth pink

because of underlining well vascularized tissue and abcense of cell tuffling secondary to pathologic proliferation. Endocervical glandular cells are seen as tiny pink-red vesicular structure with outsite smooth demarcation line of squamocolumnar junction.

2.4.2. Colposcopy Indications

The American Society of Colposcopy and Cervical Pathology (ASCCP) recommends colposcopic examination in women with a probable immediate risk of diagnosis of CIN 3+ of 4.0% or greater based on history and current results (6, 26).

High risk patients generally have cytologic report of high-grade squamous intraepithelial lesion (HSIL), low-grade squamous intraepithelial lesion (LSIL) with HPV positivity, atypic squamous cell-high grade lesion cannot be excluded (ASC-H), atypic squamous cell of undetermined significance (ASCUS) cytology with concominant any positivity of high risk HPV infection, atypic glandular cell (AGC), adenocarcinoma insitu (AIS) and atypic glandular cell favors neoplasia, HPV 16 or 18 positivity. Also colposcopy is recommended to women with two year persistence of HPV non 16-18 high risk viral infection in the presence of normal cytology. Furthermore in nonpregnant women with all categories of AGC and AIS endometrial biopsy is also recommended with endocervical sampling during colposcopy. Endometrial biopsy is also recommended for nonpregnant women younger than 35 years age at increased risk of endometrial neoplasia according to concominant clinical disease such as presence of abnormal uterine bleeding, chronic anovulation and obesity (6, 26).

Insufficient repeat cytology with high risk HPV positivity, suspicion of invasive disease as a result of post coital bleeding, vulvar itching or long standing abnormal vaginal discharge are other indications for colposcopic evaluation.

2.4.3. Colposcopic Findings

Dysplastic epithelium contains multiple proliferated cell clusters which have dense nuclear activity and becomes visible secondary to acetic acid induced protein damage and they are seen as white colored lesions. Time from application of acetic acid to the appearence of cervical lesions, degree of opacificity, properties of demarcation lines around lesions and vascular changes take attention to not to miss underlining early invasive disease. Lugol's solution (schiller test) provides dark staining of glycogen-rich cervical squamous epithelium when applied to normal cervix. Light-colored areas secondary to application of Lugol solution occurs due to low glycogen content of malign-premalign epithelial cells and it provides the

opportunity to see diseased areas during examination.

International Federation of Cervical Pathology and Colposcopy (IFCPC) 2011 guideline summarizes the minor and major colposcopic changes predicting high-grade cervical lesion/carcinoma (36). According to colposcopic findings minor colposcopic changes are known to be thin acetowhitening, fine mosaisism, fine punctation. On the contrary major colposcopic changes are listed as dense acetowhitening, coarse mosaisism, coarse punctation, sharp border, inner border sign, ridge sign, rag sign, cuffed crypt openings. Atypical vessels, fragile vessels, exophytic lesion, ulceration and necrotic lesions are suspicious for invasion. Where as leukoplakia is a non-specific sign it has intrensic malignity potential between 2-5%. Condyloma, cervical erosion and cervical polyp are miscellaneous finding during colposcopy. In addition to cervical biopsy of suspisious cervical lesions endocervical curretage is recommended in patients with type 2 or 3 transformation zone, HPV 18 positivity, AGC, AIS or atypic glandular cells of undetermined significance favors neoplasia cytology. Random biopsies are not routinly recommended and women with a normal colposcopic findings can be observed without biopsy (36).

2.5. Management Of Histological Abnormalities

2.5.1. Loop Electrosurgical Excision Procedure (LEEP)

Conisation of cervix via surgical blade is known to be frequently performed cervical excisional procedure. However it has the risk of high blood loss, cervical stenosis and cervical insufficiency. For these reasons the LEEP procedure is the most commonly used surgical procedure for the treatment of cervical dysplasia nowadays. LEEP provides excision of the histologically abnormal cervical tissue along with the transformation zone via electrical current and effective in eradicating CIN 2-3 lesions. Some specialized loop shaped wires are used for excision of cervix.

The height of the excised material and the diameter of the bottom circle affects the size of the excised material and function of remaining cervix for the reproductive functions. Generally height of less than 1.5 cm causes no increased risks on future pregnancies. On the contrary increase of height of excised tissue can result in cervical incompetence if more endocervical canal is removed (37).

The most important factor related with dysplasia recurrence after the first treatment attempt of dysplasia is the surgical margin positivity during LEEP procedure. At least 2 mm margin negativity is desired to prevent early disease recurrence. Transformation zone is known

to be involved by dysplasitic lesions which can be multicentric and multifocal. For these reasons complet excision of cervix with one pathologic speciemen is adviced. Suturing of a known part of cervix serves exact localisation of cervical lesions. ECC during LEEP procedure is performed in the presence of have type 2 or type 3 TZ and HPV 18 positivity to ensure the negativity of the endocervical surgical margin (38, 39).

ASCCP recommends immeadiate excisional procedure for nonpregnant patients, 25 years or older with a potential risk of CIN 3+ of 60% or greater based on history and current evaluation results. In the presence of confounder factors colposcopic biopsy is also acceptable. Similarly excisional procedure without previous biopsy confirmation or histologic evaluation with colposcopy and biopsy are both acceptable for nonpregnant women, 25 years or older with a potential risk of CIN 3+ 25% or greater and less than 60% (6, 26).

2.5.2. CIN1 (LSIL) Management

Patients who underwent colposcopic biopsy due to cytological ASCUS or LSIL report and whose biopsy result are hystologically reported to be LSIL should be adviced to performe co-test at the first year visit. If cotest results indicates cellular abnormality or HPV positivity repeat colposcopy should be performed. Patients whose co-test result is negative on two occasion should be converted to routine screening at fifth year (6, 26).

Patients who underwent biopsy due to ASC-H or HSIL with biopsy result of LSIL, it is necessary to reanalyze the histological, cytological and colposcopy evaluations. Further diagnostic measures can be adviced if suspicion of malignancy occurs. Women with a history of HSIL but biopsy showing LSIL, either an eargent diagnostic excisional procedure or observation with HPV based testing and colposcopy at 1 year is acceptable. Patients with ASC-H cytology but LSIL histology on biopsy can be evaluated at 1 year with HPV based co-testing if they have type 1 transformation zone, adequate colposcopic visualisation of ectocervix with absence of endocervical glandular invıolvement. Follow-up with co-test after 12 and 24 months are also be offered and patients with negative results called to have return visit at 3 years and then proceed with long-term surveillance. If any test is abnormal during the observation period repeat colposcopy is recommended in the presence of percistance HSIL cytology either at the first or second-year visit, or ASC-H cytology persist at the second-year visit (6, 26).

Situation of at least two years percistance of histologic LSIL can be managed with further observation but treatment is also acceptable according to ASCCP (6, 26).

2.5.3. CIN2, CIN3 Dysplasia (HSIL)

Treatment is recommended for all patients with a histopathological diagnosis of HSIL. Excisional procedure is preferred in patients with HSIL and ablation is not attractive. Hysterectomy should not be preferred solely because of the concominant risk of cervical malignancy and potential to do incomplete surgery due to less radical surgical attempt due to prefered simple hysterectomy (6, 26).

2.6. Follow Up Strategıes In Women With Treated Cervical Dysplasia

Long term follow-up after low grade cytology results such as HPV positive NILM cytology, ASC-US, or LSIL with biopsy results of histologic LSIL is performed by co-testing at first and second year and if results are negative on two occasion patients should be conducted to routine screening at fifth year (6, 26).

Close follow-up after treatment of hystologic HSIL is needed to prevent the development of recurrent dysplasia and cervical invasive cancer by early diagnosis of cellular alterarions. Current knowledge indicates that posttreatment HPV based testing is more sensitive than cytology alone after LEEP surgery. According to accompanying cellular alterations and high risk HPV positivity colposcopic examination can be performed throughout follow-up (6, 26). Risk of residual and recurrent disease after LEEP is known to vary between 5% and 30% in the literature. Surgical margin positivity, presence of dysplastic endocervical cytology, persistence of high risk HPV infection, endocervical glandular involvement, cigarett smoking and menopausal state are well defined factors associated with recurrent cervical dysplasia. HPV based co-testing at 6 months is preferred. If any tests are positive, colposcopy and targeted biopsies should be performed. ECC is also recommended. If surgical margins are positive for CIN 2+ or ECC involes CIN 2+ repeat excision should be suggested but close observation can also be acceptable if fertility is a special concern. ASCCP recommends annual HPV or cotesting until 3 consecutive negative tests have been obtained after the negative initial HPV based co-testing at 6 months in patients treated for histologic or cytologic HSIL. After the initial intensive surveillance period, continued surveillance at 3-year intervals is recommended for at least 25 years after treatment of HSIL. Literature data supports to advice HPV vaccine to women with the history of cervical dysplasia treatment according to knowledge of decreased rate of persistant HPV infection after vaccine administration (6, 26, 40).

2.7. Human Alpha Actinin-4 (ACTN4) And Its Potential Role In The Cervical Carcinogenesıs

ACTN is an actin-cross linking protein from the spectrin superfamily. It participates in the structure of cytoskeleton and has a role in cellular shape adaptation such as cytokinesis, adhesion, protrusion formation and cell migration (9).

ACTN has four isoforms and recides in the cytoplasm and area of focal adhesion contacts. Alpha-actinin isoforms can be grouped as muscle type (ACTN2 and ACTN3) and non-muscle type isoforms (ACTN1 and ACTN4). Muscle-type isoforms are expressed in both skeletal and smooth muscle tissue and mediate interactions with the Z-disk. On the contrary non-muscle type isoforms are expressed in non-muscle cells and mediate interactions with cell membranes for adhesion and cell migration processes (8, 9).

Nuclear localisation of ACTN4 and its interaction with nuclear proteins has been reported in the literature which may indicate its function in regulation of transcription. There are numerous studies evaluating the mechanisms and potential role of ACTN4 in cancerous cell invasion and metastasis (8).

Recently it was indicated that ACTN4 could promote epithelial-to-mesenchymal transition (EMT) and cercinogenesis via regulating Snail expression, which is known to be dependent on the Akt signaling pathway in cervical cancer (10). ACTN4 effects on cellular invasion is presented to occure through Snail-mediated matrix metalloproteinase-9 (MMP-9) expression at the tissue level. Furthermore ACTN4 expression levels correlate with stabilization of β-catenin, which in turn propose that accumulatioin of β-catenin secondary to ACTN4 induced tumorigenesis due to upregulation of genes involved in cellular proliferation such as cyclin D1 and c-myc. Literature data suggests that expression level of ACTN4 is elevated in human cervical cancer tissue compared to normal cervical tissues. Owing to the fact that ACTN4 have unique role in the potential regulation of cervical carcinogenesis it can be acceptably hypothesized that persistent cervical HPV infection and dysplasia formation may be related to alteration of cervical ACTN4 expression levels (10).

For this purpose a well conducted study was developed simply to evaluate vaginal fluid ACTN4 levels by ELISA in women with p-HPV infection and cervical cytological abnormality as well as in patients with cervical dysplasia.

3. MATERIALS & METHODS

3.1. Etichal Approval

The current prospective-controlled observational study was carried out with the decision of Basaksehir Cam and Sakura City Hospital Ethics Committee with the decision ID KAEK/2022.02.52.

3.2. Patient Selection And Formation Of Study Groups

Sample required for the study and power analysis was calculated by using G*Power 3.1 software. Considering the effect size (f) 0.4, alpha error probability 0.05, power 0.8 and the number of groups as 6, the minimum sample size of the study was calculated to be composed of a total of 90 cases, 15 cases per group, respectively. However it was decided to conduct the study with 20 patients per group, for a total of 120 cases considering possible data loss.

Patients who were admitted to our Gynecologic Oncology outpatient clinic in the Basaksehir Cam and Sakura City Hospital were asked whether or not they wanted to participate in the study. Patients whose vaginal cytological reports were positive for high risk HPV infection with or without concominant cervical cytological abnormality and who agreed to participate in the research were included in the study.

It is planned to form six study groups according to degree of SIL (LSIL or HSIL) with hr-HPV type-16-18 or hr-HPV non-16-18 positivity and with or without abnormal cervical cytology, presence of cervical squamous cell carcinoma regardless of high risk HPV type or cervical cytology report and as a control group it was decided to include patients with benign cervical cytology reports and negative vaginal high risk HPV PCR test. For these reasons inclusion and exclusion criterias were decided to be as follows:

Inclusion criteria were defined as;

- Patients with hr-HPV type -16 and/or -18 (+) and cercival cytology report ≥ ASCUS
- Patients with hr-HPV type -16 and/or -18 (+) and benign cercival cytology report
- Patients with hr-HPV type non-16/18 (+) and cercival cytology report ≥ ASCUS
- Patients with two years of persistent hr-HPV type non-16/18 (+) and benign cercival cytology report

- Patients with histologically diagnosed cervical squamous cell cancer regardless of HPV type and cytology report.
- Asymptomatic patients with negative HPV test and benign cervical cytology report

Exclusion criteria were defined as;

- Patients that do not want to participate in the study
- Patients who have missing data related with high risk HPV type, cervical cytology or cervical biopsy result
- Lack suitable pathology material for the adequate diagnosis of cervical dysplasia

Colposcopic examination was performed to all patients participated in the study as an indication according to ASCCP Guidelines. Cervical biopsy and endocervical curretage were performed if indicated. 5 ml serum physiologic fluid was administered after speculum application through vagina to perform vaginal lavage aspiration for the evaluation of vaginal ACTN4 levels. Samples were centrifuged at 3000 rpm for 10 minutes and than coded supernatants were stored in biochemisty laboratory at -80 degrees for the evaluation after the study completion. Patients in each study groups were decided to be selected according to colposcopic biopsy and ECC pathology results. After the study completion samples were planned to be transfered by preserving cold chain to Institute of Health Sciences, Department of Molecular Medicine for ACTN4 evaluation.

A total of 205 patients were admitted to the study. Owing to heterogeneous positivity of hr-HPV types in some patients such as concominant HPV -16 or -18 and non-16-18 combined infections these patients (n:101) were excluded from the study. According to pathology results it was recognized that there was no patient with the diagnosis of cervical squamous cell carcinoma. There were 8 voluntary women with negative cervical hr-HPV PCR test and benign cervical cytology report. Therefore it was concidered to form new study groups according to available data as follows:

- Group 1: Patients with hr-HPV type -16 and/or -18 (+) and cercival cytology report ≥ ASCUS
- Group 2: Patients with hr-HPV type -16 and/or -18 (+) and benign cervical cytology report
- Group 3: Patients with hr-HPV type non-16/18 (+) and cervical cytology report ≥ ASCUS

- Group 4: Patients with two years of persistent hr-HPV type non-16/18 (+) and benign cercival cytology report
- Group 5: Patients with histologically diagnosed colposcopic cervical biopsy results of HSIL regardless of hr-HPV type and cytology report.
- Group 6: Control patients with negative genital HPV PCR test and benign cervical cytology.

In the end point a post-hoc analysis performed due to alteration of the sample size of the each group with using G*Power 3.1 software. Statistical power of the study calculated as 0.99 in final the version.

Therefore it was decided to be included a total of 104 patients; n:16 patients in each group in the first four groups (Group I-IV), n: 32 patients in Group V (for the comparision between low-and high grade lesions) and n: 8 patients in Group 6 for the measurement of normal limits of vaginal ACTN4 in women with negative HPV PCR test and benign cytology, respectively (Table.1).

Patients data were rewieved retrospectively in accordance with the study protocol. Age, history of gravidity, parity, smoking, menapousal status, high risk HPV type, cervical cytology result, cervical colposcopic biopsy and ECC pathology results, vaginal and serum ACTN4 levels at the time of colposcopy, LEEP pathology results, co-test results at 6^{th} postoperative month and presence of recurrent disease if any were recorded.

GROUP DEFINITIONS	Hr-HPV STATUS* & CERVICAL CYTOLOGY	N
GROUP I	Patients with hr-HPV type -16 and/or -18 (+) and cercival cytology report ≥ ASCUS	**N: 16**
GROUP II	Patients with hr-HPV type -16 and/or -18 (+) and benign cervical cytology report	**N: 16**
GROUP III	Patients with hr-HPV type non-16/18 (+) and cervical cytology report ≥ ASCUS	**N: 16**
GROUP IV	Patients with two years of persistent hr-HPV type non-16/18 (+) and benign cercival cytology report	**N: 16**
GROUP V	Patients with histologically diagnosed colposcopic cervical biopsy results of HSIL regardless of hr-HPV type and cytology report	**N: 32**
GROUP VI	Control patients with negative genital HPV PCR test and benign cervical cytology.	**N: 8**
TOTAL		**N: 104**

Hr-HPV: High Risk HPV
ASCUS: Atypic squamous cell of undetermined significance
LSIL: Low-grade squamous cell lesion
HSIL: High-grade squamous cell lesion
N: Number of patients

Table 1: Presentation of study groups according to patient charecteristics

3.3. Cervicovaginal Fluid Human Alpha-actinin-4 (ACTN-4) Level Measurement

Human alpha-actinin-4 (ACTN-4) ELISA Kit (Shanghai Coon Koon Biotech Co., Cat No:CK-bio-25668) with a sensitivity of 10.0 pg/mL was used for cervicovaginal ACTN4 quantitative determinations in accordance to double antibody sandwich technique known as enzyme linked immunosorbent assay (ELISA).

Parallel to the instructions thawed vaginal aspiration samples and standard were added to the wells that pre-coated with alpha-actinin-4 (ACTN-4) antibody. Then HRP-Conjugate reagent was added and samples were incubated for 60 minutes at 37°C to form immune complexes. Plates were washed 5 times for the removal of unbound enzyme and chromogen solution A and B were added and mixture was awaited for 15 minutes at 37°C for the color development. Secondary to administration of acidic stop solution the bluish color of solution changed into yellow. The color depth which was correlated with the sample concentration of alpha-actinin-4 (ACTN-4) was evaluated with optic dencitometry (OD) at 450 nm within 15

minutes of stop solution administration. Using Microsoft Office Excel for Mac (ver. 16.80) a standard curve was drawn (Figure1) using the binomal equation and corresponding sample concentrations were calculated. A standard curve was generated from different sample dilutions and standard product mixtures according to the manufacturer's instructions.

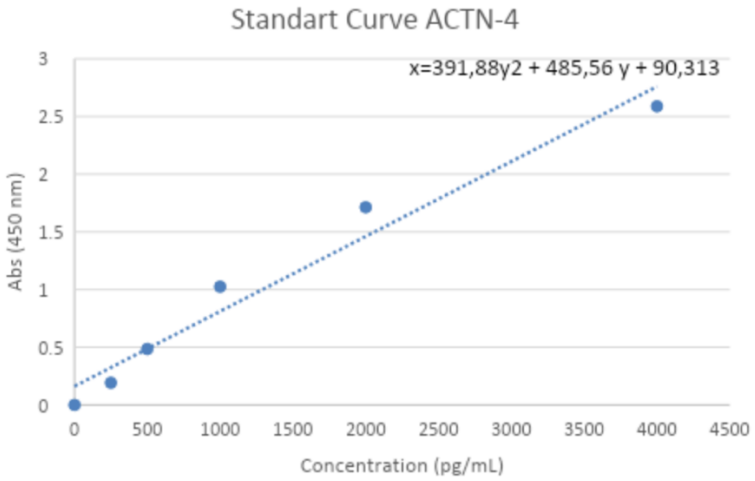

Figure1. Standard curve ACTN4

3.4. Statistical Evaluation

Statistical Analysis was performed with IBM SPSS Statistics 29 for Mac (Statistical Package for Social Sciences for Mac ver.29, IBM Corp, Armonk, NY). Test of homogenity and distribution were evaluated by Kolmogorov-Smirnov and Levene tests between the groups. According to evaluation nonparametric statistical methods were decided to be used in the statistical analysis. Demographic data was examined with descriptive statistics. Numerical data was evaluated by Kruskal Wallis test. Bonferroni correction was performed for the post hoc analysis. Chi-Square Analysis and Fisher-Freeman-Halton Exact tests were used to compare nominal data between the groups. The significance level for all analyzes was determined as $p<0.05$.

4. RESULTS

4.1. Results Of Study Parameters In All Groups:

A total of 104 patients (n: 16 in each group in the Group I-IV, n: 32 in the Group V, n: 8 in the Group VI) were included in the study. Mean age of study population was 38.57±10.86 (Mean±SD) (range: 20-72). Mean gravidity, parity and abortus rate were 2.43±0.20 (Mean±SEM), 1.68±0.15 (Mean±SEM) and 0.55±0.08 (Mean±SEM), respectively. Mean sexual partner count was 1.82±0.2 (Mean±SEM) and 18.8% (n: 18) of patients were in postmenapousal status. Vaginal ACTN4 value was reported to be 2078.28±98.42 pg/ml (Mean±SEM) in the study group.

Majority of patients 81.3% (n: 78) were asymptomatic whereas 11.5% (n: 11) had abnormal vaginal bleeding at the time of study evaluation and the remaining 7.3% (n: 7) were admitted from infertility clinic because of abnormal cervical visualisation. History of pelvic inflammatory disease (PID) was diagnosed in 2.1% (n: 2) of patients. 45.8% (n: 44) patients were current smoker and mean duration time of cigarett smoking was 12.29±1.42 (Mean±SEM) years. 9.4% (n: 9) of patients had a history of OCS usage and mean duration of oral contraceptive usage was recorded to be 2.88±1.17 (Mean±SEM) years.

44.2% (n: 46) of patients were HPV type 16-18 positive and the remaining 46.2% (n:48) were HPV non-16-18 positive. HPV status of the remaining two patients was unknown. Cervical cytological tests were reported to be benign in 39.4% (n: 41), ASCUS in 29.8% (n: 31), LSIL in 24% (n: 25), ASC-H in 3.8% (n: 4), HSIL in 1.9% (n: 2) and AGC in 1% (n: 1) of patients, respectively. Adequate colposcopic examination was performed in 71.2% (n: 74) of patients and transformation zones were recorded as follws: 44.8% (n: 43) type I, 29.2% (n: 28) type II and 26% (n: 25) type III.

A total of 91 cervical biopsy materials of 94.8% (n: 91) of patients and a total of 95 endocervical curettage materials of 99% (n: 95) of patients in all study group were evaluated pathologically for the differential diagnosis of cervical dysplasia. Cervical biopsy results were recorded to be benign in 25% (n: 24), CIN I in 42.7% (n: 41), CIN II in 19.8% (n: 19) and CIN III in 12.5% (n: 12) patients. According to two-tier cervical squamous cell intraepithelial lesion classification 32.3% (n: 31) and 42.7% (n: 41) of patients were reported to have HSIL and LSIL diagnosed by cervical biopsy. ECC results were reported to be benign in 82.3% (n: 79), CIN I

in 10.4% (n: 10), CIN II in 3.1% (n: 3) and CIN III in 4.2% (n: 4) of patients, respectively (Table.2).

HISTOPATHOLOGIC DIAGNOSIS ACCORDING TO COLPOSCOPIC BIOPSY OR LEEP	Hr-HPV STATUS*	CERVICAL CYTOLOGY	CERVICAL HISTOLOGY
GROUP I (N: 16)	Women with hr-HPV type 16/18(+)	Cervical cytology ≥ASCUS	Benign or CIN I (LSIL)
GROUP II (N: 16)	Women with hr-HPV type 16/18 (+)	Cervical cytology Benign	Benign or CIN I (LSIL)
GROUP III (N: 16)	Women with hr-HPV type non-16/18 (+)	Cervical cytology ≥ASCUS	Benign or CIN I (LSIL)
GROUP IV (N: 16)	Women with hr-HPV type non-16/18 (+)	Cervical cytology Benign	Benign or CIN I (LSIL)
GROUP V (N: 32)	Women with hr-HPV (+) (16/18 or non-16/18)	Cervical cytology Benign or ≥ASCUS	CIN II and/or CIN III (HSIL)
GROUP VI (N: 8)	Women with hr-HPV(-)	Cervical cytology Benign	Not applicable
TOTAL: 104 Women			
Hr-HPV: High Risk HPV ASCUS: Atypic squamous cell of undetermined significance LSIL: Low-grade squamous cell lesion HSIL: High-grade squamous cell lesion LEEP: Loop Electrosurgical Excision Procedure			

Table 2: Histopathological diagnosis of study groups according to colposcopic evaluation

A total of 32 patients (33.3%) were treated with LEEP and abnormal pathology results were reported to be CIN I in 12.5% (n: 4), CIN II in 28.1% (n: 9) and CIN III in 50% (n: 16) in patients undergoing LEEP procedure. 9.4% of patients (n: 3) had no recidual dysplastic lesion on LEEP material. The ratio of endocervical glandular involvement by dysplasia was 21.9% (n: 7) and positive surgical margin status was seen in 3.1% (n: 1) of patients. Surgical margin proximity was less than 2 mm in 31.3% (n: 10) patients. The rest of the patients had more than

2 mm proximity to surgical margin (n: 22, 68.8%). ECC sampling after LEEP procedure was performed in all patients and pathological results were reported as follow: benign in 84.4% (n: 27), CIN I in 6.3% (n: 2) and CIN III in 9.4% (n: 3) of patients undergoing LEEP procedure.

All the patients undergoig LEEP procedure were asked to be followed up to 6th postoperative months to obtain cervical cytology and hr-HPV PCR sample evaluation for the estimation of p-HPV infection. HPV PCR test were negative in 34.4% (n: 11) of patients, HPV 16-18 positivity was diagnosed in 3.15% (n: 1) and HPV non-16-18 infection was positive in 25% (n: 8) patients undergoing LEEP procedure. Cytological evaluation results were as follows: benign in 43.8% (n: 14), ASCUS in 12.5% (n: 4) and LSIL/coilocytic alterations in 6.3% (n: 2) patients. HPV status and cytological evaluation of 37.5% of patients (n: 12) undergoing LEEP procedure are unknown because of patients refuse to give follow up sampel.

4.2. Results Of Study Parameters In Each Groups:

4.2.1. Results In Group I: Patients With Hr-HPV Type-16 And/Or -18 (+) And Cercival Cytology Report ≥ ASCUS

Mean age of study population was 39.44±11.21 (Mean±SD) (range: 25-67). Mean gravidity, parity and abortus rate were 2.25±0.51 (Mean±SEM), 1.50±0.36 (Mean±SEM) and 0.63±0.25 (Mean±SEM), respectively. Mean sexual partner count was 1.69±0.23 (Mean±SEM) and 12.5% (n: 2) of patients were in postmenapousal status. Vaginal ACTN4 value was reported to be 1625.06±67.19 pg/ml (Mean±SEM) in this group (Table.3).

Majority of patients 87.5% (n: 14) were asymptomatic whereas 12.5% (n: 2) had abnormal vaginal bleeding at the time of study evaluation. History of pelvic inflammatory disease (PID) was absent in this group. 18.8% (n: 3) patients were current smoker and mean duration time of cigarett smoking was 19.66±8.41 (Mean±SEM) years. 18.8% (n: 3) of patients had a history of OCS usage and mean duration of oral contraceptive usage was recorded to be 2.33±0.66 (Mean±SEM) years.

All the patient had HPV type 16-18 infection. Cervical cytological tests were reported to be ASCUS in 75% (n: 12) and LSIL in 25% (n: 4) of patients. Adequate colposcopic examination was performed in 87.5% (n: 14) of patients and transformation zones were recorded as follws: 56.3% (n: 9) type I, 25% (n: 4) type II and 18.8% (n: 3) type III.

GROUP I	
DEMOGRAPHIC DATA	**N: 16**
Age	39.44±11.21 (Mean±SD)
Gravidity	2.25±0.51 (Mean±SEM)
Parity	1.50±0.36 (Mean±SEM)
Abortus	0.63±0.25 (Mean±SEM)
Sexual Partner Count	1.69±0.23 (Mean±SEM)
Menapousal Status	12.5% (n: 2) Postmenapousal
Presenting Symptom	87.5% (n: 14) Asymptomatic 12.5% (n: 2) AUB
PID	(-)
Smoking	18.8% (n: 3)
OCS Usage	18.8% (n: 3)
Hr-HPV	Hr-HPV type 16 and/or 18 (+)
Cervical Cytology	ASCUS 75% (n: 12) LSIL 25% (n: 4)
Colposcopic Examination	87.5% (n: 14) Adequate
TZ Type	56.3% (n: 9) Type I 25% (n: 4) Type II 18.8% (n: 3) Type III
Cervical Biopsy	Benign 37.5% (n: 6) CIN I 62.5% (n: 10) LSIL 62.5% (n: 10)*
ECC	Benign 81.3% (n: 13) CIN I 18.8% (n: 3)
LEEP	Not applicable
Vaginal ACTN4	1625.06±67.19 pg/ml (Mean±SEM)
SD: Standard Deviation SEM: Standard Error of Mean Hr-HPV: High Risk HPV ASCUS: Atypic squamous cell of undetermined significance LSIL: Low-grade squamous cell lesion CIN Cervical Intraepithelial Neoplasia PID: Pelvic Inflammatory Disease	ECC: Endocervical curetage AUB: Abnormal Uterine Bleeding TZ: Transformation Zone OCS: Oral Contraceptives ACTN4: Alpha Actinin 4 *According to two-tier dysplasia classification N: Number of patients

Table 3: Results of study parameters in Group I

A total of 16 cervical biopsy materials and 15 endocervical curettage materials were evaluated pathologically for the differential diagnosis of cervical dysplasia. Cervical biopsy results were recorded to be benign in 37.5% (n: 6) and CIN I in 62.5% (n: 10) of patients.

According to two-tier cervical squamous cell intraepithelial lesion classification 62.5% (n: 10) of patients were reported to have LSIL diagnosed by cervical biopsy. ECC results were reported to be benign in 81.3% (n: 13) and CIN I in 18.8% (n: 3) of patients. LEEP procedure had no indication in this group.

4.2.2. Results In Group II: Patients With Hr-HPV Type-16 And/Or -18 (+) And Benign Cercival Cytology Report

Mean age of study population was 39.94±12.09 (Mean±SD) (range: 20-71). Mean gravidity, parity and abortus rate were 2.38±0.59 (Mean±SEM), 1.75±0.47 (Mean±SEM) and 0.50±0.25 (Mean±SEM), respectively. Mean sexual partner count was 1.69±0.56 (Mean±SEM) and 18.8% (n: 3) of patients were in postmenapousal status. Vaginal ACTN4 value was reported to be 1687.93±96.22 pg/ml (Mean±SEM) in this group (Table.4).

Majority of patients 81.3% (n: 13) were asymptomatic whereas 18.8% (n: 3) had abnormal vaginal bleeding at the time of study evaluation. History of pelvic inflammatory disease (PID) was 12.5% (n: 2) in this group. 50% (n: 8) patients were current smoker and mean duration time of cigarett smoking was 12.75±3.70 (Mean±SEM) years. 12.5% (n: 2) of patients had a history of OCS usage and mean duration of oral contraceptive usage was recorded to be 1.00±0.001 (Mean±SEM) years.

All the patient had HPV type 16-18 infection. Cervical cytological tests were reported to be benign in all patients. Adequate colposcopic examination was performed in 81.3% (n: 13) of patients and transformation zones were recorded as follws: 43.8% (n: 7) type I, 37.5% (n: 6) type II and 18.8% (n: 3) type III.

A total of 16 cervical biopsy materials and 16 endocervical curettage materials were evaluated pathologically for the differential diagnosis of cervical dysplasia. Cervical biopsy results were recorded to be benign in 31.3% (n: 5) and CIN I in 68.8% (n: 11) of patients. According to two-tier cervical squamous cell intraepithelial lesion classification 68.8% (n: 11) of patients were reported to have LSIL diagnosed by cervical biopsy. ECC results were reported to be benign in 93.8% (n: 15) and CIN I in 6.3% (n: 1) of patients. LEEP procedure was only performed to a patient due to persistent CIN I lesion but there was no displastic lesion diagnosed in the permanent pathology in this group.

GROUP II	
DEMOGRAPHIC DATA	**N: 16**
Age	39.94±12.09 (Mean±SD)
Gravidity	2.38±0.59 (Mean±SEM)
Parity	1.75±0.47 (Mean±SEM)
Abortus	0.50±0.25 (Mean±SEM)
Sexual Partner Count	1.69±0.56 (Mean±SEM)
Menapousal Status	18.8% (n: 3) Postmenapousal
Presenting Symptom	81.3% (n: 13) Asymptomatic 18.8% (n: 3) AUB
PID	12.5% (n: 2)
Smoking	50% (n: 8)
OCS Usage	12.5% (n: 2)
Hr-HPV	Hr-HPV type 16 and/or 18 (+)
Cervical Cytology	Benign (n: 16)
Colposcopic Examination	81.3% (n: 13) Adequate
TZ Type	43.8% (n: 7) Type I 37.5% (n: 6) Type II 18.8% (n: 3) Type III
Cervical Biopsy	Benign 31.3% (n: 5) CIN I in 68.8% (n: 11) 68.8% (n: 11) LSIL*
ECC	Benign 93.8% (n: 15) CIN I in 6.3% (n: 1)
LEEP	6.3% (n:1)
Vaginal ACTN4	1687.93±96.22 pg/ml (Mean±SEM)
SD: Standard Deviation SEM: Standard Error of Mean Hr-HPV: High Risk HPV ASCUS: Atypic squamous cell of undetermined significance LSIL: Low-grade squamous cell lesion CIN Cervical Intraepithelial Neoplasia PID: Pelvic Inflammatory Disease	ECC: Endocervical curetage AUB: Abnormal Uterine Bleeding TZ: Transformation Zone OCS: Oral Contraceptives ACTN4: Alpha Actinin 4 *According to two-tier dysplasia classification N: Number of patients

Table 4: Results of study parameters in Group II

4.2.3. Results In Group III: Patients With Hr-HPV Type Non-16/18 (+) And Cercival Cytology Report ≥ ASCUS

Mean age of study population was 33.81±10.85 (Mean±SD) (range: 20-52). Mean gravidity, parity and abortus rate were 1.81±0.37 (Mean±SEM), 1.19±0.26 (Mean±SEM) and 0.37±0.12 (Mean±SEM), respectively. Mean sexual partner count was 1.44±0.20 (Mean±SEM) and 12.5% (n: 2) of patients were in postmenapousal status. Vaginal ACTN4 value was reported to be 1587.87±60.41 pg/ml (Mean±SEM) in this group (Table.5).

Majority of patients 87.5% (n: 14) were asymptomatic whereas 6.3% (n: 1) had abnormal vaginal bleeding and 6.3% (n:1) patient was administered upon abnormal cervical inspection at the time of infertility workup at the time of study evaluation. History of pelvic inflammatory disease (PID) was absent in this group. 68.8% (n: 11) patients were current smoker and mean duration time of cigarett smoking was 12.90±3.36 (Mean±SEM) years. 12.5% (n: 2) of patients had a history of OCS usage and mean duration of oral contraceptive usage was recorded to be 6.50±5.50 (Mean±SEM) years.

All the patient had type non-16-18 HPV infection. Cervical cytological tests were reported to be ASCUS in 37.5% (n: 6), LSIL in 56.3% (n: 9) and ACC-H 6.3% (n: 1) of patients, respectively. Adequate colposcopic examination was performed in 68.8% (n: 11) of patients and transformation zones were recorded as follws: 37.5% (n: 6) type I, 18.8% (n: 3) type II and 43.8% (n: 7) type III.

A total of 15 cervical biopsy materials and 16 endocervical curettage materials were evaluated pathologically for the differential diagnosis of cervical dysplasia. Cervical biopsy results were recorded to be benign in 31.3% (n: 5) and CIN I in 68.8% (n: 11) of patients. According to two-tier cervical squamous cell intraepithelial lesion classification 68.8% (n: 11) of patients were reported to have LSIL diagnosed by cervical biopsy. ECC results were reported to be benign in 87.5% (n: 14) and CIN I in 12.6% (n: 2) of patients. LEEP procedure had no indication in this group.

GROUP III	
DEMOGRAPHIC DATA	**N: 16**
Age	33.81±10.85 (Mean±SD)
Gravidity	1.81±0.37 (Mean±SEM)
Parity	1.19±0.26 (Mean±SEM)
Abortus	0.37±0.12 (Mean±SEM)
Sexual Partner Count	1.44±0.20 (Mean±SEM)
Menapousal Status	12.5% (n: 2) Postmenapousal
Presenting Symptom	87.5% (n: 14) Asymptomatic 6.3% (n: 1) AUB 6.3% (n:1) Referred due to cervical abnormal visualisation
PID	(-)
Smoking	68.8% (n: 11)
OCS Usage	12.5% (n: 2)
Hr-HPV	Hr-Type non-16-18 HPV (+)
Cervical Cytology	ASCUS 37.5% (n: 6) LSIL 56.3% (n: 9) ACC-H 6.3% (n: 1)
Colposcopic Examination	68.8% (n: 11) Adequate
TZ Type	37.5% (n: 6) Type I 18.8% (n: 3) Type II 43.8% (n: 7) Type III
Cervical Biopsy	Benign 31.3% (n: 5) CIN I 68.8% (n: 11) 68.8% (n: 11) LSIL*
ECC	Benign 87.5% (n: 14) CIN I in 12.6% (n: 2)
LEEP	Not applicable
Vaginal ACTN4	1587.87±60.41 pg/ml (Mean±SEM)
SD: Standard Deviation SEM: Standard Error of Mean Hr-HPV: High Risk HPV ASCUS: Atypic squamous cell of undetermined significance LSIL: Low-grade squamous cell lesion CIN Cervical Intraepithelial Neoplasia PID: Pelvic Inflammatory Disease	ECC: Endocervical curetage AUB: Abnormal Uterine Bleeding TZ: Transformation Zone OCS: Oral Contraceptives ACTN4: Alpha Actinin 4 *According to two-tier dysplasia classification N: Number of patients

Table 5: Results of study parameters in Group III

4.2.4 Results In Group IV: Patients With Two Years Of Persistent Hr-HPV Type Non-16/18 (+) And Benign Cercival Cytology Report

Mean age of study population was 43.94±7.46 (Mean±SD) (range: 29-55). Mean gravidity, parity and abortus rate were 2.94±0.47 (Mean±SEM), 2.00±0.37 (Mean±SEM) and 0.88±0.25 (Mean±SEM), respectively. Mean sexual partner count was 1.75±0.57 (Mean±SEM) and 37.5% (n: 6) of patients were in postmenapousal status. Vaginal ACTN4 value was reported to be 2067.56±70.48 pg/ml (mean±SEM) in this group (Table.6).

Majority of patients 68.8% (n: 11) were asymptomatic whereas 18.8% (n: 3) had abnormal vaginal bleeding and 12.5% (n: 2) patients was administered upon abnormal cervical inspection at the time of infertility workup at the time of study evaluation. History of pelvic inflammatory disease (PID) was absent in this group. 50% (n: 8) patients were current smoker and mean duration time of cigarett smoking was 11.00±2.47 (Mean±SEM) years. 6.3% (n: 1) of patients had a history of OCS usage and mean duration of oral contraceptive usage was recorded to be 2.00±0.01 (Mean±SEM) mounths.

All the patient had type non-16-18 HPV infection. Cervical cytological tests were reported to be benign all (n: 16) patients. Adequate colposcopic examination was performed in 68.8% (n: 11) of patients and transformation zones were recorded as follws: 31.3% (n: 5) type I, 43.8% (n: 7) type II and 25% (n: 4) type III.

A total of 12 cervical biopsy materials and 16 endocervical curettage materials were evaluated pathologically for the differential diagnosis of cervical dysplasia. Cervical biopsy results were recorded to be benign in 50% (n: 8) and CIN I in 50% (n: 8) of patients. According to two-tier cervical squamous cell intraepithelial lesion classification 50% (n: 8) of patients were reported to have LSIL diagnosed by cervical biopsy. ECC results were reported to be benign in 93.8% (n: 15) and CIN I in 6.3% (n: 1) of patients. LEEP procedure had no indication in this group.

GROUP IV	
DEMOGRAPHIC DATA	**N: 16**
Age	43.94±7.46 (Mean±SD)
Gravidity	2.94±0.47 (Mean±SEM)
Parity	2.00±0.37 (Mean±SEM)
Abortus	0.88±0.25 (Mean±SEM)
Sexual Partner Count	1.75±0.57 (Mean±SEM)
Menapousal Status	37.5% (n: 6) Postmenapousal
Presenting Symptom	68.8% (n: 11) Asymptomatic 18.8% (n: 3) AUB 12.5% (n: 2) Referred due to cervical abnormal visualisation
PID	(-)
Smoking	50% (n: 8)
OCS Usage	6.3% (n: 1)
Hr-HPV	Hr-Type non-16-18 HPV (+)
Cervical Cytology	Benign (n:16)
Colposcopic Examination	68.8% (n: 11) Adequate
TZ Type	31.3% (n: 5) Type I 43.8% (n: 7) Type II 25% (n: 4) Type III
Cervical Biopsy	Benign 50% (n: 8) CIN I 50% (n: 8) 50% (n: 8) LSIL*
ECC	Benign 93.8% (n: 15) CIN I 6.3% (n: 1)
LEEP	Not applicable
Vaginal ACTN4	2067.56±70.48 pg/ml (Mean±SEM)
SD: Standard Deviation SEM: Standard Error of Mean Hr-HPV: High Risk HPV ASCUS: Atypic squamous cell of undetermined significance LSIL: Low-grade squamous cell lesion CIN Cervical Intraepithelial Neoplasia PID: Pelvic Inflammatory Disease	ECC: Endocervical curetage AUB: Abnormal Uterine Bleeding TZ: Transformation Zone OCS: Oral Contraceptives ACTN4: Alpha Actinin 4 *According to two-tier dysplasia classification N: Number of patients

Table 6: Results of study parameters in Group IV

4.2.5. Results In Group V: Patients With Histologically Diagnosed Colposcopic Cervical Biopsy Results Of HSIL Regardless Of Hr- HPV Type And Cytology Report

Mean age of study population was 39.28±11.3 (Mean±SD) (range: 22-72). Mean gravidity, parity and abortus rate were 2.59±0.37 (Mean±SEM), 1.81±0.29 (Mean±SEM) and 0.47±0.12 (Mean±SEM), respectively. Mean sexual partner count was 2.19±0.44 (Mean±SEM)

and 15.6% (n: 5) of patients were in postmenapousal status. Vaginal ACTN4 value was reported to be 2850.12±262.19 pg/ml (Mean±SEM) in this group (Table.7).

Majority of patients 81.3% (n: 26) were asymptomatic whereas 12.5% (n: 4) had been remitted from IVF clinic and 6.3% (n: 2) of patients had abnormal vaginal bleeding at the time of study evaluation. History of pelvic inflammatory disease (PID) was absent in this group. 43.8% (n: 14) patients were current smoker and mean duration time of cigarett smoking was 10.71±2.12 (Mean±SEM) years. 3.1% (n: 1) of patients had a history of OCS usage and mean duration of oral contraceptive usage was recorded to be 2±0.1 (Mean±SEM) years.

43.8% (n: 14) of patients were HPV type 16-18 positive and 50% (n:16) were HPV type non-16-18 positive whereas the remaining 2 patients HPV status are unknown. Cervical cytological tests were reported to be benign in 3.1% (n:1) patients, ASCUS in 40.6% (n: 13), LSIL in 37.5% (n: 12), ASC-H in 9.4% (n: 3), HSIL in 6.3% (n: 2) and AGC in 3.1% (n:1) of patients, respectively. Adequate colposcopic examination was performed in 78.1% (n: 25) of patients and transformation zones were recorded as follws: 50% (n: 16) type I, 25% (n: 8) type II and 25% (n: 8) type III.

A total of 32 cervical biopsy materials and 32 endocervical curettage materials of patients in this group were evaluated pathologically for the differential diagnosis of cervical dysplasia. Cervical biopsy results were recorded to be CIN I in 3.1% (n: 1), CIN II in 59.4% (n: 19) and CIN III in 37.5% (n: 12) of patients. According to two-tier cervical squamous cell intraepithelial lesion classification 96.9% (n: 31) of patients were reported to have HSIL diagnosed by cervical biopsy. ECC results were reported to be benign in 65.6% (n: 21), CIN I in 12.5% (n: 4), CIN II in 9.4% (n: 3) and CIN III in 12.5% (n: 4) of patients, respectively.

All patients were treated with LEEP and abnormal pathology results were reported to be benign in 9.4% (n: 3), CIN I in 12.5% (n: 4), CIN II in 28.1% (n: 9) and CIN III in 50% (n: 16) in LEEP material. The ratio of endocervical glandular involvement by dysplasia was 21.9% (n: 7) and positive surgical margin status was seen in 3.1% (n: 1) of patients. Surgical margin proximity was less than 2 mm in 31.3% (n: 10) patients. The rest of the patients had more than 2 mm proximity to surgical margin (n: 22, 68.8%). ECC sampling after LEEP procedure was performed in all patients and pathological results were reported as follow: benign in 84.4% (n: 27), CIN I in 6.3% (n: 1) and CIN III in 9.4% (n: 3) of patients undergoing LEEP procedure.

GROUP V	
DEMOGRAPHIC DATA	**N: 32**
Age	39.28±11.3 (Mean±SD)
Gravidity	2.59±0.37 (Mean±SEM)
Parity	1.81±0.29 (Mean±SEM)
Abortus	0.47±0.12 (Mean±SEM)
Sexual Partner Count	2.19±0.44 (Mean±SEM)
Menapousal Status	15.6% (n: 5) Postmenapousal
Presenting Symptom	81.3% (n: 26) Asymptomatic 6.3% (n: 2) AUB 12.5% (n: 4) Referred from IVF Clinic
PID	(-)
Smoking	43.8% (n: 14)
OCS Usage	3.1% (n: 1)
Hr-HPV	43.8% (n: 14) Hr-Type 16-18 HPV (+) 50% (n:16) Hr-Type non-16-18 HPV (+)
Cervical Cytology	Benign 3.1% (n:1) ASCUS 40.6% (n: 13) LSIL 37.5% (n: 12) ASC-H 9.4% (n: 3) HSIL 6.3% (n: 2) AGC 3.1% (n:1)
Colposcopic Examination	78.1% (n: 25) Adequate
TZ Type	50% (n: 16) Type I 25% (n: 8) Type II 25% (n: 8) Type III
Cervical Biopsy	CIN I 6.3% (n: 1) CIN II 59.4% (n: 19) CIN III 37.5% (n: 12) 96.9% (n: 31) HSIL*
ECC	Benign 65.6% (n: 21) CIN I 12.5% (n: 4) CIN II in 9.4% (n: 3) CIN III in 12.5% (n: 4)
LEEP	All patients (n: 32)
Vaginal ACTN4	2850.12±262.19 pg/ml (Mean±SEM)
SD: Standard Deviation SEM: Standard Error of Mean Hr-HPV: High Risk HPV ASCUS: Atypic squamous cell of undetermined significance LSIL: Low-grade squamous cell lesion CIN Cervical Intraepithelial Neoplasia PID: Pelvic Inflammatory Disease	ECC: Endocervical curetage AUB: Abnormal Uterine Bleeding TZ: Transformation Zone OCS: Oral Contraceptives ACTN4: Alpha Actinin 4 *According to two-tier dysplasia classification N: Number of patients

Table 7: Results of study parameters in Group V

All the patients undergoig LEEP procedure were asked to be followed up to 6th postoperative months to obtain cytologic test and hr-HPV PCR sample evaluation for the

estimation of persistent HPV infection. HPV PCR test were negative in 34.4% (n: 11) of patients, HPV 16-18 positivity was diagnosed in 3.15% (n: 1) and HPV non-16-18 infection was positive in 25% (n: 8) patients undergoing LEEP procedure. Cytological evaluation results were as follows: benign in 43.8% (n: 14), ASCUS in 12.5% (n: 4) and LSIL/coilocytic alterations in 6.3% (n: 2) patients, respectively. HPV status and cytological evaluation of 37.5% of patients (n: 12) undergoing LEEP procedure are unknown because of patients refuse to give follow up sampel (Table.8).

COTEST RESULTS OF GROUP V	
DEMOGRAPHIC DATA	**N:32**
Hr-HPV postoperative 6th months	34.4% (n: 11) Negative 3.15% (n: 1) Hr-HPV 16/18 (+) 25% (n: 8) Hr-HPV non-16-18 (+) 37.5% (n: 12) Unknown
Cytology postoperative 6th months	43.8% (n: 14) Benign 12.5% (n: 4) ASCUS 6.3% (n: 2) LSIL/Coilocytic alterations 37.5% (n: 12) Unknown
Hr-HPV: High Risk HPV ASCUS: Atypic squamous cell of undetermined significance LSIL: Low-grade squamous cell lesion	N: Number of patients

Table 8: Postoperative 6th months follow-up data of Group V patients

4.2.6 Results In Group VI: Control Patients With Negative Genital Hr-HPV PCR Test And Benign Cervical Cytology

This group was composed of n: 8 patients with negative genital HPV PCR test and cervical cytology. Patients were admitted from cervical cancer screening program. Mean age of of this group was 30±0.1 (Mean±SD). Patients were completely asymptomatic, with no history of gravidity and cigarette smoking or OCS usage. Vaginal ACTN4 value was reported to be 1680.37±71.9 pg/ml (Mean±SEM) in this group (Table.9).

GROUP VI	
DEMOGRAPHIC DATA	**N: 8**
Age	30±0.1 (Mean±SD)
Menapousal Status	(n:8) Premenapousal
PID	(-)
Smoking	(-)
OCS Usage	(-)
Hr-HPV	(-)
Cervical Cytology	Benign
Vaginal ACTN4	1680.37±71.9pg/ml (Mean±SEM)
SD: Standard Deviation SEM: Standard Error of Mean Hr-HPV: High Risk HPV PID: Pelvic Inflammatory Disease	OCS: Oral Contraceptives ACTN4: Alpha Actinin 4 N: Number of patients

Table 9: Results of study parameters in Group VI

4.3. Comparision Of Parameters Between The Groups:

When all the groups were compared with each other along the study parameters there was no statistically significant difference between the groups related with degree of gravidity, parity and abortus rates, duration time of cigarette smoking and OCS usage and sexual partner count ($p>0.05$). On the contrary vaginal ACTN4 levels were statistically significantly different between the groups ($p<0.001$). There was also statistically significant difference among patients age between the groups ($p<0.006$). The mean age of Group III was statistically significantly younger than Group IV ($p=0.003$).

The mean vaginal ACTN4 values were measured as 1625.06±67.19 pg/ml (Mean±SEM) in Group I, 1687.93±96.22 pg/ml (Mean±SEM) in Group II, 1587.87±60.41 pg/ml (Mean±SEM) in Group III, 2067.56±70.48 pg/ml (Mean±SEM) in Group IV, 2850.12±262.19 pg/ml (Mean±SEM) in Group V and 1680.37±71.9 pg/ml (Mean±SEM) in Group VI, respectively. When the vaginal ACTN4 levels were evaluated by pairwise comparisons there was detected statistically significant difference among vaginal ACTN4 levels between Group I vs Group V ($p<0.001$), Group II vs Group V ($p<0.001$), Group III vs Group V ($p<0.001$), and Group VI vs Group V ($p<0.001$), respectively. Furthermore there was also statistically significant difference among vaginal ACTN4 levels between Group III vs Group IV ($p=0.002$) (Table.10).

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	P value
ACTN4 pg/ml (Mean±SEM)	1625.06±67.19	1687.93±96.22	1587.87±60.41	2067.56±70.48	2850.12±262.19	1680.37±71.9	**P<0.001* P=0.002****
ACTN4: Alpha Actinin 4 *Group I, II, III, VI vs Group V **Group III vs Group IV							

Table 10: Comparision of ACTN4 levels between study groups

Nominal data comparision gave no statistically significant difference between the groups with respect to history of PID, patients presenting symptoms, current smoking rate, history of current OCS usage, menopausal status, rate of adequate colposcopy, TZ type, cervical biopsy and ECC performation rates and histologic diagnosis of ECC materials during colposcopic evaluation, respectively. On the contrary types of high risk HPV infection rates, abnormal cervical cytology results, pathologic diagnosis of colposcopic cervical biopsy materials, LEEP operation rate was statistically significantly different between the groups as a function of study design ($p<0.001$). Abnormal cervical cytological tests results and pathologic diagnosis of high grade squamous cell lesions in cervical biopsy materials were mostly seen in Group V patients ($p<0.001$). Moreover histopathologic evaluation of cervical biopsy materials demonstrated the precence of mostly benign and low-grade squamous cell lesions in Group I-IV patients ($p<0.001$).

We also analysed the vaginal ACTN4 levels in accordance to grouping of study population parallel to hr-HPV type and grade of cervical dysplasia. To see the effects of HSIL presence on vaginal ACTN4 levels in HPV positive women we restratified the study groups with the following criteria (Table.11);

- Group I (n:32), women with hr-HPV type-16 and/or -18 positivity and benign and/or LSIL cervical histology
- Group II (n:32), women with hr-HPV non-16/-18 positivity and benign and/or LSIL cervical histology
- Group III (n:32), women with histologically diagnosed HSIL regardless of hr-HPV type and cytology report
- Group IV (n:8), women with negative genital HPV PCR test and benign cervical cytology.

GROUP DEFINITIONS	Hr-HPV STATUS* & CERVICAL HISTOPATHOLOGY	N
GROUP I	Women with Hr-HPV type-16 and/or -18 positivity and benign and/or LSIL cervical histology	**N: 32**
GROUP II	Women with Hr-HPV non-16/-18 positivity and benign and/or LSIL cervical histology	**N: 32**
GROUP III	Women with histologically diagnosed HSIL regardless of Hr-HPV type and cytology report	**N: 32**
GROUP IV	Women with negative genital HPV PCR test and benign cervical cytology	**N: 8**
TOTAL		**N: 104**
Hr-HPV: High Risk HPV N: Number of patients		

Table 11: Definition of Restratified Study Groups

According to new grouping the mean vaginal ACTN4 values were measured as 1656.06±58.00 pg/ml (Mean±SEM) in Group I, 1827.71±62.77 pg/ml (Mean±SEM) in Group II, 2850.12±262.19 pg/ml (Mean±SEM) in Group III and 1680.37±71.90 pg/ml (Mean±SEM) in Group IV, respectively. There was statistically significant difference beween Group I, II, IV and Group III ($p<0.0001$) (Table.12).

Restratified Study Groups					
	GROUP I	**GROUP II**	**GROUP III**	**GROUP IV**	**P value**
ACTN4 pg/ml (Mean±SEM)	1656.06±58.00	1827.71±62.77	2850.12±262.19	1680.37±71.90	**<0.0001***
ACTN4: Alpha Actinin 4 *Group I, II, IV vs III					

Table 12: Comparision of ACTN4 levels between the Restratified Study Groups

Furthermore when vaginal ACTN4 level comparisions were made in all women according to hr-HPV type, we found absence of statistically significant difference of ACTN4 levels between patients with hr-HPV type-16-18 and type non-16/18 ($p> 0.05$). Similarly vaginal ACTN4 evaluation according to cervical cytology results such as normal benign appearing cytology and precence of any abnormal cytological alteration (≥ASCUS) presented the absence of statistically significant difference of ACTN4 levels between patients with normal and abnormal cytology. On the contrary vaginal ACTN4 evaluation according to histopathologic alterations such as LSIL and HSIL demonstrated the presence of statistically significant difference of ACTN4 levels between patients with CIN I and CIN II-III lesions ($p<001$). According to regression analysis presence of cervical histopathologic alterations commonly HSIL (CIN II-III) was detected to be the most important predictive factor for the demonstration of elevated vaginal ACTN4 levels in patients ($p<0.001$) (Table.13).

N: 72	CIN I (LSIL) N: 41	CIN II-III (HSIL) N: 31	P value
ACTN4 pg/ml (Mean±SEM)	1736.24±57.49	2863.51±270.43	**P<0.001***
ACTN4: Alpha Actinin 4			

Table 13: Comparision of ACTN4 levels according to Cervical Histopathology

5. DISCUSSION and CONCLUSION

Cervical squamous cell lesions are commonly seen in reproductive aged women. HPV type 16 and 18 are responsible for more than two-thirds of lesions. The rate of spontaneous regression within two-three years is nearly 90%. However permanent and/or latent HPV infection in the form of re-infection or re-activation are responsible for dysplasia progression. Therefore close follow-up visits are needed in women with genital HPV infection and new alternative diagnostic methods are under development with the aim of early diagnosis of persistent HPV.

Alpha-actinin 4 (ACTN4) is a protein with an actin-binding properties and has a role in remodelling of actin cytoskeleton (9). There are numerous studies presenting the role of ACTN4 in the proliferation and invasion of different cancers (41-45). It is demonstrated that elevated vaginal ACTN4 levels might have role in cervical dysplasia progression and cervical cancer formation.

Previously An et all presented the role of ACTN4 in the progression of cervical cancer and the epithelial-to-mesenchymal transition (EMT). They found that ACTN4 induced EMT by upregulating Snail, which was dependent on the Akt signaling pathway in cervical cancer. ACTN4 induced cell migration and invasion through Snail-mediated matrix metalloproteinase-9 expression (10).

Zhu et all evaluated the value of combined serum ACTN4 and Squamous cell carcinoma antigen (SCC-Ag) detection in women with CIN3+ and demonstrated the promising effect of ACTN4 and SCC-Ag serological biomarker for CIN3 or worse lesions (46). They also presented the cervical ACTN4 mRNA levels was related with the FIGO stage, tumour size, and lymph node metastasis (46).

Ma et all observed that serum levels of ACTN4 in cervical cancer patients were statistically higher than those in CIN patients as well as healthy controls. At the optimal cutoff of 40.62 pg/mL they indicated the sensitivity and specificity were, 76.3% and 87.7%, suggesting that serum ACTN4 might be a potential diagnostic biomarker for cervical cancer. However, they presented that serum ACTN4 levels were not significantly different between the CIN group and the control group. In theiı study they evaluated a total of 52 CIN patients but in their article they did demonstrate neither the dysplasia grade nor the type of hr-HPV status (45).

Raemdonck et all pointed the potential value of vaginal ACTN4 as a promising candidate biomarker for the precancerous cervical lesions. They presented a threshold of 18 pg/ml which can be set as the highest value of ACTN4 for the non-infected condition. They found no linear correlation between the viral load and the ACTN4 concentration and pointed that ACTN4 is not just a marker for HPV infection but rather for precancerous stage (47).

Therefore, we hypothesized that vaginal ACTN4 levels may change according to hr-HPV type and concomitant cervical cytology status as well as grade of cervical dysplasia. For this purposes we formed study groups according to hr-HPV type, cervical cytology results and cervical biopsy histology to see the effects of different parameters on vaginal ACTN4 levels.

Based on demographic variables we did not demonstrate any significant difference between the study groups related with with degree of gravidity, parity and abortus rates, duration time of cigarette smoking and OCS usage as well as sexual partner count. Although high parity rate, cigaret smoking, OCS use and sexual partner count can effect the rate of cervical dysplasia formation we could not present any differences between the study groups related with these parameters.

The mean vaginal ACTN4 values were higher in this study when compared to previously published studies (46). But because of limited number of studies in the literature this should be interpreted carefully.

When the vaginal ACTN4 levels were evaluated by pairwise comparisons we found that cevical cytology has no impact on vaginal ACTN4 levels in women with benign cervical histology or low grade cervical dysplasia (CIN I) and hr-HPV type -16 and/or -18 infection. Although we found significant difference of vaginal ACTN4 levels in women with hr-HPV type non-16-18 infection with benign cervical histology or low grade cervical dysplasia (CIN I) and with or without presence of smear abnormality, similarly this difference was not related with cytological abnormality. We found that vaginal ACTN4 levels in women with benign cervical histology or low grade cervical dysplasia (CIN I) and hr-HPV type non-16-18 infection was higher in women with normal cytology which seems to be opposite to the expected. But because colposcopy indicated in patients with at least two years persistence of HPV non-16/18 infection in the presence of normal cervical cytology but immeadiate colposcopy adviced if cytologic abnormality encountered, elevated levels of vaginal ACTN4 measurements in women with normal cytology and benign cervical histology or low grade cervical dysplasia (CIN I) and hr-

HPV type non-16/-18 can be postulated to be related with persistent HPV infection rather than smear abnormality itself.

We demonstrated that there were no significant difference of ACTN4 levels between patients with hr-HPV type-16-18 and type non-16/18 or with normal and abnormal cytology. However vaginal ACTN4 values were higher in women with HSIL histology with respect to control group and women with benign cervical histology or low grade cervical dysplasia (CIN I) with hr-HPV type-16 and/or -18 with and without cervical cytological abnormality and women with benign cervical histology or low grade cervical dysplasia (CIN I) with hr-HPV type non-16/-18 infection with cytological abnormality. Furthermore vaginal ACTN4 levels in women with histological diagnosis of LSIL and HSIL were detected to be significantly higher in women with HSIL apart from hr-HPV type.

However, there are several limitations in the present study. Although the sample size was relatively higher than the studies in the literature, considering the heterogenous nature of hr-HPV infection life cycle, relation between cytologic abnormalities and personal propensity for cervical carcinogenesis it should be acknowledged that the number of cases in the study is relatively low which actualy reduce the statistical power of our results in reality. Second, because we had limited number of control case we could not analyse neither the sensitivity, specifity analysis nor positive and negative predictive values in women with HSIL. Third, we only explored the relationship between vaginal ACTN4 and HSIL, and serum evaluations were not examined, which needs to be improved in the future studies. On the contrary this study ensures the additional information of vaginal ACTN4 levels with respect to literature with its well stratified study groups including cervical cytology, hr-HPV type and grade of cervical dysplasia.

In the current case-control study, we found that ACTN4 levels in vaginal fluid were significantly elevated in patients with HSIL independent from type of hr-HPV and/or cervical cytology. Furthermore elevated levels of vaginal ACTN4 in women with normal cytology, hr-HPV type non-16/-18 positivity and benign cervical histology or low grade cervical dysplasia (CIN I) might be related with persistent HPV infection.

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7. APPENDICES

7.1. Ethical Approval

T.C. SAĞLIK BAKANLIĞI BAŞAKŞEHİR ÇAM ve SAKURA ŞEHİR HASTANESİ - İSTANBUL
BAŞAKŞEHİR ÇAM ve SAKURA ŞEHİR HASTANESİ GELEN EVRAK BİRİMİ
15.02.2022 16:41 - E-96317622-000-6410

T.C.
İSTANBUL VALİLİĞİ
İL SAĞLIK MÜDÜRLÜĞÜ
Başakşehir Çam ve Sakura Şehir Hastanesi

Sayı: 2022.02.52

Konu No: KAEK/2022.02.52

Doç. Dr. Nilüfer KOCADAL ÇETİNKAYA

Kurulumuz çoğunluğunun katılımı ile klinik araştırmalar etik kurulu toplantısı yapılmış olup; Yürütücüsü olduğunuz 'Yüksek riskli enital hpv enfeksiyonu bulunan ol ulularda servikovajinal sıvıda alph-actının-4 seviyelerinin değerlendirilmesi'konulu araştırma dosyası klinik araştırmalar etik kurulunca örüşülüp oy birliği ile uy un olduğuna karar verilmiştir.

Gereği bilgilerinize sunulur.

Başakşehir Çam ve Sakura Şehir Hastanesi Klinik Araştırmalar Etik Kurulu
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8. CURRICULUM VITAE

Personal Data

Name	Nilüfer	**Surname**	ÇETİNKAYA KOCADAL
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Education

Medical Faculty	Medical Faculty	Istanbul University, Cerrahpasa Medical Faculty, Department of Uğur Derman English Program	2000-2006
Specialization	Specialization	Yeditepe University Hospital, Department of Obstetrics & Gynecology	2006-2011
Subspecialisation	Subspecialisation	Zekai Tahir Burak Women Health Education & Research Hospital, Department of Gynecologic Oncology	2013-2016
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Work Experience

1	Zeynep Kamil Education & Research Hospital	Departmanet of Obstetric & Gynecology	2011-2012
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4	Başakşehir Çam & Sakura City Hospital	Department of Gynecologic Oncology	2020-Cont.

EDUCATION AND QUALIFICATIONS

Master Of Obstetrıcs & Gynecology Thesis:

'*Oferektomize Rat Endometriyoziz Modelinde Melatonin Doz ve Süre Çalışması'* Yrd. Doç. Dr. Rukset ATTAR

SCIENTIFIC STUDIES*:*

A.International Studies:

A1. Baykal, C., Al, A., Ugur, M. G., Cetinkaya, N., Attar, R., & Arioglu, P. (2008). Knowledge and interest of Turkish women about cervical cancer and HPV vaccine. European journal of gynaecological oncology, 29(1), 76.

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B. Internationally Accepted Congress Presentations:

B1. Cetinkaya N, Attar R, Yildirim G, yilmaz B, Ficicioglu C, Yesildaglar N, "The Effects Of Melatonin On Endometriotic Implants In An Ooferectomızed Rat Endometriosis Model", 19th International Pelvic Pain Society Annual Scientific Meeting, Istanbul, 26-29 May, 2011 (Poster Presentation).

B2. Cetinkaya N, Bas S, Bayramoglu H, Erkaya S, Gungor T, "Atypic Carcinoid Tumor: A Member Of Cervical Neuroendocrine Tumors", 7th Hellenic & 3rd Turkish joint Congress on Colposcopy and Cervical Pathology, Athens, Greece, 11-13 October, 2013 (e-Poster Presentation).

B3. Cetinkaya N, Erkaya S, Ozgu E, Sirvan L, Gungor T, "Malign Mixed Mullerian Tumor Of Cervix; A Case Report", 7th Hellenic & 3rd Turkish joint Congress on Colposcopy and Cervical Pathology, Athens, Greece, 11-13 October, 2013 (e-Poster Presentation).

B4. Cetinkaya N, Ozgu E, Akbay S, Erkaya S, Gungor T, "Illustration Of Silent Gastrointestinal Malignancy Thought As Cervical Adenocarcinoma: A Case Report", 7th Hellenic & 3rd Turkish joint Congress on Colposcopy and Cervical Pathology, Athens, Greece, 11-13 October, 2013 (e-Poster Presentation).

B5. Gungor T, Cetinkaya N, Yalcin H, Bayramoglu H, Erkaya S, "Synchronous Endometrial Adenocarcinoma With Cervical Neuroendocrine Tumor", 7th Hellenic & 3rd Turkish joint Congress on Colposcopy and Cervical Pathology, Athens, Greece, 11-13 October, 2013 (e-Poster Presentation).

B6. Baser E, Ozgu E, Erkilinc S, Yalcin H, Cetinkaya N, Sirvan L, Erkaya S, Gungor T, ''Clinical outcomes of cases with cervical dysplasia absent in cold knife conization specimens''. 7th Hellenic & 3rd Turkish joint Congress on Colposcopy and Cervical Pathology, Athens, Greece, 11-13 October, 2013 (e-Poster Presentation).

B7. Cetinkaya N, Attar R, Yildirim G, Ozkan F, Ficicioglu C, Yesildaglar N, "The Effects Of Different Doses Of Melatonin On Endometriotic Lesions In An Ooferectomized Rat Endometriosis Model", Conjoint Meeting of the International Federation of Fertility Societies

and the American Society for Reproductive Medicine, Boston, Massachusetts, 12- 17 October, 2013 (e-Poster Presentation).

B8. Gungor T, Cetinkaya N, Zergeroglu, Yalcin H, Erkaya S, Yakut H.I., "Malign Mesenchymal Tumors Of Uterus In Young Aged Women", 18th International Meeting Of The European Society Of Gynaecological Oncology (ESGO) Scheduled To Take Place In Liverpool, UK, October 19-22, 2013 (e-Poster Presentation).

B9. Gungor T, Cetinkaya N, Ozdal B, Yalcin H, Erkaya S, Yakut H.I., "Endometrial Adenocarcinoma In Young Women: Single Center Experience", 18th International Meeting Of The European Society Of Gynaecological Oncology (ESGO) Scheduled To Take Place In Liverpool, UK, October 19-22, 2013 (e-Poster Presentation).

B10. Gungor T, Cetinkaya N, Helvacioglu Y, Akbay S, Yildirim E, "Resistance Against Cancer, Took The Baby Home", 18th International Meeting Of The European Society Of Gynaecological Oncology (ESGO) Scheduled To Take Place In Liverpool, UK, October 19- 22, 2013 (e-Poster Presentation).

B11. Gungor T, Cetinkaya N, Ozgu E, Yalcin H, Bayramoglu H, Uygur D, "Gynecologic Malignancy In Women With Mullerian Anomalies: Report Of Four Distinct Cases", 18th International Meeting Of The European Society Of Gynaecological Oncology (ESGO) Scheduled To Take Place In Liverpool, UK, October 19-22, 2013 (e-Poster Presentation).

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D2. Yıldırım G, Attar R, Çomunoğlu N, Özkan F, İzmit E, Çetinkaya N, İnan Y, Karateke A, "Appendiksi Perfore Ederek Batın İçine Yayılmış Musinöz Adenokarsinoma Bağlı Bir Pseudomiksoma Peritonei Olgusu", 6. Ulusal Jinekoloji ve Obstetrik Kongresi, Belek, Antalya, 14-19 Mayıs 2008 (Poster sunumu).

D3. Arıoğlu P, Fıçıcıoğlu C, Attar R, Akçin O, Çetinkaya N, İnan Y, Yıldırım G, "Çoğul Gebelik Redüksiyonu: Fayda-Risk Analizi İle Sonuçlarımızın Değerlendirilmesi", 6. Ulusal Jinekoloji ve Obstetrik Kongresi, Belek, Antalya, 14-19 Mayıs 2008 (Poster sunumu).

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D15. N.Cetinkaya, İ. Selçuk, B. Coşkun, B. Ozdal, M.M. Meydanlı, T. Gungör, The Impact Of Tumor Diameter On Uterine Clear Cell Carcinomas, 14. Ulusal Jinekolojik Onkoloji Kongresi, 19-23 Kasım, 2014, Antalya.

D16. M. Oz, N. Cetinkaya, H. Bayramoğlu, A. Usubütün, M. M. Meydanlı, T. Güngör, Inflammatory, Myofibroblastic Tumor Of The Uterus, An Uncommon Differential Diagnosis For Pelvic Mass, 14. Ulusal Jinekolojik Onkoloji Kongresi, 19-23 Kasım, 2014, Antalya.

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D.20. Nilüfer Çetinkaya, Sevda Baş, Nilüfer Alagöz, Mine Çavdar Atlı, M. Mutlu Meydanlı Vajinal Kanser: 9 Olguluk Vaka Serisi. 12. Zekai Tahir Burak Kongresi 12-14 Kasım Ankara mariot

D 21. Nilüfer Çetinkaya, Sevda Baş, Nilüfer Alagöz, Mine Çavdar Atlı, M. Mutlu Meydanlı. Vulva Kanserinde Tek Merkez Sonuçları-Zekai Tahir Burak Kadın Sağlığı Eğitim ve Araştırma Hastanesi Deneyimi. 12. Zekai Tahir Burak Kongresi 12-14 Kasım Ankara Mariot

D22. Nilüfer Çetinkaya, Sevda Baş, Mine Çavdar Atlı, Nilüfer Alagöz, M. Mutlu Meydanlı Servikal Adenokarsinomlarda Kaba Sağ Kalım Süresi Üzerine Etkili Prognostik Faktörler. 12. Zekai Tahir Burak Kongresi 12-14 Kasım Ankara Mariot

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E. Book Chapter Translations :

E1. Kitabın Adı: Williams Obstetrics Yazar:Schorge JO, Schaffer JI, Halvorson LM, Bradshaw KD, Cunningham FG.

Çeviri Editörü: Yavuz Ceylan, Gökhan Yıldırım, Halil Aslan, Ahmet Gül, Ali Gedikbaşı. ISBN: 978-975-420-768-2

Yayınevi: Nobel Tıp/2009

Bölüm 33: Sterilizasyon Sayfa: 698

Bölüm 41: Genel Görüşler ve Maternal Değerlendirme Sayfa: 912

E2. Kitabın Adı: Yardımla Üreme Teknikleri Temel Kitabı, Yazar: David K. Gardner, Ariel Weissman, Colin Howles, Zeev Shoham.

Çeviri Editörü: Tülay İrez, Oktay Arda, Semih Kaleli, ISBN: 978-975-420-741-5

Yayınevi: Nobel Tıp/2010

Bölüm 50: Tekrarlayan İmplantasyon Başarısızlığı: Güncel Terapötik Yaklaşımlar Sayfa: 667

E3. Kitabın Adı: Williams Obstetrics, Yazar: Cunningham FG, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL, Casey BM, Sheffield JS.

Çeviri Editörü: Gökhan Yıldırım, ISBN: 978-605-335-172-6

Yayınevi: Nobel Tıp/2016

Bölüm 58: Endokrin Hastalıklar , Sayfa: 1147

Bölüm 63: Neoplastik Hastalıklar, Sayfa: 1219

Bölüm 65: Cinsel Yolla Bulaşan Enfeksiyonlar, Sayfa: 1265

F. Book Chapter Writing:

F1. Kitabın Adı: Semiyoloji

Yazar: Yaşar Küçükardalı

Editör: Yaşar Küçükardalı ISBN: 978-975-420-942-6

Yayınevi: Nobel Tıp/2013

Bölüm 15: Kadın-Doğum Sayfa: 457

H. Certificate, Course and Workshop Participation:

Certificate:

1. Uygulama Eğitimi, Ankara, Sertifika, 11.11.2015-12.11.2015 (ulusal) Sağlık Bilimleri Alanında Ar-Ge Proje Önerisi Hazırlama Uygulama Eğitimi, Ar-Ge

2. Güncel Üreme Endokrinolojisi, Yardımcı Üreme Teknikleri Kongresi ve 1. Üreme Tıbbı derneği Kongresi, Üreme Tıbbı, Sheraton Hotel Çeşme/İzmir, Sertifika, 17.04.2008-20.04.2008 (ulusal)

3.33. Zeynep KamiL JiNeko-Patoloji Kongresi, JiNekoloji-ObstetriK, Wyndham Grand İstanbul, Sertifika, 07.11.2015 -08.11.2015 (Ulusal)

4. Middle East and Turkish Gynecologic Oncology Joint Meeting, Jinekolojik Onkoloji Ankara, Sertifika, 27.02.2016 -28.02.2016 (Uluslararası)

5.19th International Meeting of the European Society of Gynaecological Oncology (ESGO), Jinekolojik Onkoloji, Nice, France, Sertifika, 24.10.2015 -27.10.2015 (Uluslararası)

6.12. Zekai Tahir Burak Jinekoloji ve Obstetrik Kongresi, Jinekoloji-Obstetrik, Ankara, Marriot Hotel, Sertifika, 12.11.2015 -14.11.2015 (Ulusal)

7.Deney Hayvanları Kullanım Sertifikası, Deney Hayvanları Kullanım Sertifikası, Yeditepe Üniversitesi/İstanbul, Sertifika, 09.11.2009 -19.11.2009 (Ulusal)

8.Palandöken Kadın Doğum, Jinekoloji-Obstetrik, Polat Erzurum Resort Hotel, Sertifika, 03.03.2016 -06.03.2016 (Ulusal)

9.Kardiyak Yaşam Desteği Teorik ve Pratik Eğitimi, CPR Eğitimi, Yeditepe Üniversitesi/İstanbul, Sertifika, 01.12.2007 (Ulusal)

10.Ulusal perinatoloji Kongresi, Perinatoloji, Rixos Premium/Antalya, Sertifika, 26.04.2007 (Ulusal)

11.6th Annual Meeting Of Mediterranean Society For Reproductive Medicine, Reprodüktif Endokrinoloji, Antalya, Sertifika, 20.04.2007 -23.04.2007 (Uluslararası)

12.Basic Fetal Echocardiography, Fetal USG, The Fetal Medicıne Foundation/Internet Based Course, Sertifika, 04.02.2011 (Uluslararası)

13.'3. Ulusal Üreme Endokrinolojisi ve infertilite Kongresi', Üreme Sağlığı, Sungate Port Royal Delux Resort Hotel/Antalya, Sertifika, 16.10.2008 -19.10.2008 (Ulusal)

14.9. Ulusal Jinekoloji Ve Obstetrik Kongresi, Jinekoloji-Obstetrik, Kervansaray Otel/Antalya, Sertifika, 17.05.2011 -22.05.2011 (Ulusal)

15.Adriyatikten Çin'e Üreme Sağlığı Toplantıları, Üreme Sağlığı, Bişkek/Kırgızistan, Sertifika, 02.03.2009 -06.03.2009 (Uluslararası)

16.Contınuing Medical Education, The 11-13 Weeks Scan Online Course, Fetal USG, Boonshoft School Of Medicıne Wright State University, Online Course, Sertifika, 29.04.2011 (Uluslararası)

17.Fetal Tıp Derneği, First Trimester Screening and Diagnosis, The 11-14 Week Scan, Antalya, Sertifika, 25.04.2007 (Uluslararası)

18.Sedasyon/Analjezi Uygulama, Ameliyathane Dışı Sedasyon Ve Analjezi Uygulama, Yeditepe Üniversitesi/İstanbul, Sertifika, 01.12.2007 (Ulusal)

19.Türk Perinatoloji Derneği, Mezuniyet Sonrası Eğitim Kursu, Antenatal Yönetim, Bostancı Green Park Otel/İstanbul, Sertifika, 03.12.2006 (Ulusal)

20.Cervical Assessment, Fetal USG, The Fetal Medicine Foundation/Internet Based Course, Sertifika, 05.02.2011 (Uluslararası)

21.The 11-13 Weeks Scan, Fetal USG, The Fetal Medicıne Foundation/Internet Based Course, Sertifika, 29.04.2011 (Uluslararası)

22.Antenatal Surveillance, Fetal USG, The Fetal Medicıne Foundation/Internet Based Course, Sertifika, 04.02.2011 (Uluslararası)

23.Ürojinekolojide Mesh Uygulaması, Ürojinekoloji, İstanbul, Sertifika, 04.03.2010 - 05.03.2010 (Ulusal)

24.11. Ulusal Jinekolojik Onkoloji Kongresi, Onkoloji Eğitim, Belek-Antalya, Sertifika, 30.04.2008 -04.05.2008 (Ulusal)

Course:

1.Arbeitsgemeınschaft Gynakologische Endoskopie, Jinekolojik Endoskopi, Age-Grundkurs 'Gynakologische Endoskopie', Kiel School, Kurs, 29.08.2011 - 02.09.2011 (Uluslararası)

2.Neonatal Resüsitasyon Programı Uygulayıcı Sertifikası, Neonatal Resüsitasyon Eğitimi, İstanbul, Kurs, 03.05.2010 -05.05.2010 (Ulusal)

3.Türk Cerrahi Derneği, Stapler Eğitimi Ve Kullanımı, Stapler Eğitimi, Ankara, Kurs, 27.12.2014 (Ulusal)

4.Yeditepe Üniversitesi Hastanesi, Fetal Kalp Tarama Kursu, Fetal USG, Yeditepe

Üniversitesi/İstanbul, Kurs, 10.02.2012 (Ulusal)

5.Kardiopulmoner Resüsitasyon (CPR), CPR Eğitimi, Zekai Tahir Burak Kadın Sağlığı Eğitim ve Araştırma Hastanesi/Ankara, Kurs, 04.12.2013 (Ulusal)

6.Kolposkopi Kursu, Kolposkopi Eğitim, Zekai Tahir Burak Kadın Sağlığı Eğitim ve Araştırma Hastanesi, Jinekolojik Onkoloji Cerrahisi Kliniği, Kurs, 16.11.2013 (Ulusal)

7.Acil Obstetrik Bakım Klinisyen Eğitimi, Acil Obstetrik Bakım, Türkiye Halk Sağlığı Kurumu, Kadın Ve Üreme Sağlığı Daire Başkanlığı, Kurs, 03.11.2014 -05.11.2014 (Ulusal)

8.TJOD Ankara Şubesi Etlik Zübeyde Hanım Kadın Hastalıkları EAH iyi Klinik Uygulamalar Kursu, İyi Klinik Uygulamalar, Ankara, Kurs, 21.01.2015 (Ulusal)

9.Jinekolojik Onkoloji Cerrahisinde Endoskopik Yaklaşımlar Kursu, 1. Basamak, Jinekolojik Onkolojide Endoskopi, Ankara Üniversitesi Tıp Fakültesi Kadın Hastalıkları Ve Doğum Anabilim Dalı, Kurs, 20.06.2015 -21.06.2015 (Ulusal)

10.Trimester Ultrasonografi Kursu, Nukal Tarama Sertikasyonu, 11. Ulusal Perinatoloji Kongresi, Kurs, 26.04.2007-29.04.2007 (Ulusal)

11.Ürojinekoloji Cerrahi Kursu, Ürojinekoloji Eğitim, Yeditepe Üniversitesi Hastanesi/İstanbul, Kurs, 27.03.2009 (Ulusal)

12.Türk Jinekoloji & Obstetrik Derneği İstanbul Şubesi, Kolposkopi Kursu, Kolposkopi Eğitimi, Harbiye Askeri Müze Kültür Merkezi/İstanbul, Kurs, 18.10.2009 (Ulusal)

13.Perinatal Tıpta 3d-4d Sonografi Kursu, Perinatal USG, 11. Ulusal Perinatoloji Kongresi, Kurs, 26.04.2007 -29.04.2007 (Ulusal)

14.Standard Training Course In Gynaecological Endoscopy, Jinekolojik Endoskopi, Kiel School Of Gynaecological Endoscopy and Reproductive Medicine, Kurs, 29.08.2011 - 02.09.2011 (Uluslararası)

Workshop:

1.Türk Jinekolojik Onkoloji Derneği, Trofoblastik Hastalıklar Derneği ˙ Batı Karadeniz Çalıştay ve Sempozyumu, Jinekolojik Onkoloji, T.C. Sakarya Üniversitesi, Merkez Kampüsü / Sakarya, Çalıştay, 15.02.2014 -16.02.2014 (ulusal)

2.19th International Pelvic Pain Society Annual Scientific Meeting, Pelvic Pain, Ceylan Intercontinental Hotel / İstanbul, Çalıştay, 26.05.2011 -29.05.2011 (Uluslararası)

3.Türk Jinekolojik Onkoloji Derneği Güneydoğu Anadolu Çalıştay ve Sempozyumu, Jinekolojik Onkoloji, Diyarbakır, Çalıştay, 30.11.2013 (Ulusal)

4.Türk Jinekolojik Onkoloji Derneği Doğu ve Güneydoğu Anadolu Çalıştay ve

Sempozyumu, Jinekolojik Onkoloji, Şanlıurfa, Çalıştay, 07.06.2014 -08.06.2014 (Ulusal)

5.Türk Jinekolojik Onkoloji Derneği Doğu Akdeniz Çalıştay ve Sempozyumu, Jinekolojik Onkoloji, Hatay, Çalıştay, 13.09.2014 (Ulusal)

6.Üreme Tıbbında Güncel Yaklaşımlar Sempozyumu, Üreme Endokrinolojisi, Ankara Hilton, Çalıştay, 20.12.2008 -21.12.2008 (Ulusal)

7.Üreme Tıbbı Derneği Jinekolojik Endoskopi Çalıştayı 'Teorik ve Uygulamalı Eğitim',Endoskopi Eğitim, Ümraniye EAH/İstanbul, Çalıştay, 03.04.2009 -05.04.2009 (Ulusal)

8.Neuroendocrinology Symposium & Workshop 'Experimental Models for Studying Neuroendocrine Disorders', Nöroendokrin Hastalıklar İçin Deneysel Modeller, Yeditepe Üniversitesi/İstanbul, Çalıştay, 23.04.2009 -25.04.2009 (Uluslararası)

9.Türk Jinekolojik Onkoloji Derneği Orta Karadeniz Çalıştay Ve Sempozyumu, Jinekolojik Onkoloji, Çorum, Çalıştay, 02.05.2015 -03.05.2015 (Ulusal)

10.Türk Jinekolojik Ve Obstetrik Derneği Ulusal Eğitim Sempozyumu, Jinekoloji-Obstetrik Güncel Konular, Antalya, Çalıştay, 25.10.2007-29.10.2007 (Ulusal)