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Analysis of Sensitivity, Specificity, and Positive and Negative Predictive Values of Smear and Colposcopy in Diagnosis of Premalignant and Malignant Cervical Lesions

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Background: This study aimed to examine the positive and negative predictive value in the diagnosis of premalignant and malignant lesions of cervical colposcopy, the sensitivity and specificity of smear, and to evaluate the correlation with histopathology of abnormal cytology and colposcopy.

Material/Methods: The criteria for inclusion of patients with unhealthy cervix in the study were: Erosion, Chronic cervicitis, and Healed lacerations, Hypertrophied cervix, bleeding on touch, suspicious growth/ulcer/polyp on the cervix, and abnormal discharges from the cervix. Women with frank carcinoma cervix, pregnant females, patients with bleeding per vaginum at the time of examination, and those who had used vaginal medications, vaginal contraceptives or douches in the last 48 h of examination were excluded from the study.
Demographic analysis was performed for 450 patients who were admitted to the clinic. Sensitivity, specificity, positive predictive value and negative predictive values of patients to identify cervical pathologies of smear and colposcopy were histopathologically calculated. The statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and Spearman's and Chi-Square tests were used for statistical analysis.

Results: Sensitivity, specificity, PPD and NDP of smear were 0.57%, 0.76%, 0.26%, 0.92% respectively. Sensitivity, specificity, PPD and NDP of colposcopy were 0.92%, 0.67%, 0.52%, 0.96% respectively. A statistically significant correlation was found between abnormal cytology and histopathology, and abnormal colposcopy finding and histopathology.

Conclusions: Women with clinical diagnosis of unhealthy cervix should be evaluated by cytology to detect any premalignant or malignant lesions. It was concluded that Pap smear, colposcopy and histopathology should be collectively evaluated to evaluate cervical findings in low socio-economic regions.

MeSH Keywords: Colposcopy • Cytological Techniques • Uterine Cervical Neoplasms

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Background

Cervical cancer is one of the biggest health problems of women around the world [1]. Premalignant lesions are characterized by abnormal cellular or epithelial architecture in the areas surrounding the junction between the squamous and columnar epithelium (transformation zone) of the uterine cervix [2].

Papanicolaou (Pap) smear cytology has remained an important tool in the screening for cervical cancer [3]. Ideally, all patients with abnormal Papanicolaou smear cytology results should undergo colposcopic examination [4].The current evidence prefers reflex human papillomavirus (HPV) testing for women with atypical squamous cells of undetermined significance (ASCUS) cytology test results. However, for women with HPV-positive ASCUS, whether from reflex HPV testing or co-testing, and women with low-grade squamous intraepithelial lesion (LSIL) cytology test results and no HPV test or a positive HPV test result, colposcopy is recommended [5]. Further, in women with a histologic diagnosis of cervical intraepithelial neoplasia (CIN) 2, CIN 3, or CIN 2/3 and adequate colposcopic examination, both excision and ablation are acceptable treatment modalities, except in pregnant women and young women [4]. CIN I corresponded to mild dysplasia, CIN II to moderate dysplasia, and CIN III corresponded to both severe dysplasia and CIS. Thus, in 1990, a histopathological terminology based on two grades of disease was proposed: low-grade CIN comprising the abnormalities consistent with koilocytic atypia and CIN I lesions and high-grade CIN comprising CIN II and III. The high-grade lesions were considered true precursors of invasive cancer [6].

2001 Bethesda System is the most commonly used and most widely accepted classification to report cervical smear samples. In Bethesda System, squamous epithelial cell abnormality is divided into five categories including Atypical Squamous Cells of Undetermined Significance (ASC-US), Atypical squamous cells – cannot exclude HSIL (ASC-H), Low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL) and Squamous cell carcinoma (SCC). In this system, mild dysplasia (CIN I) patients are included in LSIL, while moderate (CIN II) and severe (CIN III) dysplasia patients are included in HSIL. On the other hand, glandular epithelial abnormalities are divided into three categories including Atypical Glandular Cells not otherwise specified (AGC), endocervical *in situ* adenocarcinoma (AIS), adenocarcinoma (endocervical, endometrial, extrauterine and non- specified [7]. Low sensitivity of cervical smear, the cost of colposcopy and invasiveness of histopathology are the handicaps for the evaluation of lesions. Furthermore, the objective is early and accurate diagnosis of pre-malign lesions. A review of the literature found that sensitivity, specificity, PPV and NPV values of smear, colposcopy and histopathological evaluation had wide ranges. This study was carried out to evaluate these screening and diagnosis tests in a tertiary healthcare center on a patient group with low socio-economic status in a developing region in Turkey. In this context, we aimed to analyze cyto-histopathological correlations in patients with diagnosis distribution and tissue diagnosis in cervical smear samples reported to be in the epithelial cell abnormality category. Sensitivity, specificity, PPV and NPV values for Pap smear, colposcopy, and histopathology were analyzed. We aimed to evaluate the correlation of abnormal cytology and colposcopy with histopathology.

Material and Methods

The study included a total of 450 married women clinically diagnosed with unhealthy cervix who were referred to colposcopy unit of Dicle University Faculty of Medicine. Ethics committee report was received from hospital local ethics committee and informed consent of for were received from the patients. All of the patients were asked questions about their history including age, occupation, number of pregnancy, contraception method (condom, the use of OKS-RIA, coitus interruptus, depo Provera), educational status and smoking. The criteria for inclusion of patients with unhealthy cervix in the study were: Erosion, Chronic cervicitis, and Healed lacerations, Hypertrophied cervix, bleeding on touch, suspicious growth/ulcer/polyp on the cervix and Abnormal discharges from the cervix. Women with frank carcinoma cervix, pregnant females, patients with bleeding per vaginum at the time of examination and those who had used vaginal medications, vaginal contraceptives or douches in the last 48 h of examination were excluded from the study. Acetowhite area, atypical vascularization, punctuation and mosaicism were evaluated in colposcopic analysis.

Smear collection technique

After taking a detailed history and doing thorough clinical examination, Pap smear was taken using conventional method for cervical cytology in all the patients. After asking the patient to empty the urinary bladder, they were put on the examination table in dorsal position and speculum was introduced into the vagina to expose the cervix. A Medscand Cell Sampling Kit (manufactured by Medscand Medical AB, Sweden) was used for taking cervical cytology. The endocervical cell sampling was done by using the cytobrush which was introduced into the cervical os along its axis till few bristles remained outside the os. The brush was rotated by 180o, maintaining the contact with the cervical canal. The sample was unrolled onto the slide in the opposite direction from which it was collected by twirling the handle of the brush. The slide was fixed immediately in 95% ethyl alcohol in Coplin's jar. Next, the pointed end of the plastic Colin's Ayres spatula was introduced into the cervical os and rotated 360° about the circumference of the os maintaining constant contact with the ectocervix and both surfaces

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Table 1. Pap Test results of all patients.

Smear results	N	%
Normal	394	87.5
ASCUS	29	6.4
LGSIL	17	3.8
HGSIL	7	1.6
AGUS	1	0.2
Epidermoid CA	2	0.4
Total	**450**	**100.0**

Table 2. Distribution of patients according to colposcopy findings.

Colposcopy findings	N	%
Normal	226	50.2
Abnormal	97	21.6
Ectropion	76	21.6
Chronic cervicitis	45	10.0
Cervical tm.	6	1.3

of the spatula were smeared on a new slide and fixed immediately. After this, using the blunt end of the Ayres spatula, sample was taken from the posterior fornix and the material obtained was spread on a next slide and fixed immediately. All the three slides obtained were numbered as 1, 2 and 3 in the sequence in which they were prepared. After fixing, the slides were sent to the Pathology Department, stained with the Papanicolaou staining method [8]. Reporting of the slides was done according to The 2001 Bethesda system [9] by the consultant pathologist as follows.

Colposcopy technique

Sub-epithelial vascular structures were examined by damping the cervix with 10 cc serum physiologic. After applying 5% acetic acid solution, Leisegang brand BG/LED model was connected to the monitor and the cervix was analyzed using binocular colposcope (Model 1DW-LED) that comes with a drum changer with a magnification of 7.5/15/30, a convenient angular insight of 45 and is equipped with an extremely bright LED lighting.

Statistical evaluation

The statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL,USA) and Spearman 's and Chi-Square tests were used for statistical analysis.

Results

Table 1: Pap test results of all patients were evaluated. Smear results of 394 patients (87.5%) were 'Normal', 29 patients (6.4%) were 'ASCUS, 17 patients (3.8%) were 'LGSİL', 7 patients (1.6%) were 'HGSIL', 1 patient (0.2%) was AGUS, 2 patients (0.4%) were 'Epidermoid ca'.

In Table 2, of a total of 450 patients, 226 (%50.2) had normal, 97 (% 21.6) had abnormal, 76 (%16.9) had ectropion, 45 (%10) had chronic cervicitis, 6 (%1.3) had cervical Tm.

In Table 3, of a total of 450 patients 97 (21.6%) were found to have abnormal colposcopy findings. Of these patients, 18.5% had atypical vascularization, 16.4% had acetowhite epithelial + punctuation, 14.4% had atypical vascularization + punctuation, 10.3% had atypical vascularization + acetowhite epithelium, 10.3% had punctuation, 5.1% had acetowhite epithelium + punctuation + mosaicism, 4.1% had acetowhite epithelium + punctuation + mosaicism + atypical vascularization.

There was statistically significant correlation between abnormal cytology and histopathology ($r=0.273$, $p=0.000$ $p<0.05$). There was a statistically significant correlation between abnormal colposcopy finding and histopathology ($r=0.613$, $p=0.002$, $p<0.05$). Spearman's correlation test. Recommended examination and treatment to our patients have been shown in Table 4.

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Table 3. Distribution of patients according to abnormal colposcopy findings.

Abnormal colposcopy findings	N:97	%
Atypical vascularization	18	0.185
Atypical vascularization + acetowhite epithelial	10	0.103
Atypical vascularization + punctuation	14	0.144
Acetowhite epithelial	12	0.123
Acetowhite epithelial + punctuation	16	0.164
Acetowhite epithelial + punctuation + mosaicism	5	0.051
Punctuation + mosaicism	4	0.041
Acetowhite epithelial + punctuation + mosaicism + atypical vascularization	4	0.041
Punctuation	10	0.103
Total	**97**	**100.0**

Table 4. Recommended examination and treatment.

Recommended examination and treatment	N	%
Local treatment + follow-up	320	71.1
LEEP + endocervical curettage	57	12.7
Conization + fractional curettage	38	8.4
Directed cervical punch biopsy + polypectomy	32	7.1
Refused the necessary treatment	3	0.7
Total	**450**	**100.0**

Table 5. Correlation of abnormal cytology and colposcopy with histopatholgy.

Correlation	Total patients (n)	r	p
Between abnormal cytology and histopathology	450	0.273	0.000
Between abnormal colposcopy and histopathology	450	0.613	0.002

Discussion

Cancer of the cervix is a preventable disorder as the different screening, diagnostic and therapeutic procedures are effective. One of the essential responsibilities of the gynecologist is to detect neoplasm of the genital tract at the earliest. Since the introduction of cytology into clinical practice by Papanicolaou and Traut in 1944, it has become possible to detect cervical cancer in its preinvasive stages, thus reducing the morbidity and mortality from this disease.

The present study was conducted on 450 patients with the clinical diagnosis of unhealthy cervix.

Evaluation of maser results of our patients revealed that ASCUS, LGSIL, HGSIL, AGUS, Epidermoid carcinoma ratios were 6.4%, 3.8%, 1.6%, 0.2% and 0.% respectively (Table 3). According to abnormal colposcopy findings, abnormal colposcopy finding was found in 97 patients (21.6%). Of these patients, 18.5% had atypical vascularization, 16.4% had acetowhite epithelium + punctuation, 14.4+ had atypical vascularization + punctuation, 10.3% had atypical vascularization + acetowhite epithelium, 10.3% had punctuation, 5.1% had acetowhite epithelium + punctuation + mosaicism, 4.1% had acetowhite epithelium + punctuation + mosaicism + atypical vascularization. The correlation between abnormal cytology and histopathology was statistically significant (r=0.273, p=0.000 p<0.05) (Table 5). A statistically significant correlation was

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Table 6. Evaluation of negative predictive value, positive value, sensitivity and specificity of smear and colposcopy.

Method	NPD (%)	PPD (%)	Sensitivity (%)	Specificity (%)
Pap smear	0.92	0.26	0.57	0.76
Colposcopy	0.96	0.52	0.92	0.67

Table 7. Evaluation of histopathology results of the patients with abnormal pap smear.

Smear results	Results of histopathology	N	%
AGUS (n=1)	Chronic cervitis	1	100.0
ASCUS (n=28)	Normal	13	0.46
	Chronic cervitis	13	0.46
	Koilocytes changes	2	0.071
LGSIL (n=16)	Normal	4	0.25
	Chronic cervitis	7	0.43
	LGSIL	2	0.125
	Koilocytes changes	1	0.062
	CIN	2	0.125
HGSIL (n=7)	Normal	1	0.142
	Chronic cervitis	6	0.85
	CIS	1	0.142
Atypical cells (n=2)	Chronic cervitis	1	0.5
	Endometrium adeno cancer	1	0.5

found between abnormal colposcopy finding and histopathology (r=0.613, p=0.002, p<0.05).

Sensitivity, specificity, PPD and NPD of smear were found to be 0.57%, 0.76%,0.26% and 0.92% respectively in Table 6. On the other hand sensitivity, specificity, PPD and NPD of colposcopy were found to be 0.92%,0.67%, 0.52%,0.96% respectively.

ASCUS is the most common and most controversial abnormal smear result (together with LSIL) about management. Therefore, there is a large body of research on ASCUS and LSIL patients. The most important and most comprehensive study is the randomized ALTS study which was mentioned earlier. That study aimed to determine the more correct management and follow-up mode of ASCUS and LSIL results. ASCUS and LSIL patients who were included in the study were randomly divided into cytological follow-up, immediate colposcopy and HPV groups. Sensitivities of ALTS study for these three methods are presented in HPV section. The results of the study revealed that the patients should be evaluated for all three methods for ASCUS and that HPV test should be preferred if liquid based cytology is used. As for LSIL patients, colposcopy was recommended due to high HPV (+). In ALTS study, for cytological follow-up group, it was reported that it was the most commonly used method, it had an error rate (–) of 20–50% for CIN 1 and that the rate of identifying CIN2/3 with a single cytology repeat was 67–85%. Major disadvantages in cytologic follow-up were reported to be CIN2/3, potential delay for cancer diagnosis, necessity of multiple repetitions, maladjustment and anxiety in the patient. Immediate colposcopy is advantageous as it allows for rapid diagnosis and can prevent potential losses. However, it is also considered as disadvantageous due to discomfort, causing unnecessary anxiety, high cost and possibility to cause excessive diagnosis and treatment. In immediate colposcopy group in ALTS study, although CIN3 lesion was 100% diagnosed, due to the fact that colposcopy costs 3-4 times higher than HPV test in the US, it is considered as a very expensive test. The ASCUS LSIL Triage Study (ALTS) Group, a large, randomized, multicenter trial designed to compare management strategies for women with ASCUS or LSIL cytology results, obtained the following data published in 2001: colposcopic findings for CIN I lesions were found in 51.4% of cases, and only 7% of examinations were considered to be CIN II or more severe. Among participants, the underlying prevalence of histologically confirmed CINIII was 5.1%. Compare to this data, in our study we found colposcopic findings for CIN I lesion in 78.5% of cases, 10.7% of examinations were CIN II or more severe. Among LSIL participants, the underlying prevalence of histologically confirmed CIN III was 3.5%. The data obtain from our study are appropriate as value noting that in

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ALTS study values were lower because directed biopsy was performed if any CIN lesion was suspected by colposcopic examination, so a lower number of biopsies reported to a lower number of colposcopies [10].

ASCUS rate of our study was 6.4% which depicts that the standardized diagnostic criteria for cervical cytological reporting were used by cytopathologist, so that the rate of atypical squamous cells (ASC) was between 3 to 5% of cytological diagnosis for accurate reporting as reported by Kurman et al. [11].

In our study, 5 of 28 patients who had ASCUS abnormal smear result and abnormal findings in colposcopy underwent LEEP+ECC. Histopathologies of 4 patients were reported to be chronic cervicitis, while 1 patient was koilocytic changes. 4 patients underwent conization. The results of 4 patients were reported as chronic cervicitis. 5 patients underwent colposcopy-directed biopsy and all patients were found to have cervicitis. Normal colposcopic findings were monitored in 14 patients with ASCUS abnormal smear result. The patients were followed up for smear. None of 28 ASCUS patients in abnormal smear result in Table 7 were found to have CIN or cervical carcinoma. Of 16 patients with LGSIL Pap test results, 2 were found to have CIN, while 1 was found to have HPV lesion with koilocytic changes and 2 were found to have LGSIL.

Of 7 patients with HGSIL Pap test result, 1 had cervical carcinoma *in situ*. We believe that in patients with ASCUS smear result follow-up; and in patients with LGSIL and HGSIL result, histopathologic analysis is cost-effective.

1 patient who had atypical cell in abnormal smear result underwent colposcopy-directed biopsy. The result was reported as chronic cervicitis. On the other hand, further examination showed endometrium adenocarcinoma in 1 patient. In a similar study, Fallani et al. compared biopsy histologies of patients with ASCUS and SIL cytological diagnosis under colposcopy. A total of 358 of 584 women were diagnosed with LSIL. Biopsy of ASCUS patients showed CIN I in 36.3%; CIN II-III and *in situ* Ca in 15.7% and invasive Ca in 1 patient. The researchers found CIN I in 67.7%, CIN II-II and *in situ* Ca in 20.8% and Invasive Ca in 2 patients who had LSIL. Based on the results of the study, the researchers recommended colposcopic analysis for all patients with ASCUS and LSIL diagnosis [12].

In a study carried out on 118 patients who were diagnosed with HPV infected CIN, Gonzalez et al., analyzed the relationship between Bethesda system and colposcopic punch biopsy. They found 100% fit for HPV and 97% fit for CIN 1 in 82 (98.5%) LGSIL patients. They reported 84% fit for CIN II and 100% fit for CIN III in 36 (92%) HGSIL patients. The correlation was found to be 96% for PPD and 98% for NPD [13].

Women with clinical diagnosis of unhealthy cervix need cervical evaluation to detect any premalignant or malignant lesions of cervix. The Bethesda system used for cervical cytology is not only a uniform and standard method but also gives descriptive diagnosis that helps the gynecologist in individualized patient management. Communication between gynecologist and cytopathologist is required for best results of the cervical cytology reporting.

In our study, of 97 patients (21.6%) with abnormal colposcopic findings, 76 patients (16.9%) were diagnosed as ectropion, 45 patients (10%) were diagnosed as chronic cervicitis and 6 patients (1.3%) were diagnosed as cervical Tm.

Adams AL et al. noticed in their study that the true sensitivity of the whole diagnostic process of colposcopy plus biopsy is lower because biopsies were not performed for all women, and some of the biopsy specimens may not have been taken from the most severe lesion. This results in an overestimate of sensitivity [14].

Pimple SA et al., in 2010, made an evaluation of colposcopy *vs.* cytology as secondary test to triage women found positive on visual inspection test. The colposcopic impression was CIN I changes in 33.8% of cases, CIN II–III in 8.6% of cases, and invasive carcinoma in 2.7% of cases. Histopathology findings were reported as benign in 81.6%, CIN I in 5.8% of cases, CIN II in 2.9% of cases, CIN III in 2.6% of cases, and invasive carcinoma in 2.9% of cases. The estimates of sensitivity for low- and high-threshold colposcopy were 58% and 74.5%, respectively, and those of specificity were 57.5% and 92.9%, respectively [15].

Colposcopy performs better in differentiation of high-grade from low-grade disease than in differentiation of low-grade disease from normal cervix[16], and correlated with directed biopsy is described as the reference investigation or 'gold standard' for the diagnosis of cervical precancer [17].

Maziah AM et al., in 1991, in a comparative study of cytologic and colposcopic findings in preclinical cervical cancer, obtained an accuracy rate of 94% for colposcopy. The colposcopic findings rates were: 10% for CIN I, 34% for CIN II, 34% for CIN III and 12% for invasive carcinoma. Histology findings were: 10% were CIN I, 20% were CIN II, 60% were CIN III and 10% were micro-invasive carcinoma. In this study, the overall results were similar to ours, except that the diagnosis of micro-invasive carcinoma was not made on colposcopy. According to authors, poor results obtained were very likely because specific diagnosis in these techniques is very highly operator dependent and subjective and therefore a tendency to under diagnose the severity of lesions [17].

We found a statistically significant correlation between abnormal cytology and histopathology in Table 5 (r=0.273, p=0.000

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p<0.05). The correlation between abnormal colposcopy finding and histopathology was statistically significant (r=0.613, p=0.002, p<0.05).

Sensitivity, specificity, PPD and NPD of smear were found to be 0.57%, 0.76%, 0.26% and 0.92% respectively in Table 6. Sensitivity, specificity, PPD and NPD of colposcopy were found to be 0.92%, 0.67%, 0.52% and 0.96% respectively.

The fact that all cervical examinations, smear collection and colposcopic evaluation were performed by a single gynecologist, that this physician received colposcopy training, similarly, only a single pathologist was included in the study and that the pathologist worked only in the field of gynecology eliminate intra-observer difference.

The significance of the study is that this is the first study carried out in a developing southeastern Anatolia region of Turkey on patients with low socio-economic status. The limitation of the study includes relatively low number of patients, high number of patients who were evaluated as normal in colposcopy and low number of cervical tumor patients. HPV DNA was not studied due to the fact that HPV DNA was not covered by insurance institutions in Turkey in the study period and that it was not routinely used due to its high cost. In their study, Olaniyan OB et al., in 2002 conducted a metaanalysis to quantify the validity of colposcopy in the diagnosis of early cervical neoplasia. Eight longitudinal studies were selected, which compared correlation of colposcopic impression with colposcopically directed biopsy results. The prevalence of cervical disease in the studies ranged from 40 to 89%. Colposcopic accuracy was 89%, which agreed exactly with histology in 61% of cases. The sensitivity and specificity of colposcopy for the threshold of normal *vs.* all cervical abnormalities were 87–99% and 26–87% respectively. For the threshold of normal and low-grade SIL *vs.* high-grade SIL, the values were 30–90% and 67–97% [19].

In a study meant to track the management outcomes of abnormal cervical cytology and hence confer credence to the value of colposcopy in management of abnormal cervical cytology, published in 2007, colposcopic detection rates were: 68% for CIN I, 73.3% for CIN II, 81.4% for CIN III and 88.9% for invasive carcinoma [20]. When deciding which test to use for screening, specificity must be taken into account because tests with low specificity applied to a healthy population with a very low prevalence of disease will result in a high proportion of false-positive test results [21]. Moss EL et al., in 2009, in a study on 469 patients to determine whether colposcopy is reliable in diagnosing cervical intraepithelial neoplasia in women who have undergone a previous cervical excision biopsy, reported the sensitivity and specificity of colposcopy for any cervical disease were 93.9% and 51.9%, respectively [22].

Conclusions

This study demonstrated high accuracy and correlation between colposcopy and histology, comparable with results from similar studies in the literature. Women with clinical diagnosis of unhealthy cervix should be evaluated by cytology to detect any premalignant or malignant lesions. It was concluded that Pap smear, colposcopy, and histopathology should be collectively evaluated in regions with low socio-economic status in evaluation of abnormal cervical findings.

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