ORIGINAL ARTICLE

Study of 2 years follow-up of referral patients with abnormal Pap smear

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Background: Abnormal Pap smear consists of premalignant or malignant cervical lesions. Many of premalignant cervical lesions will never progress to invasive malignancy, or even may regress over the time. Thus, there is always a risk of overtreatment of patients with an abnormal Pap smear. A long-term follow-up of these patients can reveal final events associated with each subtype of abnormal Pap smear, and, therefore, help us to prevent unnecessary interventions. The aim of our study was to present 2 years follow-up of referral patients with abnormal Pap smear. **Materials and Methods:** A total of 334 consecutive women aged more than 16 who were referred with an abnormal Pap smear were entered into the study. Patients were followed with biannual Pap smear and annual colposcopy and biopsy for 2 years. **Results:** At baseline, the majority of patients with abnormal Pap smear were normal on colposcopy and biopsy (68% and 86%, respectively). Six months after first abnormal Pap smear majority of patients in each group showed a significant regress to normal or less invasive lesion ($P < 0.001$). Twelve patients (4%) had no change in Pap smear, whereas 313 (94%) had at least one stage improvement. Only nine (3%) patients had deteriorated Pap smear after 6 months. All 308 patients who underwent colposcopy and biopsy had normal Pap smear 24 months after the first abnormal Pap smear. **Conclusion:** Pap smear is associated with a high rate of false-positive results. In addition, the majority of low-grade cervical lesions can spontaneously regress. A long-term follow-up of a patient with abnormal Pap smear can help us to avoid needless interventions.

Key words: Atypical squamous cell, cervical cancer, colposcopy, Pap smear

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INTRODUCTION

As the second most prevalent malignancy among women, cervical cancer affects a large number of women worldwide; however, the majority of patients with cervical cancer live in developing countries. Cervical cancer is a treatable condition if it is diagnosed at early stages and managed appropriately.[1,2] To detect malignant changes at earliest stages, Pap smear was introduced in 1950's. Since then, Pap smear has been widely used as a low-cost and efficient screening tool for early detection of cervical lesions.[3]

Abnormal Pap smear consists of premalignant or malignant cervical lesions. Many premalignant cervical lesions will never progress to invasive malignancy - or even may regress over the time.[4,5] According to the standard guidelines, when a patient has an abnormal Pap smear, she should undergo more invasive diagnostic interventions such as colposcopy and biopsies for more accurate evaluation. However, sensitivity and specificity of colposcopy have been reported to be low especially for high-grade abnormalities.[6] Because of these, there is always a risk of overtreatment of patients with a premalignant Pap smear, who may actually need less invasive interventions or may regress spontaneously over time. Overtreatment is associated with unnecessary cost and morbidity. A long-term follow-up of patients with an abnormal Pap smear can reveal final events associated with each subtype of abnormal Pap smear, and, therefore, help us to prevent unnecessary interventions.[7,8]

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This study was designed to determine outcome a 2-year follow-up of women who were referred to a tertiary hospital with an abnormal Pap smear.

MATERIALS AND METHODS

After approval of the Ethic Committee of Isfahan University of Medical Sciences, this prospective cohort study (research project number: 391337) was conducted between January 2011 and December 2013 at Shahid Beheshi Hospital, Isfahan, Iran. Informed consent was obtained from patients who agreed to participate the study. A total of 334 consecutive women aged more than 16 who were referred with an abnormal Pap smear were entered into the study.

According to Bethesda Classification System for Reporting Cervical Cytologic Diagnoses,[9] Pap smear findings were classified to atypical squamous cell (ASC)-including ASC of undetermined significance (ASC-US) or ASC cannot exclude highgrade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (S.C.C), and atypical glandular cells (AGC).

Diagnosis of invasive cervical cancer and death due to causes other than cervical cancer were considered as exclusion criteria.

At the first visit, colposcopy and directed biopsies were done for all patients with abnormal Pap smear. Patients with Pap smear, whose colposcopy and biopsy results were consistent ASC and LSIL, were followed with Pap smear every 6 months and annual colposcopy for 24 months.

If the biopsy showed HSIL, cervical conization was considered for the next step.

Patients with HSIL whose colposcopy-biopsy and endocervical curettage (ECC) showed LSIL or less underwent cervical conization and ECC, except for nulliparous women with satisfactory colposcopy. These women were followed by cytology and repeated colposcopy.

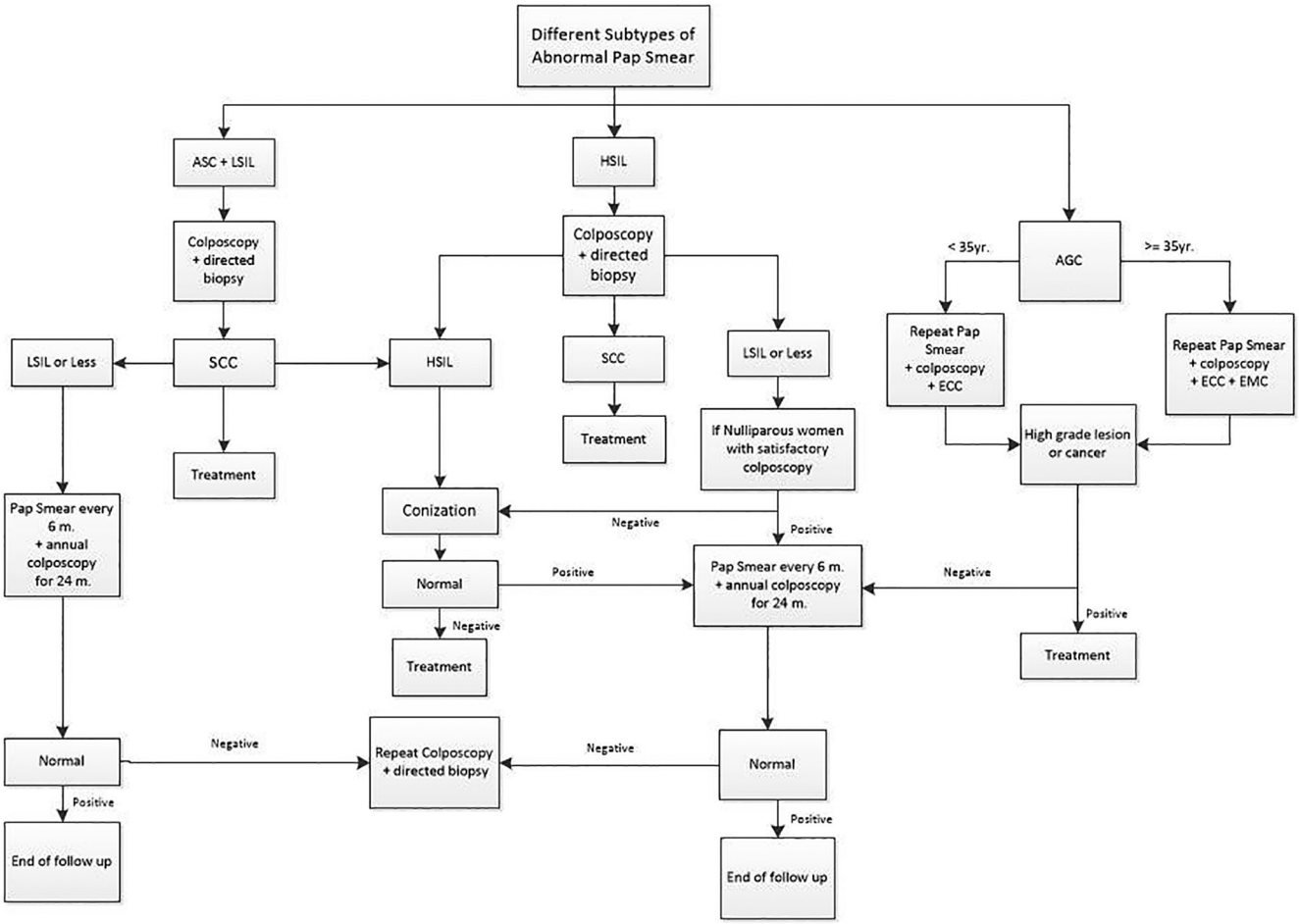

Figure 1: Scheme for management of women with abnormal Pap smear in this study (ASC = Atypical squamous cells; LSIL: Low-grade squamous intraepithelial lesion; HSIL = High-grade squamous intraepithelial lesion; SCC = Squamous Cell Carcinoma; AGC = Atypical glandular cells; ECC = Endocervical curettage; EMC = Endometrial curettage)

After conization patients were followed by cytology every 6 months for 24 months [Figure 1].

Patients aged <35 years who with an atypical glandular cell on Pap smear underwent colposcopy, Pap smear, and ECC. Those aged 35 years and more with an atypical glandular cell on Pap smear underwent more augmented endometrial curettage (colposcopy, repeated Pap smear, ECC, and endometrial curettage).

A single gynecologist performed all follow-up procedures, and an expert pathologist reviewed all samples. Patients received no treatment during this study.

After testing for normality by Kolmogorov–Smirnov test, normally distributed variables, and nonnormal variables were analyzed by ANOVA and Kruskal–Wallis, respectively. Proportions between groups were compared by Chi-square test. SPSS (version 21, SPSS Inc., Chicago, IL) was used for data analysis, and a $P < 0.05$ was considered significant.

RESULTS

Demographic data

Mean age at first abnormal Pap smear was 40.0 ± 9.9 years (minimum/maximum: 20/70 years) and mean age at first sexual intercourse was 18.0 ± 4.2 years (minimum/maximum: 10/34 years). Neither mean age at first sexual intercourse nor at first abnormal Pap smear was different between subtypes of abnormal Pap smear ($P > 0.05$).

Of 334 investigated women, 313 (94%) were multiparous and 21 (6%) were nulliparous.

In this study, we found no association between age ($P = 0.07$) and married age ($P = 0.44$) with the occurrence of high grade dysplasia. Furthermore, there was no associations among parity ($P = 0.69$), educational level ($P = 0.2$), menopausal status ($P = 0.49$), current pills used ($P = 0.61$), smoking habits ($P = 0.24$), STD history, number of vaginal delivery ($P = 0.49$), and the frequency of high grade dysplasia.

BASELINE PAP SMEAR

Patients who entered the study had various types of epithelial cell abnormalities including an abnormal squamous cell (ASC, LSIL, or HSIL) or abnormal glandular cell (AGC) [Table 1].

FOLLOW-UP PAP SMEARS

Six months after first abnormal Pap smear the majority of patients in each group showed a significant regress to normal or less invasive lesion ($P < 0.001$).

At 12 months, 22 patients left the study (19 patients were referred to treatment and three patients decided to leave the study due to personal issues), while other 312 patients were followed for more than 24 months.

Over the rest of the follow-up (from 6 to 24 months), 311 (99%) women had normal Pap smear and only 1 (<1%) patient-who initially had presented with LSIL-still had abnormal Pap smear (ASC-H).

Colposcopy and biopsy

At baseline, all patients underwent colposcopy and biopsy. Although all patients had abnormal baseline Pap smear, the majority of them were normal on colposcopy and biopsy (68% and 86%, respectively).

The majority of the rest of patients had normal findings on colposcopy and biopsy (302 [98%] and 306 [99%], respectively) [Table 2].

Table 1: Frequency of Pap smear findings during study

Pap smear	Baseline (*n* = 334) (%)	6 months (*n* = 334) (%)	12 months (*n* = 312) (%)	24 months (*n* = 312) (%)
Normal	0 (0)	309 (92)	310 (99)	311 (99)
ASC				
ASC-US	213 (64)	6 (2)	1 (<1)	0 (0)
ASC-H	9 (3)	0 (0)	0 (0)	1 (<1)
LSIL	53 (16)	3 (1)	0 (0)	0 (0)
HSIL				
Moderate and severe dysplasia	34 (10)	10 (3)	0 (0)	0 (0)
CIS	3 (1)	4 (1)	0 (0)	0 (0)
AGC	22 (6)	2 (<1)	1 (<1)	0 (0)
Total	334 (100)	334 (100)	312 (100)	312 (100)

Data presented as *n* (%); *n* = Number of patients; ASC = Atypical squamous cells; ASC-US = Atypical squamous cell of undetermined significance; ASC-H = Atypical squamous cell cannot exclude high-grade squamous intraepithelial lesion; LSIL= Low-grade squamous intraepithelial lesion; HSIL = High-grade squamous intraepithelial lesion; CIS = Carcinoma *in situ*; AGC = Atypical glandular cells

Table 2: Colposcopy and biopsy findings at baseline and 12 months after first abnormal Pap smear

Pap smear	Colposcopy		Biopsy	
	Baseline (%)	24 months (%)	Baseline (%)	24 months (%)
Unsatisfactory	38 (11)	4 (1)	N/A	N/A
Normal	225 (68)	302 (98)	288 (86)	306 (99)
MGH	0 (0)	0 (0)	4 (1)	0 (0)
CIN I	41 (12)	1 (<1)	15 (4)	1 (<1)
CIN II	17 (5)	1 (<1)	4 (1)	0 (0)
CIN III	13 (4)	0 (0)	8 (2)	0 (0)
SCC	0 (0)	0 (0)	15 (4)	1 (<1)
Total	334 (100)	308 (100)	334 (100)	308 (100)

Data presented as *n* (%); *n* = Number of patients; MGH = Microglandular hyperplasia; SCC = Squamous cell carcinoma; CIN = *Cervical intraepithelial neoplasia*; N/A = Not available

Details on colposcopy and biopsy findings of patients in each subgroup of abnormal Pap smear are presented in Tables 3 and 4, respectively.

Of four patients with unsatisfactory colposcopy, two had HSIL and two had AGC on initial Pap smear. Both patients with cervical intraepithelial neoplasia (CIN) I and CIN II on colposcopy had initially presented with HSIL.

Patients with CIN I and SCC on biopsy had initially presented with LSIL and ASC-H on Pap smear, respectively.

All 308 patients who underwent colposcopy and biopsy had normal Pap smear 24 months after the first abnormal Pap smear.

In this research, 217 patients had normal colposcopy and biopsy, and 38 patients had abnormal colposcopy and biopsy. As regards, 71 patients had abnormal colposcopy and normal biopsy, and eight patients had normal colposcopy but abnormal biopsy.

Data set showed sensitivity of colposcopy as diagnostic method was 82.6%, specificity 75.3%, positive predictive value (+PV) was 34.9%, and negative predictive value (−PV) 96.4%.

DISCUSSION

Pap smear has been more successful than all other screening test in cancer prevention. However, an accurate analysis of the hundreds of thousands of cells in each sample is not always possible. As a result, Pap smear is not still perfect and is susceptible to several limitations. Human errors are one of the most important factors that limit Pap smear accuracy. Human errors may adversely affect Pap smear at different steps: From sample collection to cytopathology assessment and interpretation.[10] Because of this, Pap smear results can be misleading in many cases, and may lead to unnecessary interventions.[10,11]

To the best of our knowledge, this is the first long-term follow-up of Iranian women with abnormal Pap smear. Our study showed that more than 2/3 of patients with an initially abnormal Pap smear had a normal colposcopy, and more than 85% had normal biopsy results. Over a 6-month follow-up, more than 90% of patients with abnormal Pap smear turned into a normal Pap smear; and after a year, almost all patients had normal Pap smear.

So, why such a significant improvement over the follow-ups was observed? This significant improvement could be attributed to two major factors: Human errors[10] and spontaneous progression of low-grade cervical lesions.[12,13]

Table 3: Colposcopic findings of patients with different subtypes of abnormal Pap smear on first colposcopy

First Pap smear	First colposcopy				
	Unsatisfactory (*n* = 47) (%)	Normal (*n* = 720) (%)	CIN I (*n* = 41) (%)	CIN II (*n* = 17) (%)	CIN III (*n* = 13) (%)
ASC-US (*n*=213)	21 (10)	156 (73)	24 (11)	9 (4)	3 (1)
ASC-H (*n*=9)	1 (11)	5 (56)	1 (11)	0 (0)	2 (22)
LSIL (*n*=53)	3 (6)	36 (68)	9 (17)	4 (7)	1 (2)
HSIL (*n*=34)	8 (23)	14 (41)	5 (15)	4 (12)	3 (9)
CIS (*n*=3)	0 (0)	0 (0)	0 (0)	0 (0)	3 (100)
AGC (*n*=22)	5 (23)	14 (64)	2 (9)	0 (0)	1 (4)

Data presented as *n* (%); *n* = Number of patients; ASC = Atypical squamous cells; ASC-US = Atypical squamous cell of undetermined significance; ASC-H = Atypical squamous cell cannot exclude high-grade squamous intraepithelial lesion; LSIL = Low-grade squamous intraepithelial lesion; HSIL = High-grade squamous intraepithelial lesion; CIS = Carcinoma *in situ*; AGC = Atypical glandular cells; CIN=Cervical intraepithelial neoplasia

Table 4: Biopsy findings of patients with different subtypes of abnormal Pap smear on first biopsy

First Pap smear	First biopsy					
	Normal (*n* = 288) (%)	CIN I (*n* = 15) (%)	CIN II (*n* = 4) (%)	CIN III (*n* = 8) (%)	MGH (*n* = 4) (%)	SCC (*n* = 15) (%)
ASC-US (*n*=213)	201 (94)	7 (3)	0 (0)	1 (1)	0 (0)	4 (2)
ASC-H (*n*=9)	7 (78)	0 (0)	0 (0)	0 (0)	0 (0)	2 (22)
LSIL (*n*=53)	43 (83)	4 (7)	0 (0)	3 (6)	0 (0)	2 (4)
HSIL (*n*=34)	19 (60)	4 (12)	4 (12)	4 (12)	0 (0)	2 (4)
CIS (*n*=3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (100)
AGC (*n*=22)	17 (77)	0 (0)	0 (0)	0 (0)	4 (18)	1 (5)

Data presented as *n* (%); *n* = Number of patients; ASC = Atypical squamous cells; ASC-US = Atypical squamous cell of undetermined significance; ASC-H = Atypical squamous cell cannot exclude high-grade squamous intraepithelial lesion; LSIL = Low-grade squamous intraepithelial lesion; HSIL = High-grade squamous intraepithelial lesion; CIS = Carcinoma *in situ*; AGC = Atypical glandular cell; CIN = Cervical intraepithelial neoplasia; MGH = Microglandular hyperplasia; SCC = Squamous cell carcinoma

First, as mentioned above, human error can affect Pap smear results significantly. Pap smear taker may fail to collects cells or cells may be are discarded with the sampling device without being transferred to the slide[10] Majority of Pap smear slides are nonrepresentative sample of cervical cells because more than 80% of cervical sample cells are discarded with the sampling device.[14] Even after perfect sampling, there are still issues with making a perfect smear. Spreading sample evenly across the slide is not easy. These factors cause the variable quality of smear and therefore, difficulty interpreting of the sample. Furthermore, cell overlapping, cell distortion, and contamination with blood, mucus or debris that may cover important parts of slide can lead to misinterpretation.[15] In addition, pathologists had lower false-positive rates in the gynecologic cytology proficiency testing program than they did in pap education, but participants were more likely to report a false-positive response (HSIL+) for negative for intraepithelial lesion or malignancy and herpes simplex virus in the gynecologic cytology proficiency test program.[16]

Second, many cervical lesions — Especially low-grades - may spontaneously regress to lower stages or to the normal condition.[17]

Since 288 out of 334 patients with abnormal Pap smear had normal biopsies (86%), we can assume that these Pap smears had been reported as abnormal by human error, and only 46 had actually abnormal Pap smear at the start point.

Melnikow *et al*. reported that up to 58% of patients with abnormal Pap smear regress over 24 months.[12] Matsumoto *et al*. also reported spontaneous regression of low-grade lesions in more than 68% of patients.[18] Moscicki *et al*. investigated 187 women with LSIL and found that 61% of them regressed over 12 months of follow-up.[8]

Based the above evidence, when a Pap smear is reported abnormal, we should keep in mind that many factors other than an actual pathology might have caused this abnormality. Given the significant role of human error, it review of slides by another cytopathologist or re-collecting a cervical sample may help us to avoid more invasive procedures. False-positive Pap smears lead to needless follow-up, more screening, more invasive unpleasant procedures such as colposcopy and biopsy and eventually waste more time and money.[11] All these more invasive tests are also imperfect and may lead to false-positive results.[11] For instance, in the present study, 63 patients with abnormal colposcopy had a normal biopsy at the same time. Even in those women with biopsy-approved abnormal Pap smear, close observation and regular follow-up for 24 months can help us to avoid unnecessary procedures. However, when dealing with a cervical lesion, it is important to consider other risk factors associated with cervical malignancy such as human papillomavirus (HPV) infection or smoking.[7] These factors may decrease the chance of regression of a low-grade cervical lesion.[19,20]

To the best of our knowledge, this is the first study that has investigated a large cohort of Iranian women for 24 months. However, the major limitation of this study is a loss of 26 patients who had an abnormal initial biopsy during our 24-month follow-up. This could be because of their — Or their health care provider's-concern regarding the risk of progression of the lesion. Furthermore, we have no data on the second review of slides or repeating the Pap smears, so we cannot evaluate the role of these potential solutions for false-positive Pap smears. Finally, since we were not able to run the HPV test for all patients, we only used the previous history of HPV testing.

In summary, although Pap smear has been recognized as a successful screening test, it can be associated with high-rate of false-positive results.

In addition, the majority of low-grade cervical lesions can spontaneously regress. These findings suggest that long-term follow-up of a patient with abnormal Pap smear can help us to avoid needless interventions. Further studies are needed to evaluate the potential role of second review of slides or repeating the Pap smear in more appropriate management of a woman with an abnormal Pap smear.

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Conflicts of interest
There are no conflicts of interest.

AUTHOR'S CONTRIBUTION

All authors contributed in the study design, conducting the study and drafting the manuscript. All authors approved the final version for submission and take the responsibility for the manuscript content.

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