

## The Frequency of Unsatisfactory Pap Smears Based on the Bethesda System-2001 and their Reasons

Zarrin Ajoodani<sup>1\*</sup>, Shahnaz Babayanazad Ahari<sup>2</sup> and Sedigheh Nasrollahi<sup>3</sup>

<sup>1</sup>Assistant Professor of Obstetrics and Gynecology, Faculty of Nursing and Midwifery, Islamic Azad University, Karaj Branch, Karaj, Iran.

<sup>2</sup>M.Sc. of midwifery, Iranian Social Security Organization, Alborz Hospital, Karaj, Iran.

<sup>3</sup>M.Sc. of midwifery, Faculty of Nursing and Midwifery, Islamic Azad University, Karaj Branch, Karaj, Iran.

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Cervical cytology is widely used for primary screening of cervical cancer. Once the Pap smear is reported as unsatisfactory for evaluation, the cervical intraepithelial lesions cannot be certainly diagnosed, which leads to false negative results. We investigated the frequency of unsatisfactory smears and their reasons. This prospective descriptive analytical study was conducted from June 2011 to September 2013, on women having Pap smear in Gynecology Clinic of Alborz Hospital in Karaj, Iran. The tests were done conventionally by cervical brush and then adequacy of specimens was assessed based on Bethesda system 2001 by one pathologist. Of 1557 cervical cytology specimens, the frequency of satisfactory and unsatisfactory smears were 445 (92.8%) and 112 (7.2%), respectively. The reasons for unsatisfactory smear included obscured by inflammation in 48 (42.9%) cases, obscured by blood in 42 (37.5%), insufficient cellularity in 11 (9.8%), and poor fixation in 11 (9.8%). The results of this study showed that sampling by trained personnel, persistent supervision and use of appropriate equipment can reduce unsatisfactory Pap smears.

**Key words:** Pap smear, Smear adequacy, Unsatisfactory smear.

Cervical cancer is considered a preventable disease<sup>1</sup> and if it is detected in its prolonged precancerous phase, progress of lesions toward invasive form can be prevented by appropriate treatment<sup>2</sup>. Cervical cytology is extensively used in cervical cancer screening<sup>3</sup>. Since 1950's, the incidence of cervical cancer has been reduced by 79% and the mortality by 70% through the Pap smear test<sup>4</sup>. Yet, 30% of new cases of cervical cancer occur in women that have already been screened, but errors occurred in sampling, preparation, or interpretation<sup>3</sup>. Two thirds of such errors are attributed to smear sampling technique

and preparation<sup>5</sup>. False negative Pap smear results are reported from 1.1% to 55%<sup>6</sup>. It is highly important to attend to adequacy of smear for cytology assessment in order to reduce false negative results<sup>1</sup>. The Third Bethesda System 2001 (TBS 2001) attempted to standardize the criteria for specimen adequacy, but much confusion including significance of unsatisfactory smears and the causes are still exist<sup>7</sup>. Detection of cervical epithelial disorders becomes impossible or uncertain with unsatisfactory specimens<sup>8</sup>. In these cases, test repeating would be needed to obtain correct results, which would lead to extra visits and higher healthcare costs<sup>6, 8</sup>. Moreover, most women dislike it and are not willing to repeat the test, which means lost screening opportunity due to unsatisfactory results<sup>9</sup>. Thus, the onus is on health care providers and physicians to create the

\* To whom all correspondence should be addressed.

right experience for patients by preparing high quality specimens. Studies on smear adequacy indicate that unsatisfactory smear rate varies according to the system used to interpret smears, sampling technique, equipment, and skill of the sampler<sup>5</sup>. In a study by Akamatsu *et al.*, the rate of unsatisfactory smears was reported 11.45%<sup>10</sup>, while it was reported less than 1% in Thamboo *et al.* study<sup>11</sup>. In Iran, very few studies have been conducted on smear adequacy, and have mostly been based on either the old Bethesda system or comparison of sampling equipment. Thus, this study aims to determine the frequency and causes of unsatisfactory smears based on Bethesda System 2001 in conventional Pap smear tests performed on women attending Alborz Hospital in Karaj.

## METHODS

This prospective descriptive analytical study was conducted on a population of women attending Alborz Gynecology Clinic for their Pap smear tests between September 2010 and May 2013. Study exclusion criteria were the following: intercourse 48 hours prior to attendance, use of vaginal drugs a week before the test, pregnancy, history of hysterectomy, history of cervical cancer, and active uterine bleeding. The sample size was calculated as about 1560 patients with a random sampling method, using frequency of unsatisfactory specimen of 5-3%. Women sequentially entered the study until sample size was completed. A questionnaire for each participant was completed about their personal details, symptoms and complaints by a midwife. Then, vagina and cervix were examined and Pap smear specimens were prepared using conventional method, involving simultaneous endocervical and exocervical sampling with a 360° clockwise rotation of the cervix-brush, and specimen obtained was immediately transferred onto a slide, and fixed with the standard spray. Next, specimen and TBS2001 form<sup>1</sup> were sent to the laboratory for Papanicolaou staining and evaluation of adequacy and interpretation by a cytopathologist. Results obtained were recorded in a form.

In terms of adequacy, specimens were divided into satisfactory and unsatisfactory

groups. Group I (satisfactory smear) was divided into 3 subgroups of I(a) completely satisfactory, I(b) satisfactory but partially obscured I(c) no endocervical cells. Group II (unsatisfactory smear) was divided into 4 subgroups according to the cause of inadequacy, including: II (a) insufficient cellularity, II(b) poor fixation II(c) obscured by blood, and II(d) obscured by inflammation<sup>13</sup>. Data collected were statistically analyzed in SPSS-13 Win software.

## RESULTS

From the 1560 specimens, 3 slides were lost or broken, reducing specimens to 1557. Participants were aged between 19 years and 82 years, with a mean age of 43 years and standard deviation of 10, parity ranged from 0 to 10, and most participants (26%) had had 2 childbirths and 1162 (76.4%) patients were non-menopausal, and 390 (25.4%) were postmenopausal. From the 1557 Pap smear specimens, 1445 (92.8%) were rated satisfactory (group I), and 112 (7.2%) unsatisfactory (group II), which are presented in Table 1 according to the causes.

## DISCUSSION

The frequency of unsatisfactory cervical cytology smears in this study was 7.2%, which was less than the frequencies reported by Treacy *et al.* (11%)<sup>12</sup> and Akamatsu *et al.* (11.45%)<sup>10</sup>, but more than the unsatisfactory rates reported in studies by Fidda *et al.* (5.3%)<sup>6</sup> and Lu *et al.* (4.5%)<sup>7</sup>. The result of this study in satisfactory group showed that 68.3% of smears were completely satisfactory, 27.7% partially obscured, and 4% had no endocervical cells.

In total, 96% of specimens were found to contain endocervical cells by using cervix-brush, which concurs with studies by Jarvi (90.7%)<sup>13</sup> and Altermatt *et al.* (98.5%)<sup>14</sup>.

The presence of endocervical cells in smear is often regarded as an indication of good smear quality. According to Solomon *et al.* report, intraepithelial lesions are better detected with the presence of sufficient endocervical cells in the specimen. Moreover, due to increased prevalence of cervical adenocarcinoma, future use of endocervical cells may change<sup>15</sup>. By comparing

**Table 1.** Frequency distribution of smear adequacy according to reasons in women attending Alborz Hospital in Karaj

Adequacy	Number	Percentage
Satisfactory		
Completely satisfactory	987	68.3%
Partially Obscured	400	27.7%
No endocervical cells	58	4%
Total	1445	92.8%
Unsatisfactory		
Insufficient cellularity	11	9.8%
Poor fixation	11	9.8%
Obscured by blood	42	37.5%
Obscured by inflammation	48	42.9%
Total	112	7.2%
Overall	1557	100%

smear specimens prepared by cervix-brush and spatula, Dyp *et al.* showed both instruments were equally efficient<sup>16</sup> while, in a study by Noel *et al.*, only 64.8% of smears prepared by spatula contained endocervical cells<sup>17</sup>. Considering application of cervix-brush, the presence of endocervical cells in smears may be increased by using this instrument and sampling more carefully. This will positively affect detection of cervical dysplasia, particularly in postmenopausal women<sup>18</sup>. In the present study, reasons of unsatisfactory smears according to the prevalence were 42.9% obscured by inflammation, 37.5% obscured by blood, 9.8% poor fixation and 9.8% insufficient cellularity. Gavranovic *et al.* reported insufficient endocervical cells and thickness of specimen as the most important reasons of smear inadequacy<sup>8</sup>. In a study by Edwards *et al.*, 16.5% of smears were unsatisfactory, and the most common cause was insufficient cells<sup>19</sup>. In Yarandi *et al.* study, the most important cause of unsatisfactory smears was found insufficient number of squamous cells<sup>20</sup>. In the present study, low prevalence of these factors appears to be due to careful sampling and use of cervix-brush. One of the advantages of cervix-brush is simultaneous endocervical and exocervical sampling, which reduces likelihood of air drying<sup>21</sup>. If trained personnel perform sampling, blood and inflammatory cells will not obscure samples, which reduce unsatisfactory Pap smear cases. An investigation in Iran has showed that a reduction in unsatisfactory smears from 17.7% to 0.4% could

be achieved by training the samplers<sup>22</sup>. Limitations in the present study included a lack of follow-up for all unsatisfactory cases and future studies should determine clinical importance of follow-up of these cases.

## CONCLUSION

Considering the frequency and reason of unsatisfactory Pap smears in this study through greater care in sampling by trained personnel and constant monitoring and use of proper instruments, we can reduce inadequate specimens as the most common cause of false-negative cytologic findings.

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