The Use of Cytology Tests in the Diagnosis of Cervical Lesions in High Risk Patients

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Abstract
Numerous comparative studies were conducted of Liquid-based Cytology (LBC) vs Conventional Pap (CP) tests for the detection of cervical carcinomas and its precursors, however, few studies were performed to compare these two methods for detection of cervical infection by microorganisms, commonly seen in cervical cytology.

The Aim of this Work: To evaluate the use of manual liquid-based cytology (MLBC) and conventional pap smear test (CP) in the diagnosis of different cervical lesions, in high risk patients.

Methods: Four hundreds women with cervical symptoms were examined by cervical smear tests (200 MLBC and 200 CP). The cytology results were confirmed by colposcopic-guided biopsy.

Results: Comparable findings were noticed by both CP and MLBC: Specific infection (Trichomonas Vaginalis and Candida Albicans) were diagnosed in 9% in CP and 7.5% by LBC, abnormal epithelial changes (CINI, II and AGC) were found in 3% in CP & 3.5% in LBC, malignant tumors were diagnosed in 1% by CP & 1.5% by LBC. There were 28% and 30% unsatisfactory tests in CP and LBC respectively. Each test showed 95% sensitivity & 35% specificity in detecting specific inflammation, and 88% sensitivity & 39% specificity in detecting malignancy in the cervix, with no statistical differences between them, as referred by biopsy.

Conclusion: MLBC is comparable to CP in detecting different cervical lesions and is more suitable to be applied in our community.

Key Words: Conventional Pap (CP) – Manual Liquid-based cytology (MLBC) – Colposcopy – Cervical cancer – Microorganism.

Introduction
FOR more than ten years, liquid-based cytology has been developed for cervical screening. The main advantages of this technique are to reduce the number of inadequate smears and to provide enough cells for the detection of HPV through molecular biology techniques [1].

Cervical cancer is still the second most commonly diagnosed cancer in women. More than 83% of annual cervical carcinoma deaths occur in developing countries of the world, and 68000 cases in Africa [2].

In developing countries, including Egypt, the incidence of cervical carcinomas is low, 6.4% [3], while that of cervical infections is relatively high, especially in low socioeconomic standard, poor females. Cervical cytology both conventional and liquid-based Pap smear are used widely for primary screening of cervical cancer and cervical intraepithelial neoplasia but few studies evaluated these tests in detecting cervical infections and different cervical lesions which are prevalent in the majority of women during their reproductive periods.

Most of currently used LBC tests: Thin Prep, CytoRich, Sure Pap, Auto Pap (computer-assisted tests), are using new technology that is expensive, and difficult to be applied, especially in our community. Moreover, regular, well established screening programs for detection of cervical epithelial abnormalities are lacking in our hospitals. In this study we choose an inexpensive, manual LBC test to be cost-effective for high risk, poor patients. The risk factors for different cervical lesions that are present in our community are early marriage, long marital duration, grand-multiparity, low socioeconomic standard of life, bad personal hygiene, lack of regular medical supervision, absence of screening programs, and poor education.

Aim of the work:
To evaluate the use of both conventional Pap and manual liquid-based tests in the diagnosis of different cervical lesions, in high risk females.
Colposcopic-guided biopsy and histopathology were performed to confirm the smear results.

**Patients and Methods**

*Patient grouping:*

Four hundred (400) women were recruited from those attended the Ob/Gyn Outpatient Clinic, Sohag University Hospital during the period from March 2010 to May 2011 with symptomatic cervical lesions. They were randomly allocated into two groups (200 MLBC) and (200 Conventional Pap). Every woman in both groups was clinically evaluated as regard age, parity, age at first marriage, duration of marriage, complaint, medical therapy, obstetric history and contraceptive methods.

*Gynecological assessment and biopsy:*

For all patients, the gynecological examination was started by bimanual examination, speculum inspection of the cervix, Pap smear either conventional or liquid-based smear, followed by colposcopic evaluation, including acetic acid (application of 5% acetic acid to the ectocervix for minutes and detect the aceto-white areas) and Schiller test (application of Logol's Iodine to the cervix to identify pale yellow abnormal lesion). A colposcopic-guided biopsy from the suspicious areas was performed after patient consent. The patients were notified about the procedure, the scheduled date for the biopsy results and full pathological reports were given to them. The research was approved by the Institutional Ethical Committee.

*Pap smear technique:*

A cytological smear was taken (either conventional Pap or LBP) according to the patient's test date. The patients were advised to be tested not during the menstrual period or after intercourse, and to avoid the use of vaginal medications, creams, douches and the sample was taken before vaginal examination was done. Adequate cervical cytology specimen should involve circumferential sampling of the ectocervix adjacent to the transformation zone, the endocervix and the cervical transformation zone (T-zone).

For conventional Pap smear, a combination of the extended-tip spatula and the endocervical brush provides sampling of the ectocervix, T-zone and the endocervix. Excessive mucus, discharge or blood was carefully removed. The slide was then rapidly immersed in 95% ethanol to avoid air drying artifact, or a special fixative spray was applied directly to the slides when available. For liquid-based Pap test, the endocervical brush and extended tip spatula was carefully rinsed in a single container with appropriate fixative solution (95% ethanol). Samples were transported to the laboratory. After centrifugation, a few drops of the sediment is drawn and applied on a microscopic slide.

*Staining technique:*

Both CP and MLBC were stained with Papanicolaou stain and the biopsy specimens were stained by routine (H&E) stain.

*Evaluation of the biopsy results:*

All the slides were examined and evaluated by single pathologist*.

The colposcopic findings were classified by the gynaecologist* according to Bethesda system, 2001 [4].

*Descriptive diagnosis:*

- Low grade squamous intraepithelial lesions (LSIL).
- High Grade squamous intraepithelial lesions (HSIL).
- Squamous cell carcinoma.
- Adenocarcinoma.
- Infection by 3 microorganism, Candida Albicans (Moniliasis), Trichomonas Vaginitis, and HPV virus were detected and recorded.

A complete histopathology report was written and a copy was given to every case.

*After study patient's care:*

The cases that diagnosed as malignant were referred to the Sohag University Oncology Center for management; Patients with pre-malignant epithelial changes were scheduled to follow-up with the Ob/Gyn at the out-patient, high-risk clinic.

Women with different cervical infections were given complete medical treatment for each specific lesion.

*Results*

400 women with symptoms related to cervical pathology were divided randomly into two groups 200 examined by CP and 200 by MLBC. The age range of the studied women was (20-70y). The patients complain of vaginal discharge, backache, pruritis, contact bleeding, and dyspareunia. Colposcopic-guided biopsy was performed to confirm the cytological findings.

The colposcopic findings that indicated tissue biopsy are shown in Table (1) as followed: Cervical erosion or ectopy in 66 cases, cervical polyp either
endocervical or ectocervical in 52 cases, cervical ulcers with bleeding and secondary infection in 17 cases, and leukoplakia in 26 cases. Positive Schiller test cases (aceto-white areas; and irregular cervical mass found in 9 cases, were biopsied and examined histologically. 226 cases were within normal limits by colposcopy.

Cytological findings were nearly comparable in both tests, Chronic non specific cervicitis was detected in 80 cases (40%) in each test; CP and MLBC, (polymorphes, eosinophiles, plasma cells, lymphocytes, cell debris, necrotic tissue and pus cells) Table (2).

Specific cervical infections caused by Candida albicans was found in 12 cases by CP and 11 cases with MLBC, Trichomonas vaginalis infection was detected in 6 cases by CP and 4 cases with MLBC. Table (3), Figs. (1,2).

![Image A: Cervical fungal infection by Candida Albicans hyphae.](image)
![Image B: Koilocytosis (HPV).](image)
![Image C: Chronic non specific cervicitis inflammatory cell infiltrate and congested BV.](image)
![Image D: Trichomonas Vaginalis infection of the cervix (CP).](image)
Squamous cell carcinoma was detected in one case by each test and was both confirmed by biopsy as Grade I tumor. Adenocarcinoma was also detected in single case by CP and in 2 cases by MLBC, and the three cases were confirmed by biopsy as well differentiated adenocarcinoma. Premalignant epithelial changes (CIN I and II were detected in 6 cases by CP and 7 cases by LBC), and biopsy examination confirmed that CIN I in 4 cases and CIN II cases in 4 cases with 9 cases diagnosed as atypical glandular cells (AGC).

Unsatisfactory for examination smear test were found in 18 cases CP smear (excessive mucus, blood, necrotic tissue, thick areas), and 19 cases MLBC smear (scant cellularity), and negative
cytology was found in 38 cases (19%) by CP and 41 cases (20.5%) in LBC.

HPV infection (koilocytosis, moderate dysplasia, acanthosis, papillomatosis, and focal hyperkeratosis was detected in 73 cases (18.5%), (38 by CP and 35 by LBC).

The findings of both CP and MLBC were comparable and the sensitivity of cytology smear tests both CP and MLBC in detecting specific cervical infection was (95%) and specificity was (35%), while the sensitivity of smear tests in detecting epithelial pre-malignant changes and malignancy was (88%) and specificity was (39%).

Table (1): Colposcopic findings of the studied cases.

<table>
<thead>
<tr>
<th>Colposcopic findings</th>
<th>CP</th>
<th>LBC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical erosion</td>
<td>32</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>Cervical polyp</td>
<td>28</td>
<td>24</td>
<td>52</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>14</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>Ulcer</td>
<td>8</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Abnormal ectocervical area</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Aceto-white areas</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Within normal limits (WNL)</td>
<td>114</td>
<td>112</td>
<td>226</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>200</td>
<td>400</td>
</tr>
</tbody>
</table>

Table (2): Cytological findings of the studied cases.

<table>
<thead>
<tr>
<th>Cytological findings</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus cells</td>
<td>17</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>266</td>
</tr>
<tr>
<td>Polymorphes</td>
<td>17</td>
</tr>
<tr>
<td>Hyphae of candida</td>
<td>23</td>
</tr>
<tr>
<td>Trichomonas organism</td>
<td>10</td>
</tr>
<tr>
<td>Koilocytic cells</td>
<td>37</td>
</tr>
<tr>
<td>Atypical glandular cells</td>
<td>12</td>
</tr>
<tr>
<td>Atypical squamous cells</td>
<td>6</td>
</tr>
<tr>
<td>Normal squamous cells</td>
<td>79</td>
</tr>
<tr>
<td>Scant cellularity</td>
<td>37</td>
</tr>
<tr>
<td>Thick areas</td>
<td>28</td>
</tr>
<tr>
<td>Blood</td>
<td>11</td>
</tr>
</tbody>
</table>

Table (3): Histopathological results.

<table>
<thead>
<tr>
<th>Histopathological findings</th>
<th>CP</th>
<th>MLBP</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC</td>
<td>80</td>
<td>80</td>
<td>40%</td>
<td>40%</td>
</tr>
<tr>
<td>Candidal albicans</td>
<td>12</td>
<td>11</td>
<td>6%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>6</td>
<td>4</td>
<td>6%</td>
<td>4%</td>
</tr>
<tr>
<td>Koilocytosis (HPV)</td>
<td>38</td>
<td>35</td>
<td>19%</td>
<td>17.5%</td>
</tr>
<tr>
<td>AGC</td>
<td>4</td>
<td>5</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>2</td>
<td>1</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Squamous cell ca</td>
<td>1</td>
<td>1</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>CIN I</td>
<td>1</td>
<td>1</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>CIN II</td>
<td>1</td>
<td>1</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Negative smear</td>
<td>38</td>
<td>41</td>
<td>19%</td>
<td>20.5%</td>
</tr>
<tr>
<td>Unsatisfactory smear</td>
<td>18</td>
<td>19</td>
<td>9%</td>
<td>9.5%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Numerous comparative studies were conducted of conventional Pap vs Liquid-based cytology for the detection of cervical carcinomas and its precursors, however, few studies were performed to compare these 2 methods for the detection of microorganisms, commonly seen in cervical cytology. In developing countries, including Egypt, the incidence of cervical cancer is low while that of cervical infections is high, specially in low socio-economic females. The incidence of cancer in North Africa is 103/100,000 with 78/100,000 mortality rate at 2008 [5].

Two automated expensive methods approved by Food and Drug Administration widely used: the ThinPrep and SurePath that add to the cost of CP. Another alternative inexpensive test; the PapSpin that uses a conventional centrifuge to deposit the cells from the liquid onto a glass slide has been reported [6].

MLBC was found to be comparable to the conventional scrap smear in cervical cytology [7] and can be used with a number of fixatives [8].

The Bethesda 2001 workshop reviewed issues regarding terminology and reporting of cervical cytology. The report includes a statement of adequacy and the diagnosis. There are two categories for specimen adequacy: Satisfactory for evaluation and unsatisfactory for evaluation. The unsatisfactory for evaluation category indicates the smear was rejected/not processed or that it was processed and examined but was unsatisfactory for evaluation of epithelial abnormality. Unsatisfactory smears are mostly due to cervical sampling and specimen collection problems [4].

In our study negative smear was noticed in 29% (38 cases negative and 18 unsatisfactory for examination) with CP and 30% (41 negative tests and 19 unsatisfactory for examination with MLBC.

The unsatisfactory smears (19%) were due to scant cells in LBC and the presence of blood, excessive mucus secretion, cell debris and thick areas in CP.

Recent cohort studies also reported that causes of unsatisfactory and satisfactory but limited test results were similar for LBC and CP with scant cellularity constituted the main cause for both [9-12].

Weynand et al., 2003 described (0.7%) of inadequate samples with PapSpin system [13], and Bergeron et al., found (0.14%) unsatisfactory sam-
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samples with the CytoScreen system [14], while Chhieng et al., 2004, described (0.8%) unsatisfactory tests [15], and Garbar et al., 2005 found it to be (0.9%) in their study [16]. The differences in the percentage of unsatisfactory samples between our study and the mentioned studies could be due to the different smear methods as they used expensive, automated LBC technique, which is more sensitive in detecting, even scanty cervical smear, than the cytocentrifugation LBC test.

In the current study, the histopathological findings of the MLBC were virtually the same as for CP, although a higher background (cell debris, inflammatory cells, mucus and blood) was observed in CP, but did not influence the overall results.

Few reports supported the fact that cytology is a valuable tool in the diagnosis and detection of cervical infection [17].

In our study, Candida Albicans was the commonest pathogenic organism in the studied cases as it was found in (6%) 12 cases by CP and in 11 cases (5.5%) by LBC test. In Bukhari et al., Candida was diagnosed in 6.5% [18] while in Sherwani et al., infectious agents were detected in 8.7% of cases by PapSpin and in 3.1% by CP [19].

In the present study, 6 cases (3%) of Trichomonas was diagnosed by CP test while with LBC, 4 cases (2%) were detected. Trichomonas vaginalis was found in 7.3% of cases in Bukhari et al., 2012 [18], while in Karaman et al., 2008 study, it was found in 0.9% of cases [20]. The variability in the percentage of different infective agents in the studies demonstrated the differences in the socio-economic and educational levels between the studied groups at different countries.

HPV was diagnosed in 19% of cases in our study (according to criteria of HPV infection in Schneider et al., 2003) [21]. In Bukhari et al., HPV was diagnosed in 7% of cases [18].

Malignant and premalignant cervical conditions were detected in 18 cases (4.5%) with both CP and LBC and only (2.5%) was confirmed by the biopsy specimens.

In our study, we diagnosed adenocarcinoma in one case by CP and in two cases by MLBC.

Several problems with conventional cytology smears are addressed by liquid-based (LBP) methods. With LBP, the sampling device is directly placed into a liquid fixative instead of being directly placed on a glass slide providing immediate fixation, thereby, decreasing air drying artifacts and thus improving specimen adequacy. Additionally, LBP improves cellular sampling, distributes the cells more evenly over the slides, reduces cell overlapping and decreases obscuring background factors such as blood and inflammatory cells often seen in conventional cytology (factors that may also affect test accuracy). Cytocentrifugation provides a proper mixing and distribution of the scraped cells. Another advantage of LBP over CP is the availability of residual material which potentially may be used for detection of HPV DNA and DNA methylation using PCR.

Many studies demonstrated the superiority of the quality of LBC in comparison with those with CP and found MLBC provides an alternative method to the currently automated technique of LBC [22-24].

In the present study, the sensitivity of smear tests (CP and LBC), in detecting inflammatory conditions of the cervix was 95% and specificity was 35% with no statistical differences between them. The sensitivity of cytological examinations in detecting malignant and pre-malignant epithelial lesions was equal for both tests (88%), and specificity was 30% for each test.

Mattosinho et al., compared the diagnostic efficiency of LBC and the CP smear in comparative study using histological results as the gold standard in 800 women of low socioeconomic outpatient population. The sensitivity and specificity of LBC for detection of cervical intraepithelial lesions and cancer were (75.3%) and (86.4%) and for CP (81.8%) and (85.2) with no statistical differences between them [25].

Sherwani et al., concluded in their study that the sensitivity and specificity of PapSpin was 97% & 50% respectively and of CP smear 53.7% and 50% [19].

Recent evidence from 2 randomized controlled trials and a meta-analysis comparing the performance of LBC and CP support the conclusion of equal accuracy for both methods [26-28].

In one meta-analysis of 47 articles, the sensitivity of the LBC (ThinPrep) was found to be (76%) and specificity (86%) as compared to sensitivity of (68%) and specificity of (79%) for conventional smear, relative to histology [29].

Conclusion:

MLBC method was found to be comparable to the CP smear. It has potential benefits of better morphology and quality of testing with preservation
of the specimen for ancillary studies. It would be worthwhile to get the advantages of LBC in our community by using a cost effective MLBC as an alternative to the automated expensive LBC method.

References


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