The City of Linzhou, Henan Province, China has a high incidence of esophageal carcinoma (129.76/100,000 in men and 102.65/100,000 in women) that apparently threatens the health and lives of its people. 1 In the late 1950s, the Cancer Institute, Chinese Academy of Medical Sciences, initiated wide-range prevention and treatment programs for patients with esophageal carcinoma. Cytologic screening in a large population enables timely treatment of many patients with curable esophageal carcinoma. However, in the research on cytologic screening as compared with biopsy diagnosis for esophageal carcinoma, the sensitivity was not favorable. 2

The liquid-based cytology with a novel smear technique is widely reported to be valuable in screening for cervical carcinoma. Current research from most laboratories have demonstrated that the liquid-based cytology has a higher sensitivity than the traditional Papanicolaou smear. 3,4 The current research brought liquid-based cytology into the cytologic screening of esophageal carcinoma to investigate its value.

Materials and Methods

Subjects

A total of 940 subjects ranging in age from 35 years to 69 years from 4 administrative villages in Yaocun Township of Linzhou City, Henan Province, were included in this examination of liquid-based cytology for esophageal carcinoma by the Cancer Institute in partnership with the US National Cancer Institute (NCI) in March 2002. Residents with esophageal varices, esophageal ulcers, bleeding gastroduodenal ulcers, severe heart disease, hypertension, acute laryngitis, or severe enervation were excluded from the research (Fig. 1).

Sampling

Cytologic screening was performed on the subjects in the morning after fasting overnight. Before being treated in the operating room, informed consent was obtained from the subjects. Dental prostheses were removed and the mouth was

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**Liquid-based cytology for esophageal carcinoma screening**

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**Abstract** Background and Objective: Cytologic screening of asymptomatic high risk individuals can detect curable esophageal carcinomas and has been used for several decades. However, the sensitivity of such screening is relatively low, which limits its widespread use and development. This study investigated the utility of liquid-based cytology in esophageal carcinoma screening. Methods: A mass screening of esophageal carcinoma was performed for asymptomatic residents in Yaocun County, Linzhou City, Henan Province, China. Esophageal samples were put into a liquid buffer for cytologic diagnosis. Endoscopic biopsies were performed on all subjects. Cytologic diagnostic categories were adapted from the criteria of The Bethesda System (TBS). The results of liquid-based cytology were compared with those from endoscopic biopsy. The sensitivity and specificity of liquid-based cytology was evaluated. Results: Carcinomas in situ and carcinomas were identified in 17 (2.4%) of the 710 subjects. Taking atypical squamous cells of undetermined significance-favor neoplasm (ASCUS-N) as the detection threshold, the sensitivity and specificity of liquid-based cytology were 76.5% and 76.0%, respectively. Conclusions: In a hospital with a high level of conventional cytology, although the liquid-based technique has not gained much improvement in screening sensitivity, the workload of reading slides has been greatly decreased.

Key words: Esophagus neoplasm, balloon cytology, liquid-based cytology, screening, biopsy
One-quarter of the concentrated cell solution was transferred to a centrifuge tube containing 12 mL of white or red AutoCyte preservative (TriPath Imaging Inc., Burlington, North Carolina, US) to produce the smears using the AutoCyte Prep machine (TriPath Imaging Inc., Burlington, North Carolina, US). Three-quarters of this concentrated cell solution were transferred to the freezing tube for cytologic smears in the future.

Preparation of the cytologic smear

The concentrated cell solution was vortexed for 10 min and centrifuged at 600 × g for 10 min. The cell pellet was resuspended with 10 mL of distilled water and was transferred to the CytoRich centrifuge tube, followed by centrifugation at 600 × g for 5 min. After the supernatant fluid was removed, the tube was placed on the vortex machine to create a homogeneous mixture. The tube rested for 15 min before being transferred to the AutoCyte Prep machine. Cytologic smear slides with a diameter of 13 mm and with Papanicolaou staining were prepared using the AutoCyte Prep machine.

Cytologic diagnosis

The cytologic smear slides and the histological slides were read by both Chinese and American experts in a blinded manner. The cytologic diagnosis was based on The Bethesda System (TBS) for the diagnosis of cervical or vaginal cancer.

The degree of satisfactory evaluation on the slides

The smear slides were considered satisfactory if they met the following criteria: complete squamous epithelial cells >10 and complete adenocytes >10 in the low-power field (×10). The smear slides were considered less than satisfactory if they met any of the following criteria: 1) 50%~75% cells degenerated; 2) 50%~75% epithelial cells were overlapped by inflammatory cells or blood cells; or 3) complete adenocytes were less than 10. The smear slides were unsatisfactory if they met any of the following criteria: 1) >75% cells degenerated; 2) >75% epithelial cells were overlapped by inflammatory cells or blood cells; or 3) epithelial cells in more than 4 low-power fields (×10) were ≤ 10.

Cytologic grading

Squamous cells were graded as WNL (within normal limits), BCC (benign cellular changes), ASCUS-R (atypical squamous cells of undetermined significance-favor reaction), ASCUS-N (atypical squamous cells of undetermined significance-favor neoplasm), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), SUSCC (suspicous for squamous cell carcinoma), and SCC (squamous cell carcinoma). Abnormal adenocytes were graded as AGCUS-R (atypical glandular cells of undetermined significance-favor reaction), AGCUS-N (atypical glandular cells of undetermined significance-favor neoplasm), SUACC (suspicous for adenomatous cell carcinoma), and ACC (adenomatous cell carcinoma). To standardize the diagnosis terminology in cytology and histology, the LSIL in TBS was equivalent to mild atypical hyperplasia and the HSIL was equivalent to moderate or severe atypical hyperplasia and carcinoma in situ.

Iodine staining and multiple-site endoscopic biopsy

At 5 days after balloon cytology, the endoscopic examination was performed with the assistance of 1.2% iodine staining. If a lesion could be observed by the naked eye, biopsy was conducted at the site. If no lesions were visualized, 2 pieces of...
tissue were obtained from the site, 26 cm from the esophagus and the cardia.

Statistical analysis

The endoscopic biopsy findings were regarded as the reference. The cytologic diagnosis was compared with the histology results of the endoscopic examination. The cytology and histology diagnoses involved both the squamous epithelium and the glandular epithelium. In the analysis of the cytologic screening capacity, the most severe diagnosis was regarded as the final in both the cytologic and histological diagnoses regarding the squamous epithelium and the glandular epithelium. All analyses were performed using SPSS version 13.0. Between-group differences of the diagnostic sensitivity were analyzed using a $\chi^2$ test. A significant difference was considered if $P<0.05$.

Results

Basic information

Biopsy findings were obtained from 740 of the 940 subjects, of whom 30 had unsatisfactory smear slides and thus were excluded from the analysis. Consequently, 710 subjects were included in the final analysis.

The degree of satisfactory evaluation for the smear slides

Of the 710 cases, 497 and 213 were considered satisfactory and less than satisfactory, respectively. Of the less-than-satisfactory cases, 77 had large blank areas or large areas of mold contamination on the slides, 110 lacked adenocytes on the slides, and 26 had inflammatory cells or blood cells overlapping epithelial cells (Fig. 2). Regarding ASCUS-N as the positive detection threshold, the sensitivity of liquid-based cytology in the diagnosis of moderate atypical hyperplasia or worse was 60.2% and 43.3% ($P=0.10$) in the satisfactory and less-than-satisfactory cases, respectively.

Comparison of the cytologic and histologic diagnoses of the endoscopic biopsies

Of the 710 cases, 235 (33.1%) were histologically diagnosed as mild atypical hyperplasia or worse, including 2 cases of squamous cell carcinoma, 5 squamous cell carcinoma in situ, 1 adenocarcinoma, 9 adenocarcinoma in situ, 34 severe squamous epithelium atypical hyperplasia, 5 severe glandular epithelium atypical hyperplasia, 82 moderate squamous epithelium atypical hyperplasia, 94 mild squamous epithelium atypical hyperplasia, and 3 mild glandular epithelium atypical hyperplasia. A total of 52 (22.0%) cases were diagnosed as LSIL or worse in the cytology. Of the 475 cases with a negative histologic diagnosis, 452 (95.2%) were diagnosed as LSIL or worse (ASCUS or negative). The overall accuracy for the cytologic diagnosis was 71.0%.

Analysis of the cytologic screening capacity

Table 1 details the sensitivity and specificity of cytologic diagnosis for carcinoma in situ or worse, and moderate atypical hyperplasia or worse. As the threshold of the cytologic diagnosis lowered, the sensitivity of the cytologic diagnosis decreased, while the specificity increased. In light of a threshold of $\geq$ ASCUS-N, the sensitivity of the cytologic diagnosis was high, while the specificity decreased insignificantly. The diagnostic sensitivity for carcinoma in situ or worse and moderate atypical hyperplasia or worse were 76.5% and 56.5%, respectively, while the specificity was 76.0% and 82.3%, respectively.

Discussion

The current research reported the use of liquid-based cytologic screening of esophageal carcinoma and analyzed the accuracy of the liquid-based cytologic diagnosis as compared with the histologic diagnosis of endoscopic biopsies. The research innovatively avoided errors arising from the natural transition of diseases during follow-up through a comparison with the histologic diagnosis and thus provided reliable data.

Liquid-based cytology is a smear technique that developed at...
the end of last century. Compared with conventional smears, it has many advantages and innovations. First, the sampling device is directly immersed in the preservative and removed after the collected cells are retained in the sample bottle through constant shaking. Second, during the smear procedure, the cells are mixed homogeneously and proportionally diluted so the chance of abnormal cells transferring to the smear slide increases. Finally, the wet fixation technique enables clear cellular structures. Liquid-based cytology has recently become a hot spot in research thanks to its advantages over the conventional smear.

In the current research, regarding ASCUS-N as the positive detection threshold, the sensitivity of liquid-based cytology in the diagnosis of carcinoma in situ or worse and moderate atypical hyperplasia or worse was 76.5% and 56.5%, respectively, which is not significantly different from the results of cytologic screening (where 59% of cytologic screening diagnosed moderate atypical hyperplasia or worse) \(^4\) \((P=0.73)\). This outcome should encourage researchers to further evaluate the value of liquid-based cytology in practice.

Theoretically, the sensitivity of the cytology is influenced by clinical sampling, laboratory smear processes, and cytologic diagnosis. Liquid-based cytology makes use of the automatic and programmed smear technique that standardizes the smear procedure in the laboratory, thus minimizing the impact of individual differences of laboratory staff on smear quality and providing high-quality smear slides for cytologic diagnosis. In addition, abnormal cellular structures on liquid-based slides are clear and easy to identify as diseased cells, which decreases qualification requirements for technicians who read the slides. As different laboratories have different levels of competence in preparing and reading slides, liquid-based cytology may have different degrees of value for them.

Regarding the cytologic diagnosis of cervical cancer, liquid-based cytology apparently increases the sensitivity of histological screening in Turkey and China, as strict systematic laboratory quality control is lacking and there is no standard screening system for cervical cancer, \(^4\) while the benefits from liquid-based cytology are not apparent, in Italy and France, as a standard screening system exists. \(^7\) This was verified by the findings from the use of liquid-based cytology in cytologic screening of esophageal carcinoma in the current research. The Laboratory of Cytology, Cancer Institute, started to conduct biologic screening for esophageal carcinoma in Linxian County in the 1950s and accumulated rich experience in conventional cytologic screening. So we expected insignificant benefits from liquid-based cytology. It is thus hypothesized that liquid-based cytology does not apparently increase the sensitivity in health care institutes with rich experience in conventional cytology, while it is more valuable in areas with substandard skills in cytology.

In the current research, liquid-based smears, while guaranteeing stable sensitivity and specificity, significantly reduced the workload required for reading the smear slides. On one hand, the counts on liquid-based slides to be read are fewer than on conventional smear slides. In conventional cytology, 4 smear slides are required for each case, each with between 1.5 \(\times\) 10^5 and 3 \(\times\) 10^5 cells, while only 1 fluid-based slide of 13 mm in diameter is needed for one case with between 4 \(\times\) 10^4 and 7 \(\times\) 10^4 cells. On the other hand, the background of liquid-based slides is transparent, rendering it easy to read. Additionally, the cell samples can be preserved for a long time for further cytologic research. It was reported that the cells in the liquid-based preservative could be used for immune and cytologic experiments even after preservation for 1 year. \(^6\)

In the clinical sampling for liquid-based cytologic diagnosis of esophageal carcinoma, several aspects may be improved. First, the esophageal carcinoma samples are often mixed with thick mucus that cannot be dissolved while in the Autocyte preservative. The existence of mucous makes the detachment of cells from the slide possible, leaving a blank area on the slide that influences the diagnosis. It is thus recommended to use the red Autocyte preservative instead. Second, the area of the esophageal mucous membrane is larger than that of the cervix mucous membrane, and people from high-risk areas have a high rate of mold infection in the esophageal mucous membrane and consequently the mold may occupy a large area on the slide. A liquid-based slide of 13 mm in diameter cannot meet the requirements for diagnosis, leading to a potential diagnosis of ASCUS (33.2\%). Increasing the number of the slides for liquid-based cytology may accordingly increase the sensitivity of the diagnosis.

In the past 40 years of cytologic screening, criteria for cytologic diagnosis have been developed. Cytologic diagnosis is not only about differentiating cancer from non-cancer, but also involves identifying precancerous cells. A five-grade classification system was established in the 1980s and is widely used in research on cytology for esophageal carcinoma in China. \(^8\) The five grades consist of normal, mild dysplasia, severe dysplasia (including Grades I and II severe dysplasia), suspicious cancer, and cancer. Summarizing previous studies on cytologic screening, we found that some cases of cancer and
cytology and TB shave been initially verified in terms of feasibility diagnosis are improved. In the current research, liquid-based diagnosis of biopsies using an esophagoscope as reference. The their research, TBS and the five-grade classification standard were compared for cytologic diagnosis with the histologic diagnosis of biopsies using an esophagoscope as reference. The overall accuracy of the five-grade classification system was 32% for mild atypical dysplasia or worse, while the overall accuracy of TBS was 66.2%, as opposed to the overall accuracy of 71.0% of cytologic diagnosis in the current research.

Cytologic screening is simple, cheap, and acceptable to patients as a valuable technique. However, its sensitivity is not favorable until the clinical sampling, smear process, and diagnosis are improved. In the current research, liquid-based cytology and TBS have been initially verified in terms of feasibility in cytologic screening. The findings may be referred to in similar research or, even, in practice.

References