Cancer Screening and Prevention **2023** vol. 2(3) | 191–195
DOI: 10.14218/CSP.2023.00008

Review Article

Strategies on Endoscopic Screening for Esophageal Cancer at Early Stage and High-risk Subjects with Esophageal Precancerous Lesions on Symptom-free Subjects in High-incidence Areas

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Received: April 12, 2023 | **Revised:** July 28, 2023 | **Accepted:** August 24, 2023 | **Published online:** September 25, 2023

Abstract

Endoscopic biopsy and histopathological examination remain one of the critical methods for high-risk subjects (HRS) screening for esophageal squamous cell carcinoma (ESCC) in symptom-free subjects (SFS) of high-incidence areas (HIA) for ESCC. Almost 90% of the symptom-free residents show normal esophageal epithelia in HIA of ESCC. Based on that, overexamination by endoscopy was found in the screening of early ESCC. Furthermore, in large-scale screening on SFS in HIA of ESCC, the application of endoscopy is limited because of the complicated protocol, high cost, and shortage of experienced endoscopists. The authors suggest a two-step screening method. The first step involves a non-invasive serological screening by which to determine the blood level of neoplasm-related molecules which indirectly reflects the esophageal epithelial lesions. Endoscopic and histopathological examinations are involved in the second step. The second step will decrease the screening cost and improve the effectiveness of endoscopic examination for large-scale screening in HIA of ESCC. It is crucial to combine the two steps within a cooperative medical system in rural villages and communities in cities for extensive application.

Introduction

At present, esophageal squamous cell carcinoma (ESCC) remains one of the main causes of tumor-related deaths in Linzhou and Anyang cities of Henan Province, an area of ESCC high-incidence worldwide. Although the 5-year survival rate of early ESCC after surgery is more than 95%, clinically over 90% of patients with ESCC are diagnosed initially at the medium and advanced stages. The main reason for this is the lack of specific clinical symptoms in patients at the early stage. Once symptoms develop, such as dysphagia, almost all cases are already at the middle or advanced stage. Therefore, it is particularly important to conduct screening of high-risk subjects (HRS) for the early detection of disease in symptom-free subjects (SFS) residing in the HIA for ESCC.[1,2] Esophageal exfoliative cytology (dragnet) is one of the important methods used for symptom-free HRS screening.[3] However, endoscopy plus iodine staining with targeted mucosal biopsy has the advantage of providing a visual field of vision that could facilitate targeted mucosal biopsy and endoscopic treatment, and as such has quickly replaced esophageal exfoliative cytology.[4,5]

Here, the authors summarize the experience of SFS screening in high-risk areas of ESCC for a period of over 10 years, and conclude that endoscopy has certain limitations in SFS screening.

Keywords: Endoscope; Screening; Esophageal cancer; High-risk subjects; Symptom free subjects.

Abbreviations: ctDNA, circulating tumor DNA; ESCC, esophageal squamous cell carcinoma; HIA, high-incidence areas; HRS, high-risk subjects; SFS, symptom-free subjects.

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How to cite this article: You D, Zhao XK, Xu RH, Lei LL, Li XS, Wang LD. Strategies on Endoscopic Screening for Esophageal Cancer at Early Stage and High-risk Subjects with Esophageal Precancerous Lesions on Symptom-free Subjects in High-incidence Areas. *Cancer Screen Prev* 2023;2(3):191–195. doi: 10.14218/CSP.2023.00008.

Limitation of endoscopy in SFS screening in high-risk areas of ESCC

Endoscopy plus iodine staining with targeted mucosal biopsy is well recognized as one of the most effective methods for early de-

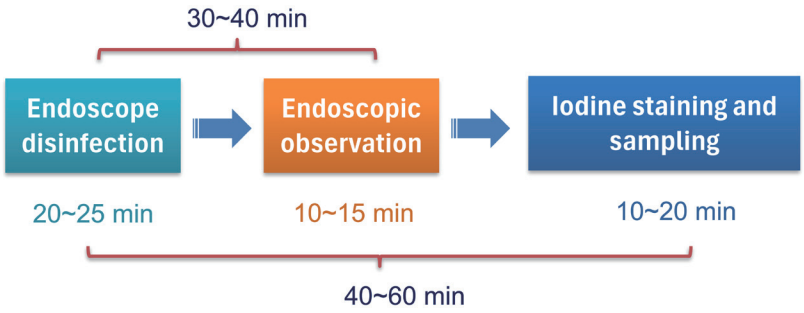

Fig. 1. Endoscope procedure. Iodine staining and biopsy may or may not be required.

tection of ESCC in SFS residing in HIA.[6–8] Generally, the most suitable screening population for HRS screening is symptom-free residents over 35 years age in HIA of ESCC. Endoscopic and mucosal biopsy histopathological examination for these residents usually identifies 2–3% of early ESCC and 6–10% of dysplasia and basal cell hyperplasia (i.e. precancerous lesions).[3,9] Regular pigment endoscopy combined with mucosal targeted biopsy and pathological examination is the most effective method to find precancerous lesions and early ESCC. At present, no alternative method has been found for the screening of HRS. For patients with early ESCC and precancerous lesions, the importance of endoscopic and mucosal biopsy histopathological examinations cannot be overemphasized.

However, that method also has its limitations. The endoscopic lesion detection rate is low and the screening cost is high. Almost 90% of the esophageal epithelial histopathology findings fall within the normal range of the symptom-free residents screening; as such, overexamination of endoscopy exists in the HRS screening.[3,10] The screening method by endoscopy plus iodine staining with targeted mucosal biopsy for 100 symptom-free residents reportedly detects 2–3 patients with early ESCC and 6–10 patients with precancerous lesions. The main reason for this phenomenon is still the "symptom-free" status. Based on that, overexamination by endoscopy exists during SFS screening. What is more, the lesion detection rate is low. In other words, nearly 90% of tested subjects are accompanied with the inspection. This is obviously too much waste to justify large-scale popularization and application. Reducing both the blindness of SFS screening and the scope of endoscopy will improve the detection rate of early ESCC, and these aims are urgent for improving screening of symptom-free HRS and early ESCC in HIA.

In addition, endoscope screening is costly, invasive and uncomfortable, and especially requires experienced endoscopists and specific endoscopic equipment. In addition, the standard procedure is complex.[11–13] The endoscope itself should be disinfected and subjected to other procedural methods for 20–25 m after use on each patient. The endoscopic observation takes 10–15 m per patient. As such, the regular schedule takes 30–40 m. Moreover, the iodine staining and biopsy procedures, if required, take an additional 10–20 m, which means that each endoscopic surgery requires an average of 30–60 m (Fig. 1). According to these numbers, an endoscope can only inspect 8–16 symptom-free residents in a working day on average, which limits the efficiency of this approach, far from the requirements of a large-scale population screening but necessitating a high cost of application and further complicated by a lack of experienced endoscopists.[14] It is very difficult to simply expand the number of endoscopes to improve the examination efficiency. Therefore, improving and shortening the disinfection time without affecting the disinfection quality, in order to improve the efficiency of endoscopy, has become an important issue. At the same time, improving the endoscopic instruments (reducing cost, reducing the diameter of the endoscope fiber, etc.) without affecting the targeted biopsy of mucosa has become another urgent problem to be solved.[15–18]

Combination of serum tumor-associated molecular and endoscopic approaches for symptom-free HRS screening of ESCC

In order to overcome the endoscopic limitations, detecting high-risk groups in SFS firstly and only carrying out endoscopic examination in high-risk groups can greatly improve the detection rate and save medical costs. Recent studies suggest that the carcinogenesis of esophageal epithelial tissue is a multi-stage evolutionary process involving multiple gene and protein changes (accumulation or superposition).[19–23] At the early stage of this carcinogenesis evolution, esophageal epithelial cells have already undergone molecular and morphological changes.[24] Moreover, most of the molecular changes occurred before the morphological changes. The tumor-related molecules with abnormal expression can be reflected in the blood in different ways, such as changes in protein levels and autoantibody responses.[25] Therefore, the changes of tumor-related molecular markers in blood can indirectly reflect the proliferative state of esophageal epithelium.[26]

Compared to the endoscopy technique alone, detection of serum tumor-related markers has greater advantages in screening symptom-free HRS of ESCC in HIA. Namely, it is non-invasive, easy to repeat, high-throughput, economic and sensitive, etc., making it especially suitable for large-scale symptom-free high-risk population screening. By detecting molecular markers related to the progression of ESCC in peripheral blood and combining with data profiles of family history and living habits, the risk of the subjects can be classified into mild, moderate and severe. Only the severe risk patients are subjected to endoscopic examination. Obviously, serological screening could be applied as the first step of screening, and endoscopic biopsy and histopathological examination as the second step. This combination approach could significantly reduce the extent of endoscopic examinations and screening costs, and improve the detection rate of HRS. We found the examined population decreased by about 80%, with only about 20% requiring the second-step endoscopic examination. The new combination method can still catch 2–3 patients with early ESCC and 6–10 patients with precancerous lesions. Liquid biopsy combined with pigment endoscopic biopsy are the best for early screening. Subsequent formulation of a reexamination plan can be made according to the first screening results; this will help, for example, patients with basal cell hyperplasia who should be examined every

Table 1. Pros and cons of serum cancer-markers in ESCC screening

Serum markers	Application in population	Pros	Cons
Autoantibodies	Yes	Stable	Low sensitivity
Circulating microRNA	No	Stable and consistently expressed	Limited studies
ctDNA	No	Stable and consistently expressed	Low sensitivity, limited studies

ESCC, esophageal squamous cell carcinoma; miRNA, microRNAs; ctDNA, circulating tumor DNA.

2 years, patients with mild and moderate dysplasia who should be examined every 1 year, and patients with severe dysplasia who should be examined every 6 months. In this way, the progression of precancerous lesions could be prevented in a timely and effective manners.[6,27–29] It is a very important research direction to focus efforts on strengthening the screening and identification of serum-related proteins in patients with early ESCC and precancerous lesions, to establish accurate screening indicators and methods for HRS.

Liquid biopsy (serum cancer-markers) application to HRS screening in high-incidence areas for esophageal cancer

With the advancement of molecular biology research and the in-depth study of esophageal carcinogenesis, more and more serum markers related to esophageal malignant progression have been discovered. These serum markers are candidate targets for liquid biopsy. Autoantibodies, circulating microRNAs, and circulating tumor DNAs (ctDNAs) have been found and are promising for application in the screening for ESCC. The advantages and disadvantages of some common serum cancer markers are shown in Table 1.

Tumor-associated autoantibodies are produced in response to mutations, overexpression, or abnormal processing by humoral immune response throughout tumorigenesis. Because of their stability in blood, autoantibodies have already shown success as biomarkers for malignancy.[30] The majority of autoantibody biomarkers show relatively low sensitivity but high specificity for ESCC, with a range from 3.9% to 93.7% and from 78.7% to 100%, respectively.[31] Due to sensitivity being limited for a single autoantibody marker, increasing research investigations have aimed to identify a suitable panel of autoantibodies. The optimized combination of various autoantibodies improves the sensitivity of diagnosis. Zhou *et al*[25] reported the sensitivity and specificity for six autoantibodies in diagnosing ESCC, reaching up to 64% and 94%, respectively. Zhang *et al*[32] assessed a combination of four autoantibodies in ESCC samples and normal controls with independent validation and found the sensitivity and specificity to be 67.9% and 86.7%, respectively. Wang *et al*[33–36] in a series of studies found the application of four groups of autoantibodies in the screening of ESCC in HIA to have a sensitivity range from 78.0% to 89.5% and a specificity range from 70.0% to 89.5%. The collective results suggested that the combination of autoantibodies is promising for liquid biopsy.

Circulating microRNAs are regulatory small non-coding RNAs of approximately 20–25 nucleotides in length. Many studies have focused on extracellular microRNAs as potential markers, since they are stable, sensitive and relatively cheap to assay.[37] The levels of circulating microRNAs have been studied for their correlation with esophageal cancer development.[38,39] The combined application of multiple microRNAs is beneficial for distinguishing early-stage ESCC patients from healthy controls.[40] Jinsei *et al*[41] identified eight microRNAs (miR-103, miR-106b, miR-151, miR-17, miR-181a, miR-21, miR-25, and miR-93) in serum specimens using three ESCC tissue miRNA datasets. The eight-miRNA signature in the other independent validation cohort distinguished ESCC patients from healthy controls (sensitivity: 93%, specificity: 89%).

ctDNA is tumor-derived fragmented DNA in the bloodstream. High level methylated ctDNA is associated with poor prognosis in ESCC.[42] Qiao *et al*[43] identified 921 differentially methylated ctDNAs between tumor and adjacent tissues in esophageal cancer, capable of discriminating esophageal cancer patients from benign and healthy controls with a sensitivity of 74.7% and a specificity of 95.9% in the independent validation cohort. Chen *et al*[44] retrospectively analyzed the pre-disease plasma samples from cancer patients and demonstrated that five types of cancer can be detected through a ctDNA methylation-based blood test up to 4 years before conventional diagnosis, including gastric cancer, esophageal cancer, etc.

In brief, noninvasive serum cancer-markers are urgently needed for the screening of ESCC, as they will play an important role in the management of patients. However, most of the serum-cancer markers defined to date are not sufficiently sensitive nor specific for liquid biopsy. The combination of multiple serum markers may be an effective method. What's more, the markers need to be evaluated as a screening test in high-risk populations in practice, to evaluate the true application value for liquid biopsy. So far, however, most studies still lack validation in the population. More population studies are needed for the ultimate clinical application of serum markers for the ESCC screening of HRS.

Conclusions

In summary, the development of liquid biopsy is the key basis of the two-step method and its successful application. Meanwhile, development of the two-step screening method is difficult to accomplish by medical scientists alone because government leadership plays a decisive role. The reason underlying this is that these populations are symptom-free and the cost of testing and population compliance must be guaranteed for this screening method to be implemented. The authors believe that an important guarantee that must be made for this type of screening implementation to be successful will involve exploring a suitable examination cost and logistical system; only in this way will the two-step screening become available within the current rural (community) new cooperative medical system.

Acknowledgments

None.

Funding

This work was supported by grants from the Key Project of National Natural Science Foundation of China (U1804262) and the Key Science and Technology Project of Henan Province (161100311300).

Conflict of interest

One of the authors, Prof. Li-Dong Wang has served as an associate editor of *Cancer Screening and Prevention* since February 2022. All other authors have no conflict of interest related to this manuscript.

Author contributions

Writing of the main body of the paper (DY), preparation of the information for writing of the paper (XKZ, RHX, LLL, XSL), and designing the paper's structure and providing revisions for important intellectual content (LDW).

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