Non-Endoscopic Screening for Esophageal Squamous Cell Carcinoma- A Review

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ABSTRACT

Esophageal cancer (EC) is the eighth most common cancer and sixth most frequent cause of cancer mortality worldwide. Esophageal squamous cell carcinoma (ESCC) is the most common type of EC. ESCC develops by progression from premalignant lesions, which are called esophageal squamous dysplasia (ESD). Prevention is the most effective strategy for controlling this disease. Generally, two methods may be defined for ESCC prevention. The aim of the first preventive method is to prevent the initiation of ESD by avoiding the known risk factors, or primary prevention. Secondary prevention focuses on detection of the disease in its early curable stage, thus preventing its progression into advanced stages. Endoscopy with iodine staining and biopsy is the diagnostic choice for ESD. However it is invasive and expensive, and not accepted by asymptomatic ESD cases. Therefore, it is necessary to find a non-endoscopic screening method. Despite the large number of studies conducted worldwide, no approved method has been developed for ESCC screening. Regarding the multi-factorial nature of ESCC, it is proposed that the use of a combination of various criteria, such as cytological examination, risk factors, genetic alteration, and molecular markers may result in the development of a comprehensive and effective ESCC screening program.

KEYWORDS
Esophageal squamous cell carcinoma; Screening; Non-endoscopic; Review.

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Epidemiology of ESCC

Esophageal cancer (EC) is the eighth most common cancer and the sixth most frequent cause of cancer mortality worldwide.[1] The highest rates for EC are reported from a geographical area that extends from northern Iran to north-central China, so-called the "Asian belt of EC".[2-4] Despite its declining trend, EC remains the most frequent malignancy with a high incidence rate in northern Iran.[5,6] Southeast Africa (Kenya, Zimbabwe), parts of South America (Brazil, Uruguay) and western Europe (France) are known as intermediate risk areas for EC.[3] Other parts of the world, including the US, are considered to have low incidence rates of EC.[1] Adenocarcinoma and squamous cell carcinoma (SCC) are the main morphologies of EC.[2,7] In the 1960s, esophageal squamous cell carcinoma (ESCC) comprised about 90% of EC cases.[3] Recent studies have suggested a declining trend in the rate of ESCC as well

as an increase in the rate of esophageal adenocarcinoma in the US and some European countries.[8-10] Currently, it is estimated that the morphology in about 70% of worldwide EC cases are ESCC.[11] But, the situation seems to be different in the developing world. Reports from developing countries have shown that the shifting pattern is not the same as western countries. For example, ESCC comprises more than 90% of EC cases in northeastern Iran.[12,13] Thus, ESCC is the most important type of EC in the developing world and a major health problem in high risk areas.

Pathogenesis of ESCC

ESCC develops by progression from dysplastic lesions within the squamous epithelium of the esophagus.[3] In other words, esophageal squamous dysplasia (ESD) is the premalignant precursor lesion for ESCC.[14,15] Various factors may cause dysplastic changes in normal epithelial cells of the esophagus, including Tobacco smoking, opium consumption, nass chewing, hot tea consumption, an excessive alcohol consumption, drinking mate, low intake of fresh fruits and vegetables, environmental factors, low socioeconomic status and viral infections.[16-21] So, ESCC is a complex and multi-factorial disease. Over months to years ESDs grow into tumor mass (ESCC).[22] The absence of true serosal layer in the esophageal wall makes ESCC a progressive cancer with relatively rapid invasion into neighboring structures.[23] The clinical manifestations in most of ESCC patients do not present in early stages and this results in delayed diagnosis of the disease. Distant metastasis and bone marrow invasion may be detected in 30 and 40 percent of ESCC cases, respectively.[24] So, ESCC cases usually present in late stages and the prognosis in most of them is poor. The overall five-year survival of ESCC patients is as low as 9%.[22]

Controlling ESCC

When a patient is diagnosed with ESCC, the appropriate treatment modality is selected according to the tumor stage.[3] In the early stages, curative therapeutic methods are considered. The therapeutic option in patients with submucosal ESCC or carcinoma in situ is endoscopic mucosal resection (EMR). Surgical resection, radiotherapy, and chemotherapy are selected for localized ESCC,[3] whereas endoscopic palliative therapies are recommended for patients with advanced stage ESCC. These include laser therapy, argon plasma coagulation, esophageal dilation, and esophageal stent replacement.[3] Although the above mentioned therapeutic modalities have been known to be useful in ESCC patients, prevention, however, is the most effective strategy for disease control. Generally, two methods may be defined for ESCC prevention. The aim of the first preventive method is to prevent the initiation of the ESD, or primary prevention. However, ESD is a multi-factorial condition whose risk factors have not been completely identified. Therefore primary prevention may not warrant complete elimination of ESD and consequently ESCC.

The second method focuses on detection of the disease in an early curable stage, preventing its progression into advanced stages (secondary prevention). Although endoscopy with iodine staining and biopsy is the diagnostic choice for ESD,[3,25] it is an invasive and expensive method not particularly accepted by asymptomatic ESD cases.[26] Therefore, it is necessary to find a non-endoscopic screening method which takes into consideration a combination of various criteria such as cytological examination, risk factors, and molecular markers.[27]

Screening Methods for ESCC

By definition, a screening program might have potential benefit for a condition if the following assumptions are true. At first, the disease in all or most cases should start from a detectable preclinical phase. Secondly, in the absence of intervention, most or all cases in the preclinical phase progress into the clinical phase.[28] Both assumptions are true for ESCC.[22] Thus, a simple, minimally invasive, accurate, and cost effective screening program will be helpful for controlling ESCC. Recently, some studies have been conducted to find a screening method for ESCC. Various criteria were used in these studies, including patients' risk factors, cytopathological

examination of esophageal secretions, serum markers, and genetic profiling. A detailed discussion about these criteria is given in the following sections.

1- Risk Factors

The risk factors for ESD are reported to be similar to those of ESCC,[29] and both may be used as possible criteria for ESCC screening. Xibin et al. have reported a negative relationship between household income, residential space, education, and EC. They also noted that consumption of beans, vegetables, and vinegar were protective against EC.[30] By measuring urine 1-hydroxypyrene glucuronide (1-OHPG), a stable polycyclic aromatic hydrocarbon (PAHs) metabolite, Kamangar et al. have suggested that high exposure to PAH may be a possible risk factor for ESCC in northeastern Iran.[31] Islami et al. have reported that dimensions of socio-economic status (SES), which included a higher level of education, wealth, and being married were inversely related to ESCC.[32] Drinking high-temperature beverages was shown to be an important risk factor for ESCC.[33] The results of a study from Iran suggested human papillomavirus (HPV) infection as a potential risk factor for ESCC in high risk areas.[34]

In addition, Nasrollahzadeh et al. showed that the risk of ESCC in individuals who smoked both cigarettes and opium substances (OR=2.35) was higher than those who smoked only cigarettes (OR=1.98) or opium (OR=2.12).[35] Wu et al. reported that the combined usage of three substances (cigarette smoking, betel chewing, and alcohol consumption) increased the risk of ESCC (OR=39.2, 95%CI: 13.2-116.1). They found that patients who used only one of these substances had a 1.5-fold (95%CI: 0.6-3.8) risk for ESCC.[36]

Wei et al. attempted to use a questionnaire and physical examination data to develop a risk model for triaging subjects for endoscopies.[29] The aim of their study was to define a screening program using previously known risk factors for ESCC. The researchers considered ten risk factors of age, sex, smoking status, ethanol patch test flushing response, number of persons in the subject's household, household income, family history of cancer, systolic blood pressure, heating stove type, and quintile of tooth loss. The sensitivity and specificity of their final model for predicting dysplasia was 57% and 54%, respectively. As such, they concluded that risk factors, alone, were not appropriate and successful measures with which to develop a screening program for ESCC. They have proposed that the addition of cytological or molecular data from subjects would result in the development of an efficient screening program.

Generally, researchers need to consider some important criteria for using a risk factor as a screening test for ESCC. The risk factor should have a strong association with ESCC. Variations in exposure to the risk factor within the population should be mentioned. The risk of disease should be considered in all quintiles of the distribution of the risk factor within the population. In other words, not only should those at both ends of the distribution of exposure (high and low) be included, but also those in the middle of the distribution.[37] If appropriately used, risk factors could play an important role in ESCC screening. Table 1 shows the characteristics of major risk factors for ESCC and ESD.

2- Cyto-Pathological Examination of Esophageal Secretion

Non-endoscopic cytological methods have been used for early diagnosis of ESCC since the 1970s.[56] Lazarus et al. have used abrasive brush cytology for early detection of EC, with a high sensitivity (90%) and specificity (99.9%) for their method.[57] Roth et al., in 1997, assessed the validity of balloon and sponge samples for detecting ESD and ESCC.[26] The sensitivity and specificity of balloon sampling for the detection of ESD was 47%, whereas it was 88% for ESCC. The sensitivity and specificity of the sponge sampler for identifying ESD or ESCC was 24% and 92%, respectively. The methods were not adequately sensitive to be used as screening programs within the community. The researchers suggested that improving samplers would increase the sensitivity of the test. In a complementary study to improve the validity of their screening test, Pan et al. have used new mechanical and inflatable balloons for identifying ESD or ESCC.[58] The mechanical balloons had a sensitivity of 39% whereas,

Table 1: **Risk factors for esophageal squamous carcinoma or esophageal dysplasia**

Risk factors		Author	Location	Statistic	
				Type	Values
Positive family history of cancer	Yes vs. no	Wei et al.[29]	China	OR (CI 95%)	1.57 (1.13-2.18)
	Yes vs. no	Wang et al.[38]	China	OR (CI 95%)	3.83 (1.13-12.97)
	Yes vs. no	Akbari et al.[39]	Iran	OR (CI 95%)	3.6 (2.3-5.7)
Relationship between parents	Related vs. no relationship	Akbari et al.[39]	Iran	OR (*p*-value)	4.1 (0.006)
Systolic blood pressure	per 10 mm Hg increase	Wei et al.[29]	China	OR (CI 95%)	1.11(1.03-1.19)
Heating stove without chimney	Yes vs. no	Wei et al.[29]	China	OR (CI 95%)	2.22 (1.27-3.86)
Oral health	12–31 vs. 0-4 teeth lost	Wei et al [29]	China	OR (CI 95%)	1.91 (1.17-3.15)
	6-15 vs. 0-5 teeth lost	Guha et al.[40]	Central Europe	OR (CI 95%)	2.84 (1.26-6.41)
	6-15 vs. 0-5 teeth lost	Guha et al.[40]	Latin America	OR (CI 95%)	2.18 (1.04-4.59)
	Extremely poor vs. good	Sepehr et al.[41]	Iran	OR (CI 95%)	4.76 (1.48-15.31)
	Decayed, missing, or filled teeth =32 vs. ≤15	Abnet et al.[42]	Iran	OR (CI 95%)	2.1 (1.19-3.7)
	No regular oral hygiene vs. daily tooth brushing	Abnet et al.[42]	Iran	OR (CI 95%)	2.37 (1.42-3.97)
Source of drinking water	Other than tap water	Xibin et al.[30]	China	OR (CI 95%)	5.49 (1.43-21.1)
Cigarette smoking	>30 pack-years vs. none	Wu et al.[36]	Taiwan	OR (CI 95%)	3.7 (1.6-8.7)
	≤ 3.5 packs/wk vs. none	Tai et al.[43]	Taiwan	OR (CI 95%)	6.08 (1.43-25.94)
	≥ 15 cigarettes/day vs. none	Vizcaino et al.[44]	Zimbabwe	OR (CI 95%)	4.3 (2.8-6.7)
	≥ 80 packs-years vs. none	Vaughan et al.[45]	USA	OR (CI 95%)	16.9 (4.1-69.1)
	> 11 cigarettes/day vs. none	Nasrollahzadeh et al.[35]	Iran	OR (CI 95%)	1.98 (1.2-3.25)
	≥15 cigarettes/day vs. none	Castelletto et al.[46]	Argentina	OR (CI 95%)	3 (1.5-5.7)
Areca (betel nut) chewing	> 495 betel/year vs. none	Wu et al. [36]	Taiwan	OR (CI 95%)	9.4 (1.8-48.3)
Alcohol consumption	> 1220 g-year vs. none	Wu et al.[36]	Taiwan	OR (CI 95%)	9.8 (4.2-22.6)
	> 158 g/wk vs. none	Tai et al.[43]	Taiwan	OR (CI 95%)	20.58 (1.72-245.62)
	≥20 vs. 0-6 drinks/week	Vaughan et al.[45]	USA	OR (CI 95%)	9.5 (4-22.3)
	≥200ml/day vs. none	Castelletto et al.[46]	Argentina	OR (CI 95%)	5.7 (2.2-15.2)
Esophageal lesions (esophagitis,…)	Yes vs. no	Wang et al.[38]	China	OR (CI 95%)	11.63 (1.13-119.33)
Helicobacter pylori **infection**	Yes vs. no	Wang et al.[38]	China	OR (CI 95%)	3.19 (1.11-9.15)
Eating breakfast	Yea vs. no	Sharp et al.[47]	UK	OR (CI 95%)	0.18 (0.07-0.48)
Aspirin consumption	Daily use vs. none	Sharp et al.[47]	UK	OR (CI 95%)	0.08 (0.01-0.56)
Fresh fruit consumption	Weekly vs. less often	Sepehr et al.[41]	Iran	OR (CI 95%)	3.18 (1.14-8.9)
Opium consumption	Yes vs. no	Nasrollahzadeh et al.[35]	Iran	OR (CI 95%)	2.12 (1.21–3.74)

Risk factors		Author	Location	Statistic	
				Type	Values
Drinking mate	Heavy drinkers of very hot vs. light drinkers of cold/warm/hot	Castellsague et al.[48]	South America	OR (CI 95%)	4.14 (2.24–7.67)
	Very hot vs. warm	De Stefani et al. [49]	Uruguay	OR (CI 95%)	5.76 (2.92-11.35)
Tea temperature	Very hot vs. warm	Islami et al.[50]	Iran	OR (CI 95%)	8.16 (3.93-16.91)
	Hot vs. others	Cook-Mozaffari et al.[51]	Iran	OR (*p*-value)	Men=1.72; Women=2.17 (<0.01)
	Hot vs. not hot	Onuk et al.[52]	Turkey	OR (CI 95%)	8.7 (2.5-30.2)
	Very hot vs. cold/warm	Castellsague et al.[48]	South America	OR (CI 95%)	3.73(1.41-9.89)
	High temperature vs. never drinking	Wu et al.[53]	China	OR (CI 95%)	4.2 (2.3-7.6)
Drinking coffee	very hot vs. cold/warm	Castellsague et al.[48]	South America	OR (CI 95%)	2.29 (1.37-3.81)
	Burning hot vs. others	De Jong et al.[54]	Singapore	OR (*p*-value)	Men=4.22; Women=4.09 (<0.01)
Interval between tea being poured nd drunk (minutes)	<2 vs. ≥4	Islami et al.[50]	Iran	OR (CI 95%)	5.41 (2.63-11.14)
Formal education	Middle school or higher vs. no school	Islami et al.[32]	Iran	OR (CI 95%)	0.2 (0.06-0.65)
Eating barbecued meat	≥ 1 vs. <1 per week	Castelletto et al.[46]	Argentina	OR (CI 95%)	2.4 (1.2-4.8)
PAH content (8E11 antibody) of the oesophageal epithelium	Fifth quintile vs. first quintile	Abedi-Ardekani et al.[55]	Iran	OR (CI 95%)	26.6 (5.21-135)

the inflatable balloons was 46%. Unfortunately, the sensitivity of the new balloons was still inadequate to be used as a population-based screening program. The result of this study emphasized the need to consider molecular markers to increase the validity of a program for detecting ESD.

Lao-Sirieix et al. used a capsule sponge (cytosponge) for cytological examination of the esophagus. They suggested that this device had good validity for early detection of Barrett's esophagus.[59] Recently, Kadri et al. have shown that coupling a Barrett's specific immunomarker with a cytosponge would improve its sensitivity and specificity for detecting Barrett's lesions; the combination would be a suitable screening program to be used in the primary care setting.[60] Therefore, coupling a cytosponge with an ESD specific marker may be considered as a promising method for developing an applicable ESCC screening program.

3- Serum Markers

In a study from Japan, the peripheral blood samples of EC patients were assessed for ΔNp63 gene expression by ΔNp63-specific RT-PCR.61 ΔNp63 mRNA was detected in blood samples of 52% of primary ESCC and in 60% of recurrent ESCC cases. No ΔNp63 expression was observed in controls. Therefore, the researchers have concluded that ΔNp63 is a good and highly specific blood marker for early detection of ESCC.[61] Hibi et al. found a high rate of promoter methylation of the p16 gene in the serum of ESCC patients, which suggested that p16 gene promoter methylation may be used as a serum marker for identifying precursor lesions of ESCC.[62]

In a study from India, Kannan et al. have con-

cluded that the Thomson–Friedenreich (TF) antigen was an appropriate marker for the early diagnosis of ESCC.[63] Yang et al. studied the expression of squamous cell carcinoma antigen 2 (SCCA2) in peripheral blood of patients with ESCC and ESD, as well as normal individuals.[64] Their results showed significantly higher SCCA2 mRNA expression in ESCC and ESD cases than normal subjects. They also compared the SCCA2 levels measured by two methods, including enzyme-linked immunosorbent assay (ELISA) and SCCA2 mRNA expression. The results showed a significant correlation between ELISA SCCA2 levels in the serum and SCCA2 mRNA expression levels in the peripheral blood. They have suggested that SCCA2 is a good biomarker for the detection of premalignant esophageal lesions.[64]

Chung et al. used multiplex tissue immunoblotting to quantify the expression of some proteins in esophageal carcinogenesis.[65] Their results have shown overexpression of secreted protein, acidic and rich in cysteine (SPARC). The possibility of measuring SPARC in serum makes it a potential biomarker for early detection of ESCC.[65] Munck-Wikland reported elevated serum levels of tumor markers carcinoembryonic antigen (CEA; 39%), CA 50 (41%), and CA 19-9 (13%) in ESCC patients. The sensitivity of considering these markers together for detecting ESCC was 59%.[66]

In a study from Japan, a proteomics-based approach was used to identify tumor antigen in an ESCC cell line (TE2) and related autoantibodies in serum of ESCC patients.[67] They found an autoantibody against peroxiredoxin VI, and suggested that it was a potential biomarker for early detection of ESCC.[67]

4- Genetic Alterations

Alterations in oncogenes, tumor suppressor genes as well as alterations in microRNA expression have been known to involve in the pathogenesis of ESCC.[68]

4-1- Oncogenes

4-1-1- Cyclin D1: Jiang et al. suggested changes in the expression of cyclin D1 gene in ESCC patients.[69]

4-1-2- Fart1: Saitoh et al. reported the overexpression of Frat1 mRNA in ESCC patients.[70]

4-1-3- HoxD9 and Pbx1: The results of a study from China showed over-expression of HoxD9 and Pbx1 genes in ESCC tissues.[71]

4-1-4- Vascular endothelial growth factor (VEGF) gene: A significant over-expression of VEGF gene was found in ESCC tissues.[72]

4-1-5- Akr1c2 gene: The up-regulation of Akr1c2 gene suggested it to play a possible role in the development of ESCC.[73]

4-2- Tumor suppressor genes

Mutations in p53[74,75] and MTS1[76] genes have been reported in ESCC cases. The p53 gene mutation was found in the esophagitis area as well as in dysplastic areas of the esophagus.[77] Gao et al.[78] and Wang et al.[79] have reported that p53 protein accumulation occurs quite frequently in ESD cases, therefore concluding that the p53 mutation is an early event in the pathogenesis of ESCC.

Xing et al. noted a loss of heterozygosity (LOH) of the retinoblastoma (Rb) gene in 55% of ESCC cases.[80] According to these researchers, Rb LOH was significantly more frequent in tumors with p53 mutations. They have concluded that concurrent alteration of Rb and p53 genes is an important mechanism for the development of ESCC.[80]

The results of a study from Japan have shown that alteration of the deleted in lung cancer 1 (DLC1) gene may be involved in development of ESCC.[81] According to other studies, changes in the p16INK4a and p15INK4b genes have been found in 68% and 50% of ESCC patients, which suggests these changes are important in the pathogenesis of ESCC.[82] Guo et al. have also found frequent methylation of the p16INK4a gene in ESCC patients.[83]

In a study from the US, loss of heterozygosity in adenomatous polyposis coli (APC) or MCC genes was found in 80% of ESCC patients. Therefore, it was proposed that alterations in the APC and MCC genes have important roles in the pathogenesis and progression of ESCC.[84] Zare et al. found APC pro-

moter hypermethylation in ESCC patients, thus indicating that it could be an appropriate candidate molecular marker in ESCC cases.[85]

Inactivation of the WW domain containing oxireductase (WWOX) gene, as reported by Kuroki et al. may be a possible mechanism in the pathogenesis of ESCC.[86]

Other genes, such as the cysteine-rich protein with Kazal motifs (RECK) gene showed significant reduction in ESCC tissues when compared with normal tissues.[72]

Mal gene: Kazemi-Noureini et al. reported down-regulation of mal gene in ESCC patients.[73] CDKN2A gene: Frequent mathylation of CDKN2A was reported in ESCC patients.[83]

S100A2 gene: Cao et al. found a significant down-regulation of S100A2 gene in ESCC tissues.[87]

P63 gene: The expression of P63 gene was significantly decreased in ESCC tissues when compared with normal esophageal epithelium.[87]

FHIT gene: Loss of FHIT tumor suppressor gene was reported in precursor lesions of ESCC, indicating its possible role in developing ESCC.[88]

4-3- Other Genetic Alterations

According to Ishii et al., DNA methylation may play an important role in the progression of ESD into ESCC.[89] Guo et al. have noted an increasing trend in the number of methylated genes along with increasing cellular atypia in EC cases.[83] In a research by Li et al, an association between increasing telomerase activity and ESCC precursor lesions was noted. Their results showed telomerase activity in 60% of esophageal metaplasia and in about 90% of esophageal dysplasia.[90]

Expression of matrix metalloproteinase-7 (MMP-7) increases in some cancers, including EC.68 In addition, MMP-26 could be a potential biomarker for the diagnosis of EC.[91] Increasing matrix MMP-3 and MMP-10 occurs due to upregulation of their genes in ESCC patients, which suggests they may also be potential diagnostic markers.[92]

In a study from China, proliferating cell nuclear antigen (PCNA) was expressed in 55% of normal esophageal tissue, in 75% of ESD and 93% of ESCC tissues. This study also indicated a positive correlation between overexpression of p53 and PCNA during different stages in the development of ESCC from normal esophageal epithelium.[93]

Ishii et al. have proposed that inactivation of the FEZ1 gene may play a role in the development of ESCC.[94] A member of the human frizzled gene (FzE3) was expressed in 86% of poorly differentiated ESCCs.[95] By decreasing expression of the APC gene and increasing the B-catenin mediated signals, FzE3 may play a possible role in the pathogenesis of ESCC.[95] Overexpression of ornithine decarboxylase (ODC) mRNA was seen in about 91% of ESCC cases, therefore it may be important in developing ESCC.[96] Liu et al has suggested a role for translocation of annexin I protein in the pathogenesis of ESCC.[97] Qi et al. suggested that annexin II protein was a suitable biomarker for screening and detecting precursor lesions in ESCC patients.[98] Overexpression of the gene amplified in squamous cell carcinoma 1 (GASC1) was also shown to be related to the development of ESCC.[99]

Yue et al. suggested the inactivation of esophageal cancer related gene 4 (ECRG4) due to hypermethylation as an important mechanism in the development of ESCC.[100] Gholamin et al. found a frequent over-expression of Interleukin-10 (IL-10) gene in ESCC patients.[101] Over-expression of transforming growth factor β (TGF-β) gene was frequent in ESCC cases.[101] Akbari et al. reported a significant association between ADH1B gene and the risk of ESCC in northeast of Iran.[102] Tsuda et al. reported high rates of amplification of hst1 and int2 genes in ESCC patients.[103] Allelic losses on chromosomes 5q [104] and 17q [105] were found in ESCC patients. Kashyap et al. analyzed the mRNA expression profiles of 20 ESCC patients by whole genome DNA microarrays and Significant downregulations were identified in the pathway of several genes in arachidonic acid metabolic including ALOX15B, GPX3 and PTGDS in ESCC.[106] Adams et al. used quantitative methylation-specific PCR techniques to evaluate the accuracy of methylation in selected genes in esophageal balloon cytology specimens for identifying ESD.[107] The results showed that the

maximum sensitivity and specificity for individual genes were 34% and 99%. To increase the validity, they used panels of multiple genes and found a sensitivity of 50% and a specificity of 65% for a panel of four genes (AHRR, p16INK4a, CLDN3, and MTIG). They concluded that further studies on gene methylation in balloon cytology specimens will result in designing more accurate and suitable screening methods for ESCC.[107] Akbari et al. found mutations in Fanconi anemia genes (including FANCD2, FANCE, FANCL and FANCA) [108] as well as in BRCA2 (FANCD1) gene [109] in ESCC cases from northeast of Iran. Eisenberger et al. used a Microsatellite DNA analysis using a panel of 12 microsatellite markers on chromosome 9p (p16), chromosome 17p (p53), chromosome 18q (DPC4-gene), chromosome 18p and chromosomes 5 p and q (APC gene) in ESCC patients.[110] Their results showed that 93% and 96% of cases had at least one microsatellite DNA alterations in their tumor and serum, respectively.[110] Sepehr et al. reported a higher frequency of polymorphisms of CYP1A1 m1, CYP1A1 m2, CYP2A6*9, and ADH2*1 genes in population with high ESCC rates in northeast of Iran.[111] The risk of ESCC may increase in rare combination of GSTT1 null genotype and GSTP1 Val/Val variant.[112] Zhang et al. used whole-genome microarray method to analyze genome-wide mRNA expression profiles in ESCC patients.[113] They found that 263 genes were significantly downregulated, including MFAP4, LYVE1, ANGPTL1, DPT, PPP1R1A, RERGL, NKX31 and SCARA5.[113] They also reported significant upregulation in 104 genes, including AURKA, CDC20, CDCA3, TTK, RHPN2, DEPDC1, RASEF, IGF2BP3, TRIP13, CTHRC1 and COL5A2.[113] In a study from India, genomewide mRNA profiling showed that fibroblast activation protein (FAP) as well as oral cancer overexpressed 2 (ORAOV2) were overexpressed in 98% and 68% of cases, respectively. In addition, Overexpression of Osteopontin (OPN) was found in 97% of the ESCC cases. So, genomewide mRNA profiling was a good approach to identify new biomarkers for ESCC.[106] Fu et al. used proteomics approache to assess overexpression of alpha-actinin 4 (ACTN4) and 67 k D a laminin receptor (67LR) in ESCC cases. ACTN4 and 67LR were overexpressed in 58.9% and 48.8% of samples suggesting them as suitable biomarkers for ESCC.[114] Galectin-7 was also showed a significant upregulation in ESCC cases using a proteomic analysis, indicating its potential role in development of ESCC. So, galectin-7 may potentially be served as a marker for ESCC.[115] In a study from China, CDC25B mRNA expression showed an increase in ESD and ESCC cases comparing to normal individuals, suggesting CDC25B protein as a potential biomarker for ESCC screening.[116] The expression of plasminogen activator inhibitor-1 (PAI-1) was reported to be higher in advanced than early stages of ESCC.[117] Abnet et al. reported high prevalence of mitochondrial DNA (mtDNA) mutation (the common deletion) in ESCC tissues, concluding that common deletion in mtDNA may be useful in developing ESCC screening programs.[118] Marjani et al. found a significant higher levels of polycyclic aromatic hydrocarbons (PAHs)-DNA adducts in ESCC tissues than normal ones, suggesting PAH-DNA adducts as possible marker for ESCC.[119]

4-4- Alterations in the Expression of micro-RNA (miRNA)

Feber et al. reported decreased expression of miRNA-203 and miRNA-205 and increased expression of miRNA-21 in ESCC patients.[120] miRNA-21 expression was elevated, whereas expression of miRNA-375 was lower in ESCC cases than noncancerous ones.[121] Ogawa et al. have suggested that miRNA-129 may play an important role in the pathogenesis of ESCC.[122] Lee et al. have shown that miRNA-373 may suppress an oncogene and play a role in developing ESCC.[123] Significant elevation of miRNA-21 expression was found in ESCC tissues.[124] Guo et al. used advanced microRNA microarray techniques to assess the expression of miRNAs in ESCC patients. Their results showed over-expression of three miRNAs (hsa-miR-25, hsa-miR-424, and hsamiR-151) as well as down-regulation of four miRNAs (hsa-miR-100, hsa-miR-99a, hsa-miR-29c, and mmu-miR-140).[125] In a study from Japan, the expression of miR-205 and

miR-10a was significantly altered, which suggested their possible role in pathogenesis of ESCC.[126]

Designing a Comprehensive Screening Program for ESCC

Regarding the multi-factorial nature of ESCC, some researchers propose that the use of a combination of the above mentioned criteria may result in the development of an effective screening program for ESCC. Nicolas Perez et al. have reported that the use of cytology or Lugol chromo-endoscopy in high ESCC risk populations or individuals will result in early detection of ESCC cases, suggesting this combination strategy to be a good screening program for ESCC.[127] In a study from Iran, individuals with GSTP1 Ile/Ile variants have more susceptibility to ESCC in smokers, while non-smokers with this genotype seem to be protected. Therefore, assessment of GSTP1 genotype together with smoking habits may be important in determining the risk of ESCC.[112] According to Montesano et al., a strong relationship between p53 mutation and tobacco smoking in ESCC patients exists.[128] In a study from Japan, Yokoyama et al. have developed a health risk appraisal (HRA) model to determine high risk individuals for ESCC. They used a genetic alteration (inactivation of aldehyde dehydrogenase-2; ALDH2) and some risk factors (alcohol drinking, tobacco smoking, vegetable and fruit consumption). The results showed that the higher sensitivity and specificity when both genetic changes and risk factors were considered in the model.[129]

Despite the large number of studies conducted worldwide, to date, no approved method has been developed for ESCC screening.[3] A possible explanation for this situation is that ESCC is a multi-factorial disease, where most research has focused on one or some limited aspects of the disease. Therefore, we need to consider more complex and multi-aspect designs for developing a comprehensive and effective ESCC screening program.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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