Esophageal Cancer in Golestan Province, Iran: A Review of Genetic Susceptibility and Environmental Risk Factors

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ABSTRACT

Esophageal squamous cell carcinoma (ESCC) is an aggressive tumor that is typically diagnosed only when the tumor has gained remarkable size, extended to peripheral tissues, and led to dysphagia. Five-year survival of advanced cancer is still very poor (19%), even with improved surgical techniques and adjuvant chemoradiation therapy. Therefore, early detection and prevention are the most important strategies to reduce the burden of ESCC. Our review will focus on the studies conducted in Golestan province, an area with a high prevalence of ESCC in northern Iran. We review three aspects of the research literature on ESCC: epidemiological features, environmental factors (including substance abuse, environmental contaminants, dietary factors, and human papilloma virus [HPV]), and molecular factors (including oncogenes, tumor suppressor genes, cell cycle regulatory proteins, and other relevant biomarkers). Epidemiological and experimental data suggest that some chemicals and lifestyle factors, including polycyclic aromatic hydrocarbons (PAHs), cigarette smoking, opium use, and hot tea drinking are associated with the development of ESCC in Golestan. HPV infects the esophageal epithelium, but so far, no firm evidence of its involvement in esophageal carcinogenesis has been provided. Some of these factors, notably hot tea drinking, may render the esophageal mucosa more susceptible to injury by other carcinogens. There are few studies at molecular level on ESCC in Golestan. Increasing awareness about the known risk factors of ESCC could potentially reduce the burden of ESCC in the region. Further studies on risk factors, identifying high risk populations, and early detection are needed.

KEYWORDS:
Genetically susceptibility; Environmental risk factors; Esophageal cancer; Golestan

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INTRODUCTION

Esophageal cancer (EC) has two main histological subtypes, esophageal adenocarcinoma (EAC), and esophageal squamous cell carcinoma (ESCC). Although these subtypes originate from the same organ, their geographical distribution and etiology are different.[1] The most common subtype of EC is ESCC, which predominantly is a disease of low- and middle-income regions such as central China and Golestan province in northeast Iran. ESCC in high-income countries are mainly related to tobacco smoking and much alcohol drinking, but in high-incidence areas, some other factors are likely to be major risk factors of the cancer. EAC is related to overweight and gastroesophageal reflux

disorder and is more common in high income communities .[2,3]

Despite more efficient surgical and chemical therapies, prognosis of EC is still poor (5-year survival=19%).[4] Many cases of EC are diagnosed at late stages, and currently no practical screening methods are available to identify early stage ECs at the population level. Therefore, identification of risk factors and preventive measures are the most important strategies to reduce the burden of cancer.

Golestan province in Iran has some of the highest reported incidence rates of ESCC in both men and women. For several reasons studies of ESCC in Golestan could be quite helpful in shedding light on the etiology of ESCC. In Golestan, the prevalence of smoking in men is moderate and alcohol drinking in both sexes and smoking among women are uncommon habits. Therefore, other risk factors of ESCC could be identified with little confounding effect from smoking and alcohol drinking.[5] We reviewed a number of epidemiological studies of ESCC in Golestan in an article published in 2009.[6] As so, some other studies of ESCC in Golestan have been published since then, we aimed to update the previous summary. In addition, we will provide more detailed information about genetic factors and ESCC, because genome-wide association studies (GWAS) and other wide-ranging genetic studies in other populations have provided more insight on genetic factors in ESCC.

Epidemiology

ESCC is much more common in the so-called Asian EC belt, which starts from central and northern China and extends through central Asia and northern India to Golestan province. Central China and Golestan have the highest reported incidence rates of ESCC.[7] Findings of some studies showed that the incidence rate of ESCC in Golestan province is 100 per 100 000 (World Standard Population) age-adjusted men and women, which is considerable.[8] Although a recent study has demonstrated some degrees of decline, the incidence rates of ESCC has remained high in both sexes.[9]

There are some other areas with relatively high incidence rates, including southeast Africa (e.g. some parts of Kenya and South Africa), parts of South America (e.g. some parts of Brazil and Uruguay), and Calvados in France.[10] The lowest rates have been reported from high income countries in Europe and North America.[11]

The incidence rate of ESCC in Golestan is fairly higher in men than women (52% in male patients) [3], which is consistent with the pattern observed in Linxian (a high risk area in China) with a sex ratio of 1.5, but absolutely contrary to the pattern of low incidence areas of Western countries (6.5 in France) where ESCC is much more common in men.[12]

Based on Golestan Population-based Cancer Registry between 2004 and 2008 the age-standardized incidence rates (ASRs) per 100,000 person-year for esophageal cancer were 24.3 in men and 19.1 in women. The findings of a study in Gonbad and Kalaleh districts, located in the eastern area of the province, showed higher incidence rate of EC than other districts in both sexes.[13]

The risk of EC at age 75 years in the first-degree relatives of Turkmen patients and controls were 34% and 14%, respectively (HR=2.3, P=3×10^{-8}). Also, 9.6% parents of the cases were related and 2.5% of the parents of the controls were related (OR=4.1, p=0.006).[14] In another case control study, Moradi and colleagues found that 21.6% of cases but no one in the control group had EC in their first degree relatives (OR=7.6, p=0.001).[15] Since the role of family history have genetic and environmental elements in the pathogenesis of EC, more studies should be considered.

Environmental Factors

There are some reports that show factors including age, ethnicity, tobacco smoking, opium use, education, oral health, family history, and tea temperature were related to ESCC. Studying the nature and roles of these factors could be helpful in clinical practice and probably useful for identifying high risk individuals.[16]

High-temperature foods and drinks

High temperature foods and drinks can cause

thermal damage to the esophagus. Such damage, if repeated, can predispose the users of high temperature food and drinks to EC.[17] This association is consistent with the findings of other studies including what was conducted in the United Kingdom, South America, and Turkey.[18]

A study that was conducted in Golestan demonstrated a higher risk of EC due to drinking very hot tea (OR=8.16, 95% CI: 3.93 -16.9) or hot tea (OR= 2.07, 95% CI:1.28 - 3.35) compared with drinking warm or tepid tea. Regarding the tea drinking habits, almost all (98%) Golestan cohort participants drank one liter of black tea per day. Some of them used to drink tea at temperature less than 60°C (39%) but, 38.9% drink tea at 60-64°C, and others (22%) at 65°C or higher .[19]

Tobacco, Alcohol, and Opium use

Although above 90% of ESCCs in Western countries were associated with smoking and alcohol consumption [20], the high risk areas of the central Asian EC belt, demonstrate less significant role in pathogenesis of ESCC.[2] Some studies have shown the same relation in Golestan .[3,18,21] Scant alcohol drinking in Golestan province indicates that it could not be a main cause of ESCC in this area,[22]similar to what was seen in rural high risk areas of China .[23]

In Golestan province, those who used tobacco (OR=1.70, 95% CI: 1.05-2.73), opium (OR=2.12, 95% CI: 1.21-3.74), or both tobacco and opium (OR=2.35, 95% CI: 1.50-3.67) were at a higher risk of ESCC compared with those who used neither tobacco nor opium .[24]

In Golestan Cohort Study, 17% of the participants reported ever opium use, with a mean duration of 12.7 years. Opium use was accompanied by increased risk of death from any digestive diseases (HR=1.55; 95 % CI: 1.24-1.93), and long term opium use, was related to higher risk of death due to both malignant and non-malignant digestive diseases.[25] In a study about sex-related opium consumption model, opium was used by men (83%) much more than women (13%).[26] This huge difference is contrary to slight superiority of ESCC incidence in men compared with women, which was shown in

another study.[13] Evaluating the exposure of women in this area to other risk factors could be useful to confirm this association.

Low socioeconomic status

A study aimed to find the relation between some socioeconomic status (SES) variables and risk of ESCC in Golestanian population based case control study. The strongest inverse association was found with education level. Compared with no education, primary education (AOR=0.52; 95% CI: 0.27- 0.98) and high school or beyond (AOR=0.20; 95% CI: 0.06–0.65) were inversely associated with risk of ESCC.[27] Consistent with the findings of Golestan, another study reported strong relation between SES and ESCC in Kashmir.[28] In USA a study showed that the adjusted odds ratios for subjects with annual low incomes versus high incomes were 4.3 (95% CI: 2.1- 8.7) for white people and 8.0 (95% CI: 4.3-15.0) for black people. This shows that low income in black people could lead to ESCC more than white people.[29] SES plays its causation role in EC through some intermediate factors including behavior, lifestyle, environmental exposure, nutrition, and access to health care.[30]

Polycyclic aromatic hydrocarbons

There are many studies about the relationship between exposure to polycyclic aromatic hydrocarbons (PAHs) and ESCC. Indoor wood burning and road traffic are the major sources of PAHs. Both the WHO and the UK Expert Panel on Air Quality Standards (EPAQS) have considered benzo(a)pyrene (BaP) as an indicative for the carcinogenic ability of the PAH mixture.[31,32] The PAHs entering to the body through breathing, swallowing, and topical absorption, will cause metabolites that bind to cellular macromolecules.[33] Cooking meat at high temperatures produces excessive amounts of PAHs and heterocyclic amines (HCAs).[17] High temperature cooking and frying may be considered as causative factors of increased risk of ESCC in Golestan.[34]

Two studies that were conducted in northeast Iran, showed some urinary levels of a PAH metabo-

lite, which indicate exposure in more than 40% of the evaluated households, independent from other factors such as area of residence, smoking, sex, and nass or opium use. This was along with the pattern of SCC incidence.[21,35] Although the amount of BaP in ordinary food and water of high risk areas (Golestan) is similar to other areas (south of Iran), control participants from Golestan show more BaP intake than people living in low risk regions. Statistics show no association with BaP intake among the family members of patients' with ESCC, whereas living in low risk area was related to a significant decline in overall BaP consumption. In conclusion, PAH could be a factor for higher risk of ESCC in Golestan.[36]

Another study in this area showed that 41% of people had 1-hydroxypyrene glucuronide (1-OHPG), a stable PAH metabolite that its levels more than 5 pmol/mL, indicates very high exposure. Only 15% of the variance in 1-OHPG was explained by age, sex, residential area, smoking, nass or opium use .[35] An investigation about the pattern of non-smoking related exposure to PAHs has shown high exposure of PAHs in the general population and suggested that certain foods (red meat, processed meat), cooking methods (making bread at home), and genetic polymorphisms (GSTT1-02 and GSTM1-02) increased exposure to PAHs.[37]

Fungus contamination of foods

The toxin of fungus like fumonisin B1(FB1) is believed to have a positive correlation with EC. The geographic areas where FB1 exists in high concentrations have been associated with high rates of EC.[38] FB1 has been classified by International Agency for Research on Cancer (IARC) as Group 2B carcinogen.[39] In Golestan province 50% of corn and 40.9% of rice samples were contaminated by FB1. The proportion of FB1 contaminated rice samples was higher in high EC risk area than low risk area ($p= 0.02$).[40] Researchers also found a positive relationship between aflatoxin level of wheat flour samples and the risk of EC in Golestan.[41] Although an earlier study did not suggest a major role for mycotoxins in EC.[42] A pilot biomarker survey of women also revealed that fusarium mycotoxins exposure was normal compared to other areas of the world.[43]

Silica

In high incidence areas of ESCC, significant silica contamination of common food stuffs has been described, for example millet bran in northern China, wheat flour in Iran, and porridge made from 'Cape Chervil' in Transkei, South Africa.[2] According to a recent study, the silica contamination of samples in Golestan was not more than the reference ranges. This change may be related to disposing of traditional milling methods or the fading of contaminating seeds in Golestan farms.[2,6]

Vitamin deficiency

Based on a survey using twelve 24-h dietary recalls administered monthly over a 13-month period, Islami and colleagues found severe inadequacy of vitamin intake in Golestan, particularly among women and rural dwellers. For example, the intake of vitamin C was below the recommended value in 41% of men and 64% of women in urban areas and 97% of men and 100% of women in rural areas. These deficiencies in nutrient intake may contribute to the higher risk of ESCC in Golestan, particularly in rural areas. Most of general population in Golestan province consumed protein more than the recommended values.[44]

Poor oral health

A study showed that increased risk of ESCC in Golestan (OR=2.37, 95% CI: 1.42-3.97) was associated with poor oral hygiene and dental health.[45] Also in another study in this area, researchers showed that coexistence of poor oral hygiene habit with gastric atrophy elevated the risk of ESCC eight times (OR=8.65, 95% CI: 3.65- 20.46).[46]

Human papilloma virus (HPV)

Among different types of human papilloma virus (HPV), serotype 16 has been suggested to have a role in the etiology of EC. Based on publication of IARC about HPV, this virus is detected more in

patients with ESCC and there are many reviews about the association between HPV and ESCC, but these findings have been very inconsistent. This report concludes that part of this inconsistency could be explained by the absence of reliable tests.[47] A study that was conducted in China concluded that findings did not support a major role for HPV16, HPV18, and HPV73 in the etiology of EC.[48] Another study showed an important causal role for HPV particularly in high risk areas. HPV detection rates were 23% by using in situ hybridization and 15% by polymerase chain reaction in ESCC and oncogenic HPV types.[49]

There are some studies in Golestan, which evaluated the role of HPV in ESCC. The rates of HPV detection in ESCC specimens for men and women were 52.8% and 43.7%, respectively, of which 54.7% were HPV-16. Results of this study support the possible role of HPV as an etiological agent in esophageal carcinogenesis.[50] In another study at the same region, there was no significant difference in HPV infection between cancerous and non-cancerous tissue specimens of esophagus.[51]

Based on pooled analysis of six case-control studies from South Africa, Australia, Central and Eastern Europe, Brazil, Iran (Golestan), and China , limited serological evidence regarding the Associations between ESCC and HPV was found by researchers. They also were not able to exclude the role of some types of HPV in small subtypes of ESCC.[52] According to aforementioned studies, the epidemiological evidence for the role of HPV in EC is unsatisfactory.

Microbial factors

Currently, the evidence that suggest the relation between microbes and EC is increasing. A study has revealed a significant association between the normal microbial flora of the upper gastrointestinal tract and esophageal squamous cell dysplasia as a precancerous lesion of esophagus.[53] Periodontal disease microbiotome that is defined as collection of all members in a complex microbial community is full of gram negative bacteria. Porphyromonas gingivalis is the most important pathogen in mi-

crobiotome, which has the ability to ruin epithelial cells and hinder immune reaction cell cycle mechanism. P. gingivalis was detected in 61 % and 12 % of cancerous and proximate tissues, respectively.[54]

A study that was conducted in China showed different amount of coordinates in ESCC compared with non-ESCC patients. This finding shows a relation between bacterial microbiota of saliva and risk of ESCC.[55] *Helicobacter pylori (H. pylori)* is another gram negative bacteria that has commonly accused to have a precancerous role in gastroesophageal cancers. This bacterium that is specifically colonize the gastric epithelium, was regarded as the causative agent in infection-related cancers, which comprise 5.5% of the global cancer burden.[56] A meta-analysis study about the relationship between *H. pylori* and EC, showed an opposite association between *H. pylori* and EAC (OR=0.41, 95% CI: 0.28-0.62) and no association with ESCC (OR=1.08, 95% CI: 0.76–1.53).[57]

Genetic susceptibility

Studies have suggested the involvement of a large number of molecular events in the development and progression of ESCC, including genetic and epigenetic alterations in DNA repair genes, oncogenes, tumor suppressor genes, cell adhesion molecules, cell cycle regulatory genes, genetic instability, and telomerase activation. Nevertheless, the role of molecular mechanisms in the initiation, progression, and prognosis of ESCC are still poorly understood.[58,59] The distinct risks that were shown in individuals exposed to the same known risk factors imply that genetic predisposition might play an important role in EC etiology.[60]

The pathological development of ESCC is a multi-step progressive process. The proliferation of esophageal epithelial cells is an early indicator of this process, which morphologically proceed to hyperplasia of basal cell, cell dysplasia, carcinoma in situ, and finally advanced EC. At the cellular level, the tumorogenesis process is related to abnormalities in the control of cell proliferation and differentiation and cell death (apoptosis). Most of cancerous cells contain genetic alterations, which indicate

failure in the control process, including transcription factors and apoptosis related proteins.[16,18] Two basic types of genetic damages are encountered frequently in cancerous cells: recessive (with targets known as tumor suppressor genes) and dominant (with targets known as oncogenes).[61,62] Genetic factors related to ESCC are summarized as follows:

Oncogenes

Most normal cells will undergo a programmed form of rapid cell death (apoptosis) when their critical functions are altered. Oncogenes turn a healthy cell into a cancerous cell. Frequent mechanisms, activate these oncogenes, include point mutations, amplification, rearrangement and over-expression.[63] Some of the oncogenes that are assessed in ESCC include:

Epidermal growth factor receptor (EGFR)

EGFR gene family translates proteins on cell membrane that act as receptors and has intracellular tyrosine kinase activity and extracellular binding domain.[64] In a meta-analysis EGFR over-expression was positive in 722 of 1,150 patients (63%) and was associated with higher depth of invasion and poor prognosis.65 Only the roles of two members of EGFR family (erbB-1 and erbB-2) have been investigated in ESCC. There is a tendency toward a worse prognosis in patients with erb-B-1 over-expression.[10] Based on a study in central Asian high incidence areas, on 152 cases of ESCC from Iran (Tehran and Golestan province) and North India (Kashmir Valley), EGFR mutations appear to be a rare event.[66]

Cyclin D1

Cyclin D1 was shown to be associated with increased presence of lymph nodes metastases, distant metastases, high proliferative activity, high tumor grade, poor response to chemotherapy, and poor patients' survival.[67] Because cyclin D1 has not been investigated in Golestan province, studying its expression could be informative and useful.

Tumor suppressor genes

Tumor suppressor genes are supportive genes.

Regularly, they control cell growth by monitoring cell division, repairing DNA mismatch, and controlling cell death. When a tumor suppressor gene is mutated, cells grow without control and may eventually form a tumor. Many tumor suppressor genes and the nuclear factors of the oncogenes control the expression of proteins that act as cell cycle regulators. The process can be inhibited by products of the proteins from the tumor suppressor genes such as p15, p16 , p21, p27, p53 (indirectly through p21), and enhanced by c-myc protein.[58] Some of tumor suppressor genes that have been studied in ESCC include:

P53

Over 100 ESCC mutations have been gathered in the IARC TP53 mutation database. The incidence of p53 mutation in ESCC is around 50% and the frequency of mutations appears to be higher in high incidence areas. Accumulation of p53 in areas with esophagitis, suggested that the loss of suppressor function of p53 might be an early event in carcinogenesis of the esophagus. P53 is involved in multiple cellular pathways including transcriptional regulation, apoptosis, and cell cycle control. P53 single nucleotide polymorphisms(SNPs) reduce apoptosis and are associated with increased risk, earlier age of onset, chemotherapy resistance, and ECs relapse. In Japan, Kihara and colleagues found that the prognosis of patients with p53 mutated EC was significantly poorer (chemotherapy resistant) than patients whose tumors had no p53 mutations (p=0.006). Although some results showed that patients with p53 mutations were likely to have a shorter survival but its relevance as a prognostic factor is still controversial .[10,58,62,68-71] In a recent study in Iran, no significant difference was found in the prevalence or pattern of p53 mutations between EC patients from northern Iran (Golestan) and Tehran, the capital of Iran, which is a low- to moderate-risk area for ESCC.[72] But in a case control study in Golestan, it was shown that ESCC had the highest rate of TP53 mutations (89.9%) ever reported in any cancer anywhere. These transversions include 40% at bases known as site of mutagenesis by PAHs. Mmutations showed different patterns according to tea consumption, but

no variation in ethnicity, tobacco or opium use, alcoholic beverage consumption or urban versus rural residence. The heterogeneous mutation pattern is highly suggestive of a causative role for multiple environmental carcinogens, including PAHs. The temperature and composition of tea may also affect mutagenesis .[73,74]

P21

P21 protein is a cell cycle regulator that induces G1 arrest, leading to DNA repair or apoptosis. In response to DNA damage, wild type p53 assembles several downstream target genes including p21. The preliminary data suggested that p21 polymorphisms were not associated with development of ESCC in northeastern Iran, and gene-environment interaction analysis showed that cigarette smoking might have synergistic interactions with P21 C/A and p21 C/T genotype in ESCC carcinogenesis in this region.[75]

P16INK4a

Tumor suppressor genes, p16INK4a and p53, and the proto-oncogene, MDM2, are considered as essential G1 cell cycle regulatory genes whose loss of function is associated with ESCC carcinogenesis. Abnormal expression of p16 and p53, not MDM2, were significantly higher in the tumor tissue of ESCC patients in Golestan province. P16 gene silencing caused by hypermethylation of CpG islands may be a major mechanism in the ESCC development and is associated with overexpression of p53 protein. This is the first study demonstrating the relationship between p16 hypermethylation and overexpression of p53 protein .[76]

BRCA2

BRCA2 is a nuclear protein with tumor suppressor activity through repairing DNA. A previous study from China suggested that BRCA2 might play a role in the etiology of ESCC.[77] In Golestan six mutations that were screened in the coding region of BRCA2 gene in the germline DNA of Turkmen patients with ESCC, were judged to be pathogenic. In total, a suspicious deleterious BRCA2 variant was identified in 7.6% of patients with ESCC.[78]

Single nucleotide polymorphisms (SNPs)

Adding phase I SNPs and nucleotide excision repair (NER) capacity provided the best fit, and could explain 17% of the variation in adduct levels more than the environmental model of ESCC in Golestan cohort study. Female non-smokers in this population had PAH-related DNA adduct levels three to four times higher than smokers and occupationally-exposed groups in prior studies, with large inter-individual variation, which could best be explained by a combination of phase I genes and NER capacity .[79]

Genetic polymorphisms in enzymes

Genetic polymorphisms may affect the activity of enzymes that metabolize carcinogens and make individuals more or less susceptible to the carcinogenic effects of the carcinogenic exposure.[80] Cytochrome P450 (CYPs) is primarily responsible for the bioactivation of many low molecular weight carcinogens, including certain nitrosamines. Glutathione S-transferases (GSTs) are involved in detoxifying many other carcinogenic electrophiles including benzo(a)pyrene and other carcinogens in tobacco smoke, and alcohol dehydrogenases (ADHs)/aldehyde dehydrogenases (ALDs), which are alcohol-metabolizing enzymes. The data on genetic polymorphisms at the current status were still insufficient.[10] ADH oxidizes ethanol to acetaldehyde, which is itself oxidized to aldehyde by ALDH. Acetaldehyde has been demonstrated to be carcinogenic and therefore genetic variations resulting in functional differences in ADH and ALDH activity, which lead to increased levels of acetaldehyde in drinkers may be important. A number of ADH and ALDH families have been implicated in the pathogenesis of ESCC.[2]

An association was shown between inactive polymorphism of the GSTM1 gene and the presence of esophageal squamous dysplasia, in a PAH-exposed population in China. Also, a significant higher expression of aryl hydrocarbon receptor (AhR) gene, which codes for the cell membrane receptor for PAHs, was reported in subjects from the same population who had a positive family history

of upper gastrointestinal cancers. This indicates that these individuals might be more susceptible to PAH-related carcinogenesis.[81]

Among three Iranian ethnic groups, the frequencies of polymorphisms in ten genes that have been hypothesized to have a role in risk of EC were compared. Turkmens had higher frequency of four alleles that are speculated to favor carcinogenesis (CYP1A1 m1, CYP1A1 m2, CYP2A6*9, and ADH2*1) compared with Zoroastrians. These results are consistent with an influence of these allele variants on the population risk of EC. However, none of these four alleles had a high enough prevalence in Turkmens to explain the high rates of EC in this ethnic group. Three of these four alleles (CYP1A1 m1,CYP1A1 m2, CYP2A6*9) were less frequent among Turkmens than in some Asian populations with lower risks of EC.[82] There are no case-control studies addressing the risk associated with these polymorphic genes and EC in Iranian patients, although a correlational study did not find a higher prevalence of susceptibility alleles of these genes among the high-risk Turkmens.[83]

22 functional variants (and 130 related tag SNPs) from 15 genes that have been associated previously with the risk of ESCC were evaluated in a case control study in Golestan province. The histidine allele at codon 48 of ADH1B gene was associated with a significantly decreased risk of ESCC in the joint data set under a recessive model (OR=0.41, 95% CI: 0.29-0.76, *p*=0.0004). The allele of the rs7087131 variant of MGMT (methyl guanine DNA methyltransferase) gene was associated with a decreased risk of ESCC under a dominant model (OR=0.79, 95% CI: 0.64-0.96, *p*=0.02). These results support the hypothesis that genetic predisposition plays a role in the high incidence of ESSC in Iran.[84] Also in another study in Golestan, it was demonstrated that general population had high exposure to PAHs. The study suggested that certain foods (red meat intake, processed meat intake), cooking methods (making bread at home), and genetic polymorphisms (GSTT1-02, CYP1B1-07, GSTM1-02) increased exposure to PAHs.[85]

NQO1(quinone oxidoreductase1)

NQO1 is mainly a cytosolic enzyme, which catalyzes the reductive activation of quinoid chemotherapeutic agents and environmental carcinogens such as heterocyclic amines, nitrosamines, and condensates of the smoke like PAH. However, in the process of these metabolic reactions, some unstable PAH metabolites are highly reactive and may attack the DNA molecule, which results in the formation of PAH-DNA adducts .[86]

In a meta-analysis it was shown that NQO1 C609T polymorphism increased the risk of ESCC (OR=2.03, 95% CI=1.29-3.19).[87] In a study that was conducted in Golestan about the association between NQO1 and ESCC, predisposing role of NQO1 T allele for ESCC was not concluded. This study also revealed the role of PAHs as a risk factor of ESCC and the potential of PAH-DNA adducts for being a biomarker of risk for ESCC.[88] Another study confirmed that the production of PAH-DNA adducts (DNA binding products), is a crucial step in PAH-initiated carcinogenesis.[33] A study in Linxian showed PAH-DNA in esophageal biopsies and concluded that PAH-DNA adducts were a permanent indicator of PAH exposure. They recommended this indicator could be used in large studies looking for a relationship between PAH-DNA adduct and EC risk.[89]

Fanconi anemia genes (FANC)

Researchers sequenced the entire coding regions of 12 FANC in the germline DNA of Turkmen ESCC cases and identified three heterozygous insertion/deletion mutations. Both heterozygous and homozygous mutations in several FANC predisposing genes are associated with an increased risk of ESCC in Golestan province.[90]

Matrix metalloproteinases 2(MMP-2)

MMPs have a main role in cell life such as proliferation, migration, differentiation, angiogenesis, apoptosis. These enzymes have ability to destroy the proteins of extracellular matrix. Researcher induced inhibitory effects on the invasion and migration of the EC cell by suppressing MMP-2 expres-

sion.[91] Conversely, another study demonstrated that activating MMP-2 was associated with lymph node metastasis and lymphatic or vascular invasion.[92]

Telomerase

Repetitive telomere sequences at the ends of eukaryotic chromosomes protect the ends from damage and rearrangement. Progressive shortening of telomeric sequences is associated with cell division and DNA replication. Telomerase is a ribonucleoprotein reverse transcriptase that utilizes its own RNA template for the addition of telomeric sequences to chromosomal ends in order to maintain telomeric length. Li and colleagues showed that the positive rate of telomerase activity in ESCC cases was 83.3% and increased telomerase activity was associated with the progression of ESCC. Telomerase activity of ESCC cases in grade I (metaplasia), grade II (dysplasia), and grade III (high grade dysplasia) were 60%, 90%, and 91%, respectively. The poorer differentiation, and patients with lymphatic metastasis showed a higher telomerase activity than those without such findings.[93] There is not any study about this important aspect in Golestan as a high risk area.

Proteins

Keratins:

Keratins are intermediate filament forming cytoskeletal proteins with 30 indentified subtypes (CK 1–30). The diagnostic sensitivity of serum CK19 was correlated with tumor size, stages, and responsiveness to treatment and introduced CK19 as a potential useful prognostic marker for ESCC.[94] Two representative tumor-associated proteins, HSP70 and HMGB1(especiallyHSP70), can induce autoantibody response in ESCC sera and have higher expression in ESCC tissues.[95]

Hyaluronic acid and laminin

They are non-collagenous components of the extracellular matrix, which are involved in several functions of cell, including proliferation, differentiation, migration, and cell adhesion. Several studies have reported increased serum levels of hyaluronic

acid and laminin in various cancers and the correlation of the levels with poor prognosis. But, small data on the use of serum hyaluronic acid and laminin levels for early detection of EC are available .[96,97] In Golestan province, mean serum hyaluronic acid and laminin concentrations in incidental cancer cases were higher than in controls in crude analyses and substantial associations were indicated between hyaluronic acid levels and ESCC (beta coefficient=0.332, p=0.05) in multivariate models .[98]

Proteomics

According to the first study on the proteomics of ESCC from Iran, Rastgar and co-workers showed that multiple proteins, which are generally affected in other digestive organs, are affected in ESCC, which may indicate a common molecular mechanism for digestive system tumor genesis and putative protein candidate biomarkers. Among the 14 differentially expressed proteins identified in this study, they observed loss of ß-tropomyosin (TMβ) in all tumor samples. Furthermore, this study additionally supports the involvement of TSs in the etiology of ESCC.[99]

MicroRNAs (miRNA)

Recently, a class of small non-coding RNAs, called miRNA, has been discovered, which can post transcriptionally silence protein expression. Some studies showed that different miRNAs play an important role in cell growth and carcinogenesis.[100] Also miRNA acts in other biological and pathological processes including proliferation, differentiation, and apoptosis, and behaves as a biomarker for determining the types and sources of cance.[101,102]

There are few published data on miRNAs in ESCC. Some of miRNAs, which were associated with poor prognosis of ESCC are miR-103/107, miR-21, and MiR-27a.[103-106] The detection of miRNAs will not only provide us with potential biomarkers for the early detection and prognostic assessment of human cancer but will also increase our understanding about their role in esophageal carcinogenesis.[100] So the role of microRNAs in

Table 1: Summary of evidence about the association between environmental factors and ESCC

Risk factors (RF)	Study Type (reference)		Association and risk (reference)	
	Other region	**Golestan**	**Other regions**	**Golestan**
SES	-Population based case control study[29]	Population-based case control study [5, 27, 72]	-Low annual incomes: AOR=4.3 for white people AOR=8.0 for black people -Incidence rate(exposure to the same RFs: black people>white people -Never married: OR=3.9 - Low educational: OR=3.1 - Low occupation: OR=4.2 -Low socioeconomic: OR= 1.8 [29]	-Compared with no education: primary education: AOR=0.52 ≥high school: AOR = 0.20[27] -Residence in urban areas: HR:0.7 Non-Turkmen ethnic : HR=0.76[5] -No significant difference between Turkmen and non-Turkmen ESCC cases in the prevalence of exposure[72]
Smoking	-population based case control study [29]	-Cross sectional[3] -Case control[24]	Tobacco use in ESCC : OR:3.1 white males OR:2.5 black males [29]	-Only 27% of ESCC patients had ever smoked (similar to other cancer and non-cancer groups) - OR=1.70; 95%CI:1.05-2.73[24]
Alcohol consumption	-Population based case control study [29]	- Cross sectional [3] - Case control [24]	It is main RF: OR:6.8 white males OR:8.3 black males [29]	-Only one of the 144 ESCC cases had ever consumed alcohol, so alcohol cannot be considered a risk factor for ESCC [3] -Alcohol consumption was seen in only 2% of the cases and 2% of the controls, and was not associated with ESCC risk [24]
Opium consumption	-------------	-Case control[107,24] - Cohort [25]	-----------------	- OR=2.12; 95% CI:1.21-3.74[24] - Opium use was associated with a significantly increased risk of ESCC: AOR 1.77, 95% CI 1.17-2.68 [107] - AHR for all mortality causes associated with use of opium was 1.86 (95% CI, 1.68 - 2.06)[25]
PAH exposure	-Review[17]	-Case control [36] -Cross sectiona[l35]	- Intakes are thought to be relatively high in Europe, although measures are only available from a few, generally high-income countries.[17]	- BaP daily intake in controls: high-risk area (Golestan)>low-risk area (91.4 vs. 70.6 ng/day, p<0.01) [36] -41% of people in Golestan (1-OHPG)>5 pmol/mL (very high exposure) [35]
Fungus contamination of foods	-Cross sectional (ecological study)[38]	-Cross sectional (ecologic study) [40] -Cross sectional (ecologic study)[41]	FB1 and FB2 levels were significantly higher in high risk area (p=0.01)[38]	-FB1-contaminated rice: High-risk area (75%)>low risk (21.4%) (p=0.02) [40] -AF level of wheat flour: Total AF and AFB1 were significantly higher in samples obtained from high risk area [41]

ESCC, from providing non-aggressive biomarkers to their potential role in producing modern therapeutic measures make them attractive to researchers.

Risk factors (RF)	Study Type (reference)		Association and risk (reference)	
	Other region	Golestan	Other regions	Golestan
Oral and dental hygiene	Population based Case control study [29]	Population-based Case control study[45,46]	-Rarely visited a dentist in ESCC cases: OR=1.8 for white people OR =1.7 for black people -Rarely visited a dentist at or below the poverty level: OR=2.6 for white people OR=4.2 for black people [29]	- Poor oral hygiene in ESCC: OR=2.37[45] -Poor oral hygiene with gastric atrophy elevated ESCC risk: OR=8.65[46]
HPV	- Review[10] - Pooled from six case control studies [52]	-Cross sectional[50] -Case control[51]	HPV in ESCC: 1- United States: 3%, - High risk areas in Asia (Japan, China, Hong Kong, India, Pakistan, and Korea), South Africa, Alaska, and Australia: 13- 63% (overall 22%.)[10] 2- South Africa, Australia, Central and Eastern Europe, Brazil, Iran, and China: -E6 antibody: HPV16: OR=1.89 HPV6: OR=2.53 [52]	1- HPV in ESCC: - men: 52.8% -women : 43.7% positive cases HPV-16: 54.7%[50] 2- No significant difference between cancerous and non-cancerous [51]
Low level of vitamin A&C intake	-Systematic review[59]	-Cross sectional in healthy participants in Golestan [44]	- A decreased risk associated with high retinol and β-carotene intake (combined OR=0.66, 95% CI:0.54-0.81) [59]	-Severe deficiency in vitamin intake among women and rural dwellers in Golestan: Daily intake (rural women) < LTI (lowest threshold intakes): vitamin A: 67% (p< 0.01) vitamin C : 73% [44]
High temperature cooking and frying (dietary intake of heterocyclic amines)	Population-based Case control study	Case control	Heterocyclic amine intake might be associated with an increase in risk of ESCC (50-70% increased risk)	-Frying index: p<0.01 ESCC cases:18.2:1 High risk controls: 12.8:1 Low risk controls: 2.6:1 -Cooking oil reuse: p<0.05 ESCC cases: 37.5% High risk controls: 25% Low risk controls: 7.5%
Hot beverage consumption	---------------	Case control	---------------	Risk compared to warm : -hot black tea (OR: 2.07) -very hot black tea (OR:8.16)

SES: socioeconomic status, AOR: adjusted odds ratio, OR: odds ratio, ESCC: Esophageal squamous cell carcinoma, AHR: djusted Hazard Ratio, PAH: polycyclic aromatic hydrocarbons, BaP: benzo(a)pyrene, FB1 and FB2: fumonisin B1& B2, P: P.value, HPV: Human papilloma virus, CI: confidence interval

CONCLUSION

EC is still one of the most lethal diseases despite the improved approaches for diagnosis, prevention, and treatment. EC pose a considerable medical and public health challenge in many parts of the world. The development of EC is a multifactorial process associated with a variety of risk factors. The high incidence of EC in certain parts of the world indicates a role for environmental as well as habitual factors in addition to genetics. Therefore, greater effort is desired to comprehensively understand the molecular biology of esophageal carcinogenesis especially in high risk regions like Caspian littoral.

The insight into cancer biology could be translated into practical approaches for the diagnosis, treatment, and prevention of EC. Low socioeconomic status, certain nutritional deficiencies, cigarette smoking, nass chewing, opium use, hot tea

Table 2: Summary of evidence about the association between genetic factors and ESCC

Genetic factors	Location	Tumor abnormality	Risk factors may be associated with gene expression	Study Type/method (reference)		Association and Risk)Reference)	
				other regions	Golestan	other regions	Golestan
EGFR	17p13	-Oncogene -Amplification/ overexpression	-Benzopyrene-diol epoxide (BPDE), a carcinogen present in tobacco &environmental pollution - bile acid (a tumor promoter in gastrointestinal cancer)	Systematic review/meta-analysis	Direct sequencing	In EGFR overexpression: -positive in 722 of 1, 150 - higher depth of invasion - vascular invasion - poor prognosis [65]	- EGFR mutations: rare in high incidence areas -Rare activating mutations (2.6%), frequent EGFR protein over-expression (65%) [66]
Cyclin D1	11q13	-Oncogene -Amplification	--------------	Immuno-histochemistry [108]	--------------	In cyclin D1 positive tumors: -prevalence: 86.7% - adventitia invasion: 88.2% -lymph node metastasis :87.5% -poorly differentiated :92.9% [108]	--------------
P53	17p13	Tumor suppressor genes: Point mutation; LOH	-Environmental carcinogens (PAH) -Temperature and composition of tea -Cigarette smoking	PCR and DNA sequencing[71]	Immunohisto-chemical staining [73,76,109,110]	- p53 gene mutations in 56.5% tumors [71]	-TP53 mutations in 89.9% of ESCC[73] -P53protein overexpression in 74.2% of ESCC cases[76] - one or more p53gene point mutations in 65% [109] - Positive expression of p53 protein in 56.2% of ESCC cases [110]
P21	6p21.2	Tumor suppressor	Cigarette smoking	Immu-nohisto-chemical	-Case control[75] -Immunohistochemical staining[110]	-The incidences of grade 3 were 67% and 20% in the cases with a positive and negative p21 expression, respectively (p=0.0213) [111]	-None of the p21 genotypes were significantly associated with risk of ESCC (p=0.52, OR=1.24, 95% CI: 0.63-2.42) [75] -p21 overexpression was associated with poorer clinical outcome in ESCC (p= 0.009) [110]

consumption, and poor oral hygiene are the environmental risk factors that are associated with ESCC risk in Golestan province. Other suggested risk factors are exposure to PAHs, nitrosamines, silica fibers, and infectious agents. HPV infects the esophageal epithelium, but so far, no firm evidence of its involvement in esophageal carcinogenesis has been provided. Most of these factors render the esophageal mucosa more susceptible to injury by carcinogens. The effect of environmental factors is

Genetic factors	Location	Tumor abnormality	Risk factors may be associated with gene expression	Study Type/method (reference)		Association and Risk)Reference)	
				other regions	Golestan	other regions	Golestan
p16	9p22	Tumor suppressor: homozygous loss, promoter methylation	Roasting meat intake	-Immunohistochemical staining [112] -case control [113]	Immunohistochemical staining [76]	-The expression of p16INK4a was 88% in ESCC cases [112] -Daily roasting meat intake was related to the risk of ESCC (p<0.01) and the mean CpG methylation rates of p16 promoter (p<0.01). [113]	- p16INK4a gene aberrant methylation in 62% of esophageal tumor samples [76]
NQO1	16q22	Enzymes involved in metabolism of carcinogens	Environmental carcinogens: heterocyclic amines, nitrosamines and cigarette smoking	Systematic review/ meta-analysis [87]	Case control study	-NQO1 C609T polymorphism increases the risk of ESCC (OR=2.03, 95% CI=1.29-3.19) in a meta-analysis [87]	NQO1gene expression was high in normal esophageal tissues [88]

Location: gene's "address" on a chromosome. EGFR: Epidermal growth factor receptor, NQO1: quinone oxidoreductase1

not independent of cancer genes.

Overall, molecular research on ESCC focuses on the factors related to pathogenesis and prognosis. These include topics like etiopathogenesis (cancer susceptibility genes), keratins expression, tumor related genes (oncogenes, tumor suppressor genes, genes involved in metastasis, and apoptosis genes), proliferation-related factors (nuclear proteins), and factors related to metastases (cell adhesion molecules and enzymes related to degradation of extracellular matrix).

As tables 1and 2 show, we summarized evidence, which were found in Golestan province and compared them with other evidence, which were found in other similar regions in the world. We have a lot of evidence about environmental factors but there are a few studies in molecular level in Golestan. So there is an obvious need for further studies not only for screening ESCC in curable phase but also for developing the treatment. Future molecular epidemiological studies would be helpful in identifying high risk populations, early screening and diagnosis, and guiding individual clinical treatment of EC.

CONFLICT OF INTEREST:

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

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