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Review

Oesophageal cancer in Golestan Province, a high-incidence area in northern Iran – A review

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ABSTRACT

Golestan Province, located in the south-east littoral of the Caspian Sea in northern Iran, has one of the highest rates of oesophageal cancer (OC) in the world. We review the epidemiologic studies that have investigated the epidemiologic patterns and causes of OC in this area and provide some suggestions for further studies.

Oesophageal squamous cell carcinoma (OSCC) constitutes over 90% of all OC cases in Golestan. In retrospective studies, cigarettes and hookah smoking, nass use (a chewing tobacco product), opium consumption, hot tea drinking, poor oral health, low intake of fresh fruit and vegetables, and low socioeconomic status have been associated with higher risk of OSCC in Golestan. However, the association of tobacco with OSCC in this area is not as strong as that seen in Western countries. Alcohol is consumed by a very small percentage of the population and is not a risk factor for OSCC in this area.

Other factors, such as polycyclic aromatic hydrocarbons, N-nitroso compounds, drinking water contaminants, infections, food contamination with mycotoxins, and genetic factors merit further investigation as risk factors for OSCC in Golestan. An ongoing cohort study in this area is an important resource for studying some of these factors and also for confirming the previously found associations.

1. Introduction

Oesophageal cancer (OC) is the eighth most common cancer and the sixth most common cause of death from cancer worldwide.[1] International variation in the incidence of OC is striking, with over 20-fold differences between high-risk and low-risk areas of the world (Table 1). Golestan Province, located in the south-east littoral of the Caspian Sea in northern Iran (Fig. 1), has some of the highest rates of OC in the world.[2] A series of etiological and mechanistic studies of OC in Golestan Province began in the 1970s, but these studies were discontinued after the sociopolitical changes in Iran in

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Table 1 – Age adjusted incidence rates (per 10^5 World Standard Population/year) of oesophageal cancer in selected areas of the world.

Area	Male	Female	% SCC
Iran, Caspian littoral other than Golestan-1970s[2]	13 to 28	2 to 20	Not reported
Iran, Golestan (western parts of province)-1970s[2]	54 to 84	39 to 77	Not reported
Iran, Golestan (eastern parts of province)-1970s[2]	81 to 166	60 to 195	91[6]
Iran, Golestan (western parts of province)-1990s[a77]	144	49	Not reported
Iran, Golestan (whole province)-1990s[b5]	43	36	Not reported
Iran (north-west), Ardabil Province[78]	15	14	85[79]
Iran (central), Semnan Province[80]	12	9	Not reported
China, Linxian[81]	109[c]	109[c]	~ 100[82]
China, Beijing[1]	10	4	90
Osaka, Japan[1]	10	2	90
Sweden[1]	3	1	69
UK, England[1]	8	4	41
Uruguay, Montevideo[1]	11	3	84

[a] A screening study between 1995 and 1997, using exfoliative balloon cytology on 4192 asymptomatic adults in western parts of Golestan Province.
[b] A retrospective cancer surveillance.
[c] The ASR was adjusted for gender and was not reported for males and females separately.

Fig. 1 – Geographic location of Golestan Province in the Caspian littoral of Iran.

1979. A new series of studies, collectively known as the GastroEsophageal Malignancies in Northern Iran (GEMINI), including Golestan Case–Control Study, Golestan Cohort Study, and a series of ancillary studies started from 2001. In this review, we summarise the available data on the epidemiologic features and etiology of OC in Golestan, with particular focus on the recently published results that were not included in earlier reviews. We also compare the results to those found in other areas of the world, especially Linxian, China, where incidence rates of >100/10^5 have been reported. Our aim is to review the current status of knowledge and to provide suggestions for further research.

2. Epidemiologic features

The incidence rates of OC are highly variable across geographic areas. Whereas rates are relatively low in many parts of the world, exceptionally high-incidence rates have been reported from some parts of China. Moderate to high incidence rates have been reported from other areas or populations, including parts of Central Asia, South Africa, South America, northern France, and among African-Americans in the United States.[3,4] A cancer registry, conducted between 1969 and 1971, reported age-adjusted rates over 100 per 10^5/year for OC in eastern parts of Golestan Province (Table 1), comparable to rates found in high-risk areas of China. Moving approximately 500 km to the west, the rates in Gilan Province, in the western part of the Caspian littoral, fell to ~10 per 10^5/year, which resembled those seen in low-incidence areas of the world. A marked change in the male to female ratio of age-adjusted incidence rates was also observed; the male to female ratio was approximately 3:1 in Gilan Province but approximately 1:1 in Golestan.[2] This pattern resembled what had been observed in

other parts of the world: in low-incidence areas men are more likely to be diagnosed with OC but in high-incidence areas of China and Africa the sex ratio is close to unity.[1]

Turkmen are the main ethnic group residing in the high-risk areas of the Caspian littoral. In contrast, there are very few Turkmen in the low-risk areas of the Caspian littoral.[3] However, within the high-risk areas, it is unclear whether Turkmen have a higher risk than other ethnic groups.

More recent studies (Table 2) have shown that although the incidence rates have approximately halved compared to the 1970s, they are still high. A retrospective cancer surveillance between 1996 and 2000 in Golestan demonstrated that OC was the most common cancer (excluding skin cancers) among both sexes; the reported ASR (per 10^5/year) in the whole province was 43 for males and 36 for females.[5] It should be noted that misclassification of other types of upper gastrointestinal cancers, including gastric cardia cancers, as OC might have contributed to the very high incidence measured in the early 1970s, since many cases were diagnosed only on clinical or radiological examination,[2] whereas in recent surveys most cases were diagnosed on the basis of histologic examination of endoscopy-based biopsies.[5,6]

Squamous cell carcinoma is the predominant histologic type of OC in the world.[4] Nevertheless, a shift in proportion of OC from squamous cell carcinoma to adenocarcinoma has been reported in Western countries, notably from the 1970s in the United States and from the 1980s and early 1990s in some European countries.[7–9] The reason for this shift is not clear, but it may be related to several factors, including transitions in lifestyle and diet, overweight, and declining rates of *helicobacter pylori* infection in the Western world.[8,10] In Europe, while incidence of oesophageal squamous cell carcinoma (OSCC) has remained stable or declined during the past few decades, the incidence of oesophageal adenocarcinoma has been rising.[8,9] This increase has been more prominent in Northern Europe, notably in the United Kingdom and Ireland, but smaller increases have also been reported from other parts of the continent.[8,9] In Northern Europe and among Southern European women, oesophageal adenocarcinoma has outnumbered OSCC. The only exception to the recent trend was observed among women in Western European countries, including France, the Netherlands, and Switzerland, among whom the ratio of OSCC to oesophageal adenocarcinoma seems to have increased in recent decades.[9] In other parts of the world, particularly in high-incidence areas, OSCC is much more common than oesophageal adenocarcinoma (Table 1).[4] In Golestan, OSCC and oesophageal adenocarcinoma constitute 91% and 9% of oesophageal malignancies, respectively; other oesophageal tumours are rare.[6] Earlier OC studies in Golestan provided little information on histological subtypes. Therefore, it is not clear whether the ratio of OSCC to oesophageal adenocarcinoma in Golestan has changed during the past few decades, when the incidence of OC declined considerably there. Since the majority of oesophageal malignancies in Golestan are of squamous cell type, the results reported in this review mainly apply to OSCC.

3. Environmental risk factors

Tobacco use: Risk of OSCC is increased 3- to 7-fold among current smokers in many parts of the world,[4,11] and the International Agency for Research on Cancer (IARC) has classified tobacco smoking as a known cause of OSCC.[12] Smokeless tobacco use has also shown an association with risk of OSCC in Swedish studies.[13] In the pilot phase of the Golestan Cohort Study, which was conducted on 1057 randomly selected inhabitants in both rural and urban areas, 30% of rural men, 39% of urban men, 1% of rural women, and 3% of urban women had ever smoked cigarettes.[14] The corresponding percentages for ever use of nass (a chewing tobacco product containing a mixture of tobacco, lime, and ash) were 19%, 2%, 1%, and 0%, respectively. In Golestan, although all forms of tobacco use (cigarettes, hookah, and nass) have been associated with higher risk of OSCC,[15] the strength of association (relative risk of ~1.7) is less than what is seen in low-incidence

Table 2 – Prevalence of polymorphic alleles in three Iranian populations and Japanese or other Asian populations with low oesophageal cancer incidence.

Polymorphism	Turkmen %	Turks %	Zoroastrians %	*P* value	Japanese% [a]
CYP1A1 (T3801C) (m1)	29	19	9	<0.001	21[83]
CYP1A1 (A1506G) (m2)	11	2	1	<0.001	21[b]
CYP1A1 (T3205C) (m3)	0	0	0	–	9 (Asian)[84]
*CYP2A6*9* (T–48G)	14	5	4	<0.001	19[85]
CYP2E1 c2 (G–1293C)(*RasI*)	4	6	6	0.40	21[b]
*GSTM1*0/*0* (Homo deletion)	41	57	66	0.001	53 (Asians)[84]
*GSTT1*0/*0* (Homo deletion)	22	20	19	0.84	47 (Asians)[84]
GSTP1 (A313G)	23	26	20	0.21	9 [b]
*ADH2*2*	51	46	68	<0.001	74 [b]
*ADH3*2* (A350G)	20	22	13	0.06	5[86]
*ALDH2*2*	1	2	5	0.04	26[86]
O6-MGMT (C290T) (Codon 84)	24	16	11	0.02	18[b]

The study participants were high-risk Turkmen from Golestan Province, medium-risk Turks from Ardabil, and low-risk Zoroastrian Persians from Tehran.

[a] The allele frequencies among Japanese population were first searched in HapMap data; if the data were not available, the figures were presented from other studies.

[b] Data from HapMap, retrieved from SNP database of PubMed website: (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp&TabCmd=Limits).

areas. This finding is comparable with those from Linxian, where the relative risk for ever-smoking cigarettes was 1.3.[16]

Alcohol use: Similar to tobacco smoking, alcohol drinking is a strong risk factor for OSCC in many parts of the world[4] and has been classified as a known cause of this disease by the IARC.[17] Alcohol drinking and tobacco smoking increase OSCC risk in a multiplicative manner.[17] In Golestan, however, alcohol drinking is negligible.[15,18] Similarly, in Linxian, alcohol drinking has not been associated with higher OSCC risk.[16]

Opium use: Ecologic studies conducted in the 1970s in Golestan suggested that opium use may be a risk factor for OSCC, and *in vitro* studies showed mutagenicity, genotoxicity, and transforming activities of sukhteh (the opium dross found after burning opium in the pipe), but not crude opium.[19] These effects were attributed to some of the compounds present in opium dross, such as aromatic hydrocarbons and amines.[20] In the GEMINI studies, a questionnaire for tobacco and morphine use was validated against urine cotinine, codeine, and morphine level.[21] In the pilot phase of the Golestan Cohort Study, 22% of rural men, 8% of urban men, 5% of rural women, and 1% of urban women had ever used opium.[14] In the Golestan Case–Control study, 30% of cases and 18% of controls were opium ever-users, and crude and refined opium use were associated with 1.6-fold and 3.4-fold increases in risk of OSCC, respectively.[15] This effect was independent of tobacco use.

Hot tea consumption: An association between drinking tea and other hot beverages and oesophageal cancer risk has been reported in studies from different parts of the world.[22] Any such association seems to be related to thermal irritation of the oesophageal mucosa by these drinks.[22] Green and black tea leaves have shown cancer prevention activity in animal models; however, such activity has not been convincingly shown in humans.[23]

In the 1970s, an ecological study showed that Golestan inhabitants drank tea at a higher temperature and larger quantity than people living in a nearby low-incidence area,[24] and a case–control study reported increased risk of OSCC associated with higher temperature, but not quantity, of tea drinking.[18] Of 48,582 participants in the Golestan Cohort Study, 97% reported drinking black tea regularly with a mean volume of 1180 ml/day, and 60% drank their tea at temperatures of 60 °C or higher.[25] In the Golestan Case–Control Study, drinking hot and very hot tea were associated with 2-fold and 8-fold increases in risk, respectively.[25] Based on tea temperature measurements in the Golestan Cohort Study, hot and very hot tea were approximately corresponded to temperatures of 60–65 °C and 70 °C or more, respectively. Likewise, compared to drinking tea 4 or more min after pouring it, drinking tea 2–3 min or less than 2 min after pouring was associated with 2-fold and 5-fold increases in risk, respectively. No association between amount of consumed black tea or frequency of green tea drinking and risk of OSCC was found.[25]

Foods and nutrients: An inverse association between fruit and vegetable intake and risk of OSCC has been reported in epidemiological studies worldwide.[26] This inverse association has been partly attributed to vitamins and minerals found in fruits and vegetables. Although chemoprevention trials using vitamin supplements in Western countries have shown harmful results with respect to some cancer outcomes, the supplements in Linxian could decrease the incidence or mortality of OSCC.[27] It has been suggested that chemoprevention may be beneficial only for individuals whose intake of nutrients is deficient or borderline.[27] Several studies, mainly from Linxian, have suggested that some minerals such as selenium and zinc may reduce risk of OSCC.[11]

A case–control study conducted in Golestan Province in the 1970s showed an inverse association between intake of fresh fruit and vegetables and OSCC risk.[18] A more recent study compared data collected through 12 24-h dietary recalls during a 1 year period between rural and urban dwellers and between Turkmen and non-Turkmen ethnics. After adjustment for energy intake, this study showed significantly lower intake of most food groups and nutrients by rural dwellers, compared to urban dwellers, and severe deficiency of vitamin intake among women and rural dwellers.[28] Daily intake of vitamins A and C was lower than lowest threshold intakes (the level of nutrient intake below which nearly all individuals will be unable to maintain metabolic integrity) in 67% and 73% of rural women, respectively. In terms of vitamin intake, no significant difference was observed between Turkmen and non-Turkmen ethnic groups. Protein intake was not deficient in the study sub-populations.[28]

A study in Golestan found that the median selenium levels among participants of the Golestan Cohort Study was higher than the levels in subjects from three low-incidence provinces of Iran and above the level needed to saturate serum selenoproteins.[29] Therefore, selenium deficiency is unlikely to contribute to high risk of OSCC in this area.

Water supply: Several studies from China have reported an association between drinking ground water and OSCC.[30–33] The elevated risk may be related to the presence of carcinogenic compounds, such as nitrates, or absence of potential protective substances, such as certain trace elements, in the drinking water.[34,35] A prospective study in Linxian has also reported an inverse association between drinking water piped into the home, compared to other sources, and OSCC.[16] In the 1970s, access to piped water was limited in residential places in Golestan,[36] and a study showed no significant difference in source of drinking water between cases and their matched neighbourhood controls.[18]

Polycyclic aromatic hydrocarbons (PAHs): PAHs are produced by incomplete combustion of organic materials. The major sources of PAHs are tobacco smoking, occupational exposures, air pollution, and diet, especially charbroiled or fried foods.[11,37] Ecological studies in Linxian and in Golestan Province indicate high exposure of the people in these areas to PAHs, thus implying a possible role of these organic compounds in the etiology of OSCC.[11] Other indications of a link between PAHs and OSCC are the association between tobacco smoking and OSCC and an increase in risk of OC among chimney sweeps, which has been attributed to their exposure to PAHs,[38] but there is relatively little data from well-conducted case–control or cohort studies for this association.

An ecological study in the 1970s found no unusual levels of PAHs in samples of the main foodstuffs in Golestan[36] but it was not reported which food items were investigated in the study. However, a more recent study found very high levels of a biomarker of PAH exposure in the urine of people of

Golestan. In this study, urinary levels of 1-hydroxy pyrene glucuronide (1-OHPG), which is a metabolite of pyrene, a prototypical PAH, ranged from 1 to 5 pmol/ml in 42% of subjects. These levels are indicative of moderate PAH exposure typically observed in smokers. The 1-OHPG levels in 41% of subjects were above 5 pmol/ml, indicative of very high exposure.[39] The high exposure levels were observed among non-smokers as well as smokers; and only 15% of the variance observed in urine 1-OHPG levels was explainable by age, sex, place of residence, smoking, nass consumption, and opium use.

The sources of exposure are not entirely clear. As an attempt to find the sources, another study collected cooked rice, bread, and drinking water samples from households of 40 OSCC cases and 40 healthy controls in Golestan and 40 healthy controls in a low-incidence area of Iran.[40] Whereas the mean concentrations of benzo[a]pyrene (another prototype of PAHs) in the food and water samples were similar across study groups and within standard levels in both high- and low-risk areas, cases consumed more of the diet that had higher PAH levels. For example, cases consumed more bread than low- and high-risk controls. When the daily intake of benzo[a]pyrene from the above food items in each area was estimated by multiplying intake of each food by the corresponding concentration of benzo[a]pyrene, the daily intake in Golestan was higher than in the low-risk area.[40] The search for finding the major source of PAH exposure in this area continues.

N-nitroso compounds (NNCs): Humans are exposed to NNCs from both exogenous intake and endogenous formation of the compounds. Major exogenous sources are tobacco smoking, occupational exposures, and some foods, such as processed meat and fish and dried and pickled vegetables.[11] Vegetables and drinking water may contain substantial amounts of nitrate.[11] Of the entire nitrate absorbed from the diet or produced endogenously, 25% is taken up by the salivary glands and secreted into the mouth, where bacteria convert 10–90% of this nitrate in saliva to nitrite.[41] High concentrations of nitrite are present in the mouth and the entire oesophagus. When swallowed, saliva meets acidic gastric juice, the nitrite is converted to nitrosating species that can react with nitrogenous compounds to form NNCs.[41] This process contributes to 45% to 75% of the total exposure to NNCs. NNCs cause OC in animals.[42] A meta-analysis has shown a consistent association between eating processed meat, a major source of NNCs, and risk of OC.[43] A positive association has also been reported between the amount of NNCs in gastric juice and dysplasia and neoplastic changes in the oesophageal epithelium.[44]

A chemical analysis of foodstuff from high- and low-incidence areas of the Caspian littoral found no evidence for high dietary exposure to NNCs.[36] A pilot study, however, reported much higher levels of salivary nitrite in the high-incidence area, and higher nitrite levels tended to be found more often in the lower age groups. This high nitrite concentration was attributed to difference in endogenous metabolism of these compounds.[45] Both of the above studies were conducted in the 1970s, and this topic has not been pursued in more recent studies.

Silica: Several observational studies have reported an association between long-term exposure to silica dust and increased OC risk in industry.[11] In the 1970s, biogenic types of silica fibres were found in some food items in high-risk areas of China and Golestan.[46,47] In Golestan, flour was contaminated with sharply pointed silica fibres which originated from the seeds of the common Mediterranean grass *Phalaris minor*. This fibre stimulated the proliferation of the 3T3 mouse fibroblast line more than 100-fold.[46] In a recent study on flour samples collected from all wheat mills in Golestan, morphology of the possible contaminants was not investigated, but silica concentration in the samples was not higher than the reference ranges.[48] The above potential change during the past few decades may be related to disposing of traditional milling methods or the fading of contaminating seeds in Golestan farms.

Mycotoxins: Fusarium mycotoxins, such as fumonisins and nivalenol, are known to cause cancer in a number of experimental animals. Ecological studies, mainly from South Africa and China, have reported higher rates of food product contamination with *Fusarium* and *Alternaria alternate* fungi in high- rather than low-incidence areas of OC.[11] Contamination with mycotoxins is seen mainly in corn, but this can occur in other agricultural products such as wheat, barely, or black tea.[49,50] There is little data from observational studies for an association between mycotoxins and OC.

Three ecological studies in Golestan showed no prominent contamination of grain samples with carcinogenic fungi or fumonisins,[14,51,52] but the number of samples was limited.

Infections: Oncogenic types of *human papillomavirus (HPV)*, mainly types 16 and 18, have an important role in the etiology of some epithelial cancers, such as cervical and oropharyngeal cancers.[53] Results on the association between this virus and OSCC, however, are highly controversial.[11] *Helicobacter pylori* is another infectious organism studied in relation to OSCC but the results have been inconsistent and overall there does not seem to be an association.[54]

In a case-series, *HPV*-DNA was identified by polymerase chain reaction in approximately 50% of 85 OSCC biopsy samples from Golestan.[55] In a case–control study, the percentage of positive OSCC samples for *HPV* (36.8%) in surgically excised OSCC samples in two referral centres for thoracic surgery in Tehran, the capital of Iran, was significantly higher than that of endoscopy-based biopsies collected from controls (13.2%), who were referred to a private gastroenterology clinic in Tehran and had normal endoscopy.[56] However, the study subjects might have been from different geographical areas of Iran, and the cases and controls may not be comparable.

In a study in northeastern Iran, HTLV-1 infection was found in only two out of 85 OSCC cases and one out of 48 controls.[57]

Low body mass index: Unlike oesophageal adenocarcinoma and some other cancers, a significant inverse association between elevated body mass index (BMI) and OSCC risk has been reported in a systematic review, which included four prospective and seven case–control studies.[58] However, at least in recent years, BMI in Golestan Province is not low. In the Golestan Cohort Study, the adjusted prevalence rates of overweight (BMI $\geqslant$ 25) and obesity (BMI $\geqslant$ 30) were 62.2% and 28.0%, respectively.[59]

Gluten sensitive enteropathy: This disorder is a predisposing condition for OSCC.[4] However, it is unlikely to have a major

impact on the prevalence of OSCC in Golestan; the reported prevalence for this disorder among 1400 participants in the Golestan Cohort Study was ~1%, which was not different from that in other areas of Iran with low incidence of OSCC.[60]

Poor oral hygiene: Several epidemiologic studies have reported an increased risk of OC or its precursor lesions in those with poor oral hygiene.[11] A study in Linxian showed that teeth brushing was protective and tooth loss was associated with an elevated risk.[61] This has been attributed to alterations in oral bacterial flora and subsequent increases in the *in vivo* production of carcinogens such as NNCs.[61] However, confounding by other factors related to low socioeconomic status (SES) is difficult to exclude.

A study in Golestan Province showed that poor oral health had a dose-response association with the development of squamous dysplasia, the precursor lesion of OSCC.[62] In the Golestan Case–Control study, subjects with no teeth had a nearly 2-fold higher risk of having OSCC compared to subjects with at least 20 remaining teeth. Failure to use any routine oral hygiene practices led to a greater than 2-fold higher risk of OSCC compared to people who brushed their teeth daily.[63]

Gastric atrophy: At least four case–control studies have shown a strong association between gastric atrophy, diagnosed by serological markers or histopathology, and risk of OSCC.[11] The potential increased risk associated with gastric atrophy may be related to overgrowth of bacteria in the stomach which may increase the production of some carcinogens, such as NNCs.[11] No study in Golestan has examined the association between gastric atrophy and OSCC.

Non-steroidal anti-inflammatory drugs: A protective association of these medications against OSCC has been shown in several studies and in a meta-analysis.[64] No data on medication history and OSCC are available from Golestan.

Socioeconomic status (SES): Studies in both low- and high-risk areas of the world have consistently found significant associations between lower SES and higher risk of OSCC.[4] In the 1970s, several indicators of low SES were associated with risk of OC in the Caspian littoral, including the number of years at school, number of living rooms, and number of cows owned at the age of 25.[18] In the Golestan Case–Control Study, education, being married, and wealth were all inversely related to OSCC, and duration of residence in rural areas was associated with an increased risk.[65] In the multivariate analyses, education level of middle school or higher, versus no schooling, was associated with a 5-fold lower risk and being married, versus being widowed/divorced, was associated with a 2-fold decreased risk, but the inverse association between wealth and risk of OSCC became statistically non-significant, mainly as a result of adjustment for vegetable intake. Residence in rural areas for more than 20 years was associated with an approximate 3-fold increased risk. Owning an automobile was also inversely associated with risk of OSCC. This inverse association persisted after adjustment for wealth status, which suggests that owning a car was also a proxy for some other more direct factors, such as providing convenient access to food shopping or enhancing social contacts.[65] The role of SES is compatible with the temporal trend in OSCC in Golestan, which parallels an improvement in socioeconomic conditions in the province.

4. Familial studies

These studies suggest a strong familial component to the etiology of OSCC among people of Turkmen ethnicity.[66,67] In a study in the 1970s, 47% and 15% of 427 OC patients of Turkmen ethnicity reported a positive family history of OC or of other cancers, respectively.[66] In the Golestan Case–Control Study, using detailed family pedigrees of the 167 OSCC cases and 200 controls of Turkmen ethnicity, the cumulative risks of OC in the first-degree relatives of cases and controls were 34% and 14%, respectively (relative risk = 2.3). The cumulative risk of OC in the siblings of the cases was 19% for patients with none or one affected parent, and 46% for patients with two affected parents.[67]

5. Genetic studies

Polymorphisms: A large number of studies, mainly in East-Asian countries, have investigated the association between polymorphisms in numerous genes and risk of OSCC. Most of the results are inconclusive, but certain polymorphisms in the *aldehyde dehydrogenase-2* gene, which determines blood acetaldehyde concentration after alcohol drinking, the *methylenetetrahydrofolate reductase* gene, a central enzyme in folate metabolism, and the *CYP1A1* gene, which is involved in carcinogen activation, have shown a more consistent association with OSCC.[68]

In the Golestan Case–Control Study, a nonsense variant of BRCA2 was associated with higher OSCC risk, with a frequency of 4.6% among cases and 0.8% among controls.[69] Results of an ecologic study that compared the frequencies of polymorphisms in 10 genes that have been hypothesised to have a role in risk of OSCC among Turkmen from Golestan and two other Iranian ethnic groups are shown in Table 2.[70]

Tumor protein 53 (TP53) mutations: The *TP53* tumour suppressor gene is involved in several cellular functions, including control of cell growth, DNA replication, and DNA repair. In addition to endogenous factors, some environmental factors have been reported to induce *TP53* mutations.[71]

The presence of mutations in exons 5 to 8 of the *TP53* gene in OSCC tumour specimens was investigated in two studies in referral hospitals in Tehran and northern Iran. In both studies, more than half of cases had at least one *TP53* mutation, with a high prevalence of C>T transitions, mostly at CpG dinucleotides.[72,73] Elevated levels of cyclooxygenase-2 were observed in ~70% of the samples in both studies.

6. Conclusions

The most important known or possible risk factors of OSCC are summarised in Table 3. In Golestan, tobacco and opium consumption, low intake of fresh fruit and vegetables, drinking hot tea, poor oral hygiene, and low socioeconomic status have thus far been associated with higher OSCC risk, though tobacco use is less prevalent than in Western countries and is therefore not as strong a risk factor as in Western countries. Studies suggest that alcohol consumption and selenium deficiency are not risk factors of OSCC in this area. Golestan

Table 3 – Strength[a] of evidence for the association between select factors and risk of OSCC in Western countries and in high-risk areas of China and Iran

Risk factor	Western countries	Linxian, China	Golestan, Iran
Tobacco smoking	Strong	Probable	Probable
Alcohol consumption	Strong	None	None
Opium consumption	Possible	Possible	Probable
Hot drinks	Possible	Possible	Probable
Nutritional deficiencies			
Low fruit and vegetable intake	Strong	Strong	Probable
Zinc deficiency	Possible	Possible	Possible
Selenium Deficiency	Possible	Probable	None
Water supply	Possible	Possible	Possible
PAHs	Possible	Possible	Possible
N-nitroso compounds	Possible	Possible	Possible
Silica fibres	Possible	Possible	Possible
Mycotoxins	Possible	Possible	Possible
HPV infection	Unclear	Unclear	Possible
Oral Hygiene	Possible	Probable	Probable
Gastric atrophy	Probable	Probable	Possible
Low socioeconomic status	Strong	Strong	Strong
Genetic background	Possible	Possible	Possible

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; *HPV*, *human papillomavirus*.

[a] None: present evidence shows no association; Possible: not enough data but there are some indications of an association; Probable: current evidence favours an association; Strong: there is strong evidence for an association; Unclear: large numbers of studies show conflicting results.

inhabitants, even never-smokers, seem to be highly exposed to PAHs.

In low-incidence areas, OSCC is usually much more common among men than in women. This has been attributed to higher rates of tobacco and alcohol use among men in those populations.[11] We may also expect higher incidence rates of OSCC for men in Golestan, since more men than women use tobacco and opium there, though the consumption rate for tobacco is not as high as in Western countries. More severe vitamin deficiencies among women compared to men, particularly in rural areas, may partly explain why the incidence rates in Golestan are similar in both sexes. More importantly, the similar incidence rates among men and women in high-incidence areas indicate that some common factor(s) are involved in the carcinogenesis of OSCC in those areas. The factor(s) can be environmental, genetic, or both.

The effect of duration of residence in rural versus urban areas on risk of OSCC in the same population and declining incidence rate of OSCC during the past decades, which may be related to changes in lifestyle and improved SES, suggest that environmental factors may be the main cause of high incidence of OSCC in Golestan. Examples of the changes observed during the past few decades include increased access to fresh fruit and vegetables, an increase in access to safe water and in the availability of electricity and refrigeration that may reduce exposure to some food contaminants such as NNCs,[65] an increase in the rate of schooling that may help people to acquire health-related knowledge,[65] improvements in oral hygiene, and improvements in housing conditions and cooking methods that may reduce exposure to PAHs. Agriculture is the mainstay of Golestan Province's economy, and there is little industrial air pollution. High exposure levels to PAHs even among non-smokers in urban areas, where natural gas is now used for heating and cooking, suggest that diet and particularly cooking method can be one of the possible sources of the high exposure. Frying foods, including deep frying, seems to be common in the region. However, the potential association of cooking methods with risk of OSCC needs to be investigated in future studies. Similarly, the possibility of high exposures to NNCs as a result of high intake of nitrate and NNCs, high rates of endogenous formation, and some predisposing conditions such as gastric atrophy warrant further investigations. High prevalence of *TP53* CpG transitions in Iranian OSCC specimens may suggest the possible role of oxidative stress, particularly nitric oxide, in the development of OSCC in Iranian populations. The pattern of a high prevalence of C>T transitions, which may occur as a result of exposure to some potential carcinogens, such as PAHs and NNCs, differs from that reported in some Western countries in which high prevalence of A:T base pair mutations suggests the possible role of acetaldehyde metabolism.[74] This pattern, however, needs to be confirmed in larger studies on OSCC samples from Golestan.

On the other hand, although a few polymorphisms with potential association with higher OSCC risk were more common among Golestan inhabitants than in residents of low-risk areas of Iran, none were extremely prevalent, and most of them were less frequent in Golestan inhabitants than in Japanese a with lower risk of OC. Therefore, they are unlikely to be the main reasons for observing high OSCC rates in Golestan. However, genetic factors may make some individuals more susceptible, to some degree, to the effect of environmental factors. Although the observed familial component among Turkmen may be due to possible effects of shared etiologic environmental factors in a family environment, it may also suggest the combined effect of environmental factors operating on individuals who are already genetically susceptible (gene-environment interactions).

The high incidence of OSCC in Golestan Province may be due to a single strong risk factor. The strongest risk factor that has been found in Golestan, in term of magnitude of association and prevalence of the risk factor, is hot tea drinking. The mechanism by which thermal injury can cause oesophageal cancer is not clear. Some authors have suggested that the inflammatory processes associated with chronic irritation of the oesophageal mucosa by local hyperthermia might stimulate the endogenous formation of reactive nitrogen species, such as nitric oxide, and subsequently, NNCs.[22] On the other hand, and more likely, the high incidence rates may be attributable to the interactions of several risk factors, rather than a single strong risk factor. Thermal injury may also impair the barrier function of the oesophageal epithelium;[22] if so, potential dietary carcinogens, such as PAHs or NNCs, may pass more easily through the epithelium and cause high level of DNA damage in oesophageal cells. In the presence of certain nutritional deficiencies, such as low fruit and vegetable intake, the DNA damage may not be repaired appropriately. Certain genetic factors might augment susceptibility to these carcinogenic processes. Further studies are needed to investigate these interactions.

None of the published studies conducted in Golestan Province have been prospective. Since case–control studies are subject to various biases, the above associations need to be confirmed in the ongoing Golestan Cohort Study.[75] For nutritional studies, a FFQ has been validated for the region within the cohort study.[76] Using the baseline data and data from repeated exposure measurements in long-term follow-ups within the cohort, transitions in the exposure to potential risk factors could also be investigated precisely. Trends in incidence of OSCC and oesophageal adenocarcinoma, as well as their potential relations to transitions in lifestyle, warrant further investigations. In addition, further studies on the association between the following exposures or conditions and OSCC are warranted: nutritional deficiencies other than low fruit and vegetable intake, mycotoxins (using biologic markers of exposure), infections, PAHs (verifying high exposure and finding the source), NNCs (examining the source of endogenous formation), drinking water contaminants, and gastric atrophy. As discussed above, studies on potential carcinogens that can exist in the upper gastrointestinal lumen and may influence the oesophageal epithelium locally, such as PAHs and NNCs, seems to be of particular importance. Examples of such studies could be studies on situations that increase intra-luminal presence of those compounds, and examination of oesophageal tissue samples for PAH-related DNA adduct. Further studies on genetic factors, and their interaction with environmental factors, are also indicated.

Conflict of interest statement

None declared.

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