

High Exposure to Polycyclic Aromatic Hydrocarbons May Contribute to High Risk of Esophageal Cancer in Northeastern Iran

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Abstract. *Background:* The northeastern region of Iran has some of the highest rates of esophageal squamous cell carcinoma (ESCC) in the world. *Materials and Methods:* To investigate the role of polycyclic aromatic hydrocarbons (PAHs) in the etiology of ESCC in northeastern Iran, we measured urine 1-hydroxypyrene glucuronide (1-OHPG), a stable PAH metabolite, in 99 inhabitants of this area. *Results:* The median urine 1-OHPG in participants of this study was 4.2 pmol/ml. Forty-two subjects (42%) had levels ranging from 1 to 5 pmol/ml, indicative of moderate PAH exposure, and 41 (41%) had levels above 5 pmol/ml, indicative of very high exposure. Further analysis showed that 1-OHPG levels were high in all subgroups of our study subjects, including both sexes, rural and urban dwellers, and smokers and non-smokers. Only 15% of the variance in 1-OHPG was explained by age, sex, residence, smoking, nass, or opium consumption. This pattern of PAH exposure parallels the ESCC incidence pattern seen in this area. *Conclusion:* We conclude that people in northeastern Iran are exposed to widespread and very high levels of PAH, largely from unknown sources, and this may contribute to the high rates of ESCC observed in this area.

The northeastern region of Iran, near the coast of the Caspian Sea, has some of the highest rates of esophageal

squamous cell carcinoma (ESCC) in the world, approximately 100/10⁵/year (1-3).

Epidemiological features of ESCC in northeastern Iran are similar to those in other high-risk areas, such as Linxian, China, but are different from what is seen in Western countries (4). In most Western countries, ESCC has a low incidence rate, is much more common in men than in women, and is mainly (> 90%) attributed to high tobacco and alcohol consumption (4, 5). In contrast, in northeastern Iran and in Linxian, ESCC has a very high incidence, is nearly equally distributed among men and women, and is only minimally related to smoking or alcohol consumption (4, 6-8).

The etiological reasons for such high rates of ESCC in northeastern Iran and Linxian are only partially recognized. In the 1970s, the International Agency for Research on Cancer (IARC) and the Tehran University Institute of Public Health Research (IPHR) conducted a series of ecological and nutritional studies to investigate the epidemiological features and etiology of ESCC throughout the Caspian Littoral, including Golestan Province. These studies found geographical associations of incidence with a number of variables (9). A subsequent case-control study investigated these associations further and established some risk factors, especially poverty and a restricted diet very low in fresh fruit and vegetables (6), factors that have since been shown to be associated with an elevated risk for esophageal cancer in almost all countries where diet has been studied (4). These risk factors, however, can only partially explain the very high incidence of ESCC observed in northeastern Iran, and further investigation to identify other risk factors is warranted.

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Table I. Comparing 1-hydroxyglucuronide levels in the study subgroups.

	Median 1-OHPG (20th- 80th percentiles) pmol/ml	Mean Log 1-OHPG (\pm SD)*	Unadjusted Mean Difference	<i>p</i> -value for unadjusted analysis**	Adjusted *** Mean Difference (\pm SE)	<i>p</i> -value for adjusted analysis**** (\pm SE)
Age						
< 55 years (n=50)	5.0 (2.1-11.6)	1.4 (\pm 1.5)	0.8 (\pm 0.3)	0.02	0.7 (\pm 0.4)	0.07
\geq 55 years (n=49)	2.4 (0.7-7.2)	0.6 (\pm 1.9)				
Sex						
male (n=71)	4.8 (1.5-11.5)	1.2 (\pm 1.8)	0.6 (\pm 0.4)	0.15	0.4 (\pm 0.4)	0.35
female (n=28)	2.4 (0.7-5.5)	0.6 (\pm 1.6)				
Residence						
urban (n=37)	5.1 (1.4-12.8)	1.4 (\pm 1.4)	0.6 (\pm 0.3)	0.06	0.7 (\pm 0.4)	0.08
rural (n=62)	3.8 (1.0-8.9)	0.8 (\pm 1.9)				
Smoking status						
current smoker (n=30)	6.9 (1.7-10.6)	1.4 (\pm 1.6)	0.6 (\pm 0.4)	0.12	0.1 (\pm 0.5)	0.92
former smoker (n=20)	2.7 (0.9-16.1)	1.0 (\pm 2.1)	0.2 (\pm 0.5)	0.65	0.3 (\pm 0.5)	0.51
never smokers (n=49)	3.7 (1.1-7.2)	0.8 (\pm 1.7)	baseline		baseline	
Nass consumption						
users (n=12)	6.8 (0.9-14.7)	1.2 (\pm 2.2)	0.2 (\pm 0.7)	0.76	0.7 (\pm 0.6)	0.26
non-users (n=87)	4.0 (1.3-8.8)	1.0 (\pm 1.7)				
Opium use						
current users (n=16)	8.9 (2.0-20.2)	1.6 (\pm 2.1)	0.6 (\pm 0.5)	0.25	0.4 (\pm 0.5)	0.44
former users (n=6)	2.1 (0.1-4.4)	-0.3(\pm 2.0)	-1.3(\pm 0.9)	0.20	-1.3(\pm 0.8)	0.13
never users (n=77)	4.0 (1.3-8.8)	1.0 (\pm 1.6)	baseline		baseline	

* Each unit of Log 1-OHPG is equal to (80th percentile – 20th percentile)/2.

** *P*-values were calculated using *t*-tests.

*** Adjusted for other factors in this table (age, sex, residence, smoking, nass consumption, opium use)

**** *P*-values were calculated using Wald tests.

Polycyclic aromatic hydrocarbons (PAHs) are environmental carcinogens produced during incomplete combustion of organic materials, including tobacco, which have been shown to be etiologically associated with upper aerodigestive cancers, including esophageal cancer (10). Previous studies in Linxian have shown histopathological evidence consistent with high exposure to PAHs in ESCC cases (11), presence of high levels of carcinogenic PAHs in staple foods (12), high concentrations of 1-hydroxypyrene glucuronide (1-OHPG), a PAH metabolite, in urine samples (10), and significantly positive nuclear PAH-DNA adducts in esophageal tissue samples (13). Due to the epidemiological similarities of ESCC between Linxian and northeastern Iran, we hypothesized that one possible reason for high ESCC incidence in Golestan may be high exposure to PAHs from sources other than tobacco use.

As a preliminary step to investigate the possible contribution of PAHs to the high risk of ESCC in

northeastern Iran, we measured urine 1-OHPG in selected participants of a recent pilot for a cohort study in this area. 1-OHPG is a stable PAH metabolite that reflects recent (within the past 24 hours) exposure to mixed PAHs (10). We used questionnaire data to assess tobacco consumption, including the smoking of cigarettes and the chewing of nass (a mixture of tobacco, lime and ash), and opium use. In addition, we measured urine cotinine to validate questionnaire responses about tobacco consumption.

Materials and Methods

Subjects for the pilot study, ages 35-75 years old, were selected from Gonbad city in Golestan Province and the three surrounding villages. In all, 645 individuals from Gonbad City and 682 individuals from the three surrounding villages were invited to take part in the pilot study, of whom 438 individuals (68%) from Gonbad City and 619 individuals (91%) from the three villages agreed to participate. For this study, a sample of 100 pilot participants, over-

sampled for current (n=30) and former (n=20) cigarette smokers, were selected. Spot urine samples from these 100 participants were shipped to the Johns Hopkins University, USA, for measurement of 1-OHPG levels, using immunoaffinity chromatography and synchronous fluorescence spectroscopy (10,14).

Tobacco smoking or chewing (in the form of nass) or consumption of Sukhteh (pyrolyzed opium) are known sources of PAH exposure. Therefore, we investigated use of tobacco or opium in the study subjects to evaluate the effect of these exposures on the urine 1-OHPG levels. During the pilot study, a trained local doctor used a structured questionnaire to ask all participants about their demographic characteristics, tobacco use, including the smoking of cigarettes and the chewing of nass, and opium use. To evaluate the validity of the questionnaire responses related to tobacco use, we also measured urine cotinine using NicoMeter® strips (Jant Pharmaceutical Corp., Encino, CA, USA).

The study was approved by the Institutional Review Boards of the US National Cancer Institute, the Digestive Disease Research Center, and the International Agency for Research on Cancer.

Results

Urine samples were adequate for 1-OHPG analysis in 99 subjects and for cotinine analysis in 96 subjects. The median 1-OHPG for all study participants was 4.2 pmol/ml, and the 20th and 80th percentiles were 1.3 and 10.4 pmol/ml, respectively. Forty-two subjects (42%) had urinary 1-OHPG levels ranging from 1 to 5 pmol/ml, indicative of moderate PAH exposure typically observed in smokers, and 41 (41%) had levels above 5 pmol/ml, indicative of high exposure comparable to steel blast furnace or coke oven workers who also smoke (14). Extremely high values of 1-OHPG (as high as 85.7 pmol/ml) were observed. Urine cotinine was positive in 46% of our subjects and showed a high correlation with self-reported tobacco use ($\kappa=0.72$). Our previous work also showed that urine opiate metabolites and self-reported opium use were highly correlated in this same group of subjects ($\kappa=0.83$) (15). Thus, we used questionnaire data on tobacco and opium use for further analyses.

We compared 1-OHPG levels in subgroups of our study participants, including men *versus* women, older (≥ 55 years) *versus* younger (< 55 years) subjects, urban *versus* rural subjects, current and former smokers *versus* never-smokers, nass users *versus* non-users, and current and former opium users *versus* never-users (Table I). The median 1-OHPG was high in all of the subgroups of this study, showing widespread exposure to PAHs in Gonbad and its surrounding area. Even in subjects who did not report using tobacco or opium in any form, mean and median 1-OHPG were 4.7 and 2.8 pmol/ml, respectively, suggesting that high PAH exposure in this area is due to other factors (*e.g.*, dietary factors) not measured in our study. Since the distribution of 1-OHPG was highly skewed, we used the natural logarithm of 1-OHPG (Log 1-OHPG) to test for significant differences in 1-OHPG levels among subgroups (Table I). One unit change in Log 1-

OHPG was approximately equal to half the difference between the 80th and 20th percentiles. Although there were some differences in mean Log 1-OHPG among subgroups of the study, none of the differences were significant after adjusting for age, sex, residence, smoking, nass consumption, and opium use. A linear combination of age, sex, residence, smoking, nass consumption, and opium use explained only 15% of the variance observed in urine 1-OHPG levels (Pearson's $r^2=0.15$).

Discussion

A cancer registry maintained by the IARC and IPHR in the 1970s (2) and a more recent study (3) both showed that Golestan Province in northeastern Iran has very high rates of ESCC. Since smoking in this area is uncommon (10% of the total population) (16) and alcohol consumption is rare, it seems likely that this population's high ESCC rates are attributable to other factors. The IARC and IPHR studies established low socioeconomic status and low intake of fresh fruits and vegetables as risk factors for ESCC in this area (6). However, these risk factors cannot fully explain the very high risk of ESCC observed in this area, and the reasons for such high rates are still not completely understood. The similarity of epidemiological features of ESCC in Golestan and Linxian, another area of the world with very high rates of ESCC, and the results of recent studies showing high PAH exposure in the inhabitants of Linxian, encouraged us to study PAH exposure in the residents in Gonbad, a large city in Golestan, and surrounding villages.

In all, 83% of our study subjects showed evidence of moderate (42%) or high (41%) exposure to PAHs. The median PAH exposure in all of our subjects (4.2 pmol/ml) was comparable to levels seen in steel blast furnace workers who also smoke (14). These levels are much higher than those seen in populations who are at low risk of ESCC. For example, mean 1-OHPG concentrations in 40 Koreans (20 smokers and 20 non-smokers) and 10 Americans (all non-smokers) who worked in offices were 0.38 and 0.23 pmol/ml, respectively (14,17).

PAH exposure was high in all subgroups of the study, including both sexes, older and younger subjects, rural and urban dwellers, and smokers and non-smokers. These findings show that PAH exposure in Gonbad and its surrounding villages is high and widespread. Interestingly, this pattern of PAH exposure parallels that of ESCC incidence, which is also common in both sexes, in both urban and rural areas, and in smokers and non-smokers (2,8). Age, sex, residence, smoking, nass consumption, and opium use explained only 15% of the variance observed in urine 1-OHPG levels. Thus, smoking and opium consumption are unlikely to be the main sources of PAH exposure in this area. Studies in the 1970s showed low levels

of PAHs in staple food samples from Golestan (6,18). Thus, food is possibly not the main source of PAH exposure in Golestan Province. The most important sources of exposure are currently unknown.

We conclude that the people of Gonbad and its surrounding villages are exposed to widespread and very high levels of PAH, which may contribute to the high rates of ESCC observed in this area. This high PAH exposure is only partially explained by smoking, nass, or opium use, and further investigation is needed to find other sources of PAH exposure in this area.

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