**Background**

Hookah smoking and cancer risk. Historically, hookah smoking has been prevalent in North Africa and West and Central Asia, and a small number of studies have been published on health outcomes of hookah smoking from these regions [Akl et al., 2010; Raad et al., 2011]. Hookah smoke contains many toxic compounds found in cigarette smoke [Eissenberg & Shihadeh, 2009; Monzer et al., 2008; Ghasemi et al., 2010], and hookah smokers appear to be exposed to the same agents as cigarette smokers, probably at higher levels: each puff from hookah has been reported to deliver 12-times as much smoke as a single cigarette puff [Eissenberg & Shihadeh, 2009].

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| **Table 1**. Epidemiologic studies of hookah smoking and risk of selected cancers |
| Study | Country | Type of controls | N ca/co | N exp ca/co | OR | 95% CI |
| **Lung cancer** |  |  |  |  |  |  |
| Gupta et al., 2001 | India | HV | 265/525 | 12/31 | 2.56 | 1.17-5.61 |
| Koul et al., 2011 | India | R | 251/500 | 120/100 | 5.83 | 3.95-8.61 |
| Aoun et al., 2013 | Lebanon | HV | 50/500 | 10/4 | 6.00 | 1.78-20.26 |
| *Meta-analysis* |  |  |  |  | *4.58* | *2.61-8.03* |
| **Esophageal cancer** |  |  |  |  |  |  |
| Nasrollahzadeh et al., 2008 | Iran | HP | 300/571 | 20/23 | 1.70 | 0.92-3.15 |
| Malik et al., 2010 | India | HV | 135/195 | 106/38 | 15.1 | 8.78-25.98 |
| Khan et al., 2011 | India | Pop | 100/100 | 66/15 | 9.11 | 4.44-18.72 |
| Dar et al., 2012 | India | HP | 700/1663 | 420/699 | 2.02 | 1.69-2.42 |
| Shakeri et al., 2012 | Iran | HP | 30/260 | 11/17 | 1.32 | 0.60-2.91 |
| *Meta-analysis* |  |  |  |  | *3.63* | *1.39-9.44* |
| **Head and neck cancer** |  |  |  |  |  |  |
| Khlifi et al., 2013 | Tunisia | HP | 169/351 | 40/37 | 2.63 | 1.61-4.30 |
| **Bladder cancer** |  |  |  |  |  |  |
| Bedwani et al., 1997 | Egypt | HP | 309/613 | 12/24 | 2.00 | 0.81-4.96 |
| OR, odds ratio; CI, confidence interval; HV, hospital visitors; R, relatives; Pop, population-based controls; HP, hospital patients |

Consistent with above findings, results from limited available epide-miological studies condu-cted in populations where hookah smoking has been prevalent for decades indicate harmful health effects. Current evidence suggests an association between hookah use and respi-ratory diseases, low birth-weight, and periodontal disease [Akl et al., 2010]. In addi-tion, some data suggest that hookah smoking may increase the risk of spreading infectious diseases, such as tuberculosis, hepatitis and herpes [Urkin et al., 2006; Knishkowy & Amitai, 2005]. Associations between hookah smoking and cancers of the head and neck, esophagus, lung and bladder cancer have been reported in a few studies (**Table 1**): the results are stronger for esophageal cancer. In general, however, these studies suffer from limitations including (i) suboptimal choice of controls (e.g., relatives), (ii) small number of exposed cases and controls (e.g., no more than 20 exposed cases in 5 of the available studies), (iii) inclusion of cigarette smokers with hookah smokers, (iv) lack of results (in most studies) on duration or amount of hookah smoking.

Carcinogens in hookah smoke and in biological samples of hookah smokers. Tobacco smoke contains a large number of carcinogen compounds including polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs), such as acrolein, crotonaldehyde, acrylonitrile, benzene and propylene oxide. PAHs are ubiquitous environmental contaminants formed in all processes involving incomplete combustion of organic matter [ECSCF, 2002], and occur in mixtures that include highly carcinogenic compounds, such as benzo[*a*]pyrene, as well as weakly or non-carcinogenic compounds, such as phenanthrene [ECSCF, 2002]. VOCs are also combustion products that are present in tobacco smoke [Stevens & Maier, 2008]. Benzene, benzo[*a*]pyrene, and other mixtures of PAHs are considered carcinogenic to humans [IARC, 2012]; acrolein, acrylonitrile, crotonaldehyde and propylene oxide are suspected human carcinogens. These agents, however, have mainly been studied in smoke derived from cigarettes, while their presence in smoke from other tobacco products, including hookah, are limited, although a role for them is highly suspected [Schubert et al., 2015].

Whereas similar nicotine levels in plasma have been reported following hookah and cigarette smoking, carboxyhemoglobin levels were three-fold higher after hookah than after cigarette smoking [Eissenberg &.Shihadeh, 2009]. Carbon monoxide levels following hookah smoking are similarly higher than after cigarette smoking, as the majority of the excess carbon monoxide comes from the burning charcoal [Jacob et al., 2011]. In a small clinical study of hookah smokers, Jacob et al. [2011] found increased urinary concentrations of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL), a biomarker of exposure to the tobacco-specific nitrosamine (TSNA) and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and some PAH metabolites. Two other small studies have also reported total NNAL levels in the urine of hookah smokers [Schubert et al., 2011; Ghada et al., 2013]. No data are available on exposure of hookah smokers to other carcinogens present in tobacco smoke, such as VOCs.

Increasing prevalence of hookah smoking globally. Following a reduction in prevalence of hookah smoking through most of the 20th century in areas where the habit has historically occurred, including West Asia, its popularity has risen again among youth from these regions since the 1990s [Rastam et al, 2004]. Surveys have reported current hookah smoking prevalence of over 25% in university students [e.g., Jawaid et al., 2008; Ghafouri et al., 2011], while the use is less prevalent among the older individuals. Recently, a high prevalence of hookah smoking has been reported among young people from areas where hookah use has not been a longstanding popular habit, including Canada [Chan et al., 2011] and United Kingdom [Jackson et al., 2008]. Past data on hookah smoking prevalence from these regions have been limited, probably due to scarcity of this habit, which generally hinders the ability to make valid estimations regarding the increase in use over time. However, results from one recent international survey on time trends of tobacco use during 1999–2008 conducted among more than half a million 13–15 year-old in 95 countries support an increase in hookah use globally. While cigarette smoking was either stable or declining during that decade, prevalence of hookah smoking showed a rising trend [Warren et al., 2009]. An estimated 100 million people regularly smoke hookahs worldwide [Ward et al., 2005].

**Study Population**

The proposed research will efficiently use unique resources to study of health effects of hookah smoking: the IROPICAN case-control study.

IROPICAN Study. The Iranian Study of Opium and Cancer (IROPICAN) is a multi-center case-control study started in 2017 in four provinces of Iran (Tehran, Kerman, Fars and Golestan) to evaluate the association between behavioral risk factors, including opium and tobacco consumption, and risk of cancers of the lung, colorectum, bladder, and head and neck. The goal is to recruit 800 cases for each cancer type, and a matched group of 800 controls from each cancer site, while collecting detailed data to assess dose-response analyses for opium and tobacco use, controlling for potential confounders, and careful evaluation of reverse causality. Patients with one of four types of cancer admitted to the cancer hospitals in the study areas, are recruited as cases. Controls are recruited controls among visitors (relatives or friends) to patients treated at referral hospitals and clinics in each province for chronic diseases other than cancer. This approach was shown to increase response rate, while maintaining a validity in the exposure assessment [Mendonça & Eluf-Neto, 2001]. During 2017, a pilot study was conducted, which included 185 cases and 176 controls. The prevalence of opium use among controls was 19.3% [Hadji et al., submitted], which was close to previously reported data from the general population (14.4%) [Nakhaee et al., 2008]; that of hookah smoking was 21.5%. The validity of responses to questions regarding opium use (a potential source of report bias) was assessed by testing opium metabolites in the urine. The sensitivity of self-report in response to the questionnaire among controls was 69% [Hadji et al., submitted].

A detailed questionnaire, adapted from the Golestan Cohort Study [Pourshams et al., 2010], is administered to all cases and controls, which includes questions on age, ethnicity, education, rural/urban status, occupational history, socioeconomic status (assessed by ownership score [Islami et al., 2009]), physical activity (using the international physical activity questionnaire [Vasheghani et al., 2011]), oral health, female reproductive history, history of disease, personal and family history of cancer, tobacco consumption, including cigarette, hookah, pipe, naswar (a chewing product), and chopogh (a special pipe), lifelong alcohol consumption, opiate use and use of other drugs (i.e., diphnoxiylate, methadone, codeine, etc.), use of opioids including non-medical morphine, raw opium, shireh (i.e. a refined condensed extract of remnants of smoked opium) [Amin‐Esmaeili et al., 2016], sukhteh (remnants of smoked opium), and other drugs. A validated and reproducible 125-item food frequency questionnaire [Nematy et al., 2014], similar to that used in the Golestan Cohort Study [Malekshah et al., 2006] is used to collect dietary information. All cases and controls undergo a physical exam— including standardized measurements of height, weight, blood pressure— performed by trained personnel. Blood and saliva samples are collected from each cancer case and controls; samples are aliquoted and stored at the Cancer Research Center of the Cancer Institute of Iran (CII) at -80C. The PI of this proposal, P. Boffetta, is a co-investigator of the IROPICAN study.

**Research plan**

The proposed research has the Overarching Goal to elucidate the carcinogenicity of hookah smoking in humans, through this Specific Aim:. To conduct a detailed analysis on the risk of cancer from hookah smoking in a multicenter case-control study from Iran. Specifically, we will test the hypothesis that hookah smoking increases the risk of head and neck, colorectal, lung and bladder cancer. In addition, we will explore the combined effect of cigarette and hookah smoking and the interaction between hookah smoking and opium use, in determining the risk of these cancers.

Recruitment of study subjects. The methodology for recruitment of cases and controls in the IROPICAN study was described under ‘Study Populations’ above. Recruitment is currently on going: a total of 200 cases of each type of cancer and a comparable number of matched controls are expected to be recruited by June 2019, based on current funding from local sources. The final goal (800 case-control pairs for each type of cancer, corresponding to a total of 6,400 study subjects) will be reached with an additional 3 year recruitment (200 cases of each type of cancer and matched controls per year), subject to funding of the proposed research.

Data management. Baseline data from the IROPICAN Study will be transferred to the Department of Medical and Surgical Sciences of the University of Bologna. Only de-identified dataset will be shared between the partnering institutions. Data will be checked for internal consistency; potential mistakes will be clarified with the parent institution (CII).

Statistical analysis. The first step of the statistical analysis will consist of a series of cross sectional analyses among controls to identify correlates of hookah smoking to be included as potential confounders: a preliminary list includes ethnicity, urban residence, socio-economic status, tobacco smoking, opium use, family history of cancer, as well as alcohol drinking (for head and neck cancer), body mass index (for colorectal cancer). A liberal significance level (e.g., p<0.15) will be used. Subsequently, we will test the hypothesis that hookah smoking is associated with higher risk of developing each of the four cancers included in the study by fitting conditional logistic regression models to calculate ORs and 95% CIs. Potential confounding will be assessed by adjusting for the factors identified as described above, in addition to sex, age and study center that are included as matching factors. In secondary analyses, we will consider the effect of amount and duration of hookah smoking by fitting categorical (e.g., tertiles of exposure distribution among controls) and continuous (linear) variables. We will also explore the presence of a non-linear relation with cancer risk by adding non-linear terms (splines) to the regression models, In additional exploratory analyses, we will consider cancer subtypes (i.e., histologic types of lung cancer, subsites within the head and neck and the colorectum), and a possible effect modification by gender, age, and cigarette and opium smoking. Specific attention will be given to the analysis of the interaction between hookah and cigarette smoking, since dual users represent the majority of hookah smokers in high-income countries.

Sensitivity analysis of regression dilution. The availability of results on biomarkers of tobacco exposure from Specific Aim 2 will allow conducting a sensitivity analysis aimed at testing the hypothesis that adjustment for regression dilution [Clarke et al., 1999] will result in a stronger association between hookah smoking and cancer risk than that based on self-reported exposure alone. Specifically, we will estimate in subjects included in Aim 2 the correlation coefficient between self-reported hookah smoking and urinary cotinine level. The resulting reliability coefficient will be used to correct for regression dilution the risk estimates obtained in Aim 1 [Clarke et al., 1999].

Statistical power. The analysis of each cancer site will be based on 800 cases and 800 controls and will have >80% statistical power to detect as statistically significant (at α=0.05) an OR of 1.35 for current hookah smoking assuming a prevalence of 20% among controls. This OR is substantially lower than those detected in previous studies of cancer risk from hookah smoking (**Table 1**), and is comparable to the risk of colorectal cancer from cigarette smoking [Liang et al., 2009], the cancer included in the analysis with the weakest association with cigarette smoking (no results on colorectal cancer from hookah smoking are available in the literature). The analysis of the interaction between cigarette smoking and hookah smoking will be conducted in the combined series of 3200 case-control pairs. Assuming an OR of 3 for cigarette smoking and 2 for hookah smoking, with respective prevalence of 40% and 20%, the analysis will have 80% to detect an interaction OR in the order of 1.25. This interaction OR is substantially lower than that detected for the interaction between cigarette smoking and other carcinogens, such as alcohol drinking for head and neck cancer [Hashibe et al., 2009].

**Fellow’s Tasks**

Fellow will work in the management of data to be acquired from the IROPICAN Study and in the analysis of the data, in collaboration with the data provider. They will also collaborate with other partners involved in the Hookah and Cancer Risk Project, including laboratories for biomarker analysis, Specifically, they will be engaged in conducting descriptive and analytical statistical analyses on cancer risk from hookah smoking using standard epidemiology methods (e.g., multivariable logistic and Cox regression) under SAS, STATA or R