Molecular Screening for PTEN Gene Mutation Among Cigarette Smokers and Assessment of Knowledge of the Link Between Tobacco Use, and Cancer Among Saudi Population

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ABSTRACT

Tobacco use accounts for at least 30% of all cancer deaths, and 87% of lung cancer deaths. Therefore, the aim of this study was to screen for Phosphatase and Tensin homolog (PTEN) gene mutation among cigarette smokers and to assess population awareness towards tobacco carcinogenicity among Saudi population. This is a cross-sectional survey involved 2099 Saudi civilians living in Northern Kingdom of Saudi Arabia (KSA). PTEN gene mutation molecular detection was performed for 100 tobacco users, DNA extracted from buccal cells. Out of the 2099, participants, 669/2099(31.8%) were smokers and 1430/2099 (68.2%) were non-smokers. Data regarding smoking type and status were available for 399 cases of whom, 229/399 (57.4%) were currently cigarette smokers, 36/399 (9.2%) were currently shisha smokers and 134/399 (33.6%) were ex-smokers. PTEN gene mutations were identified among two current smokers. Cigarette smoking is a public concern in KSA, which requires control strategies. There is no significant correlation between PTEN gene mutation and cigarette smoking.

Keywords: PTEN gene mutation, Cigarette smokers, Cancer, Saudi population

ÖZET

Suudi Populasyonunda Sigara içenlerde PTEN gen mutasyonu Moleküler Taraması ve Tütün Kullanımı ile Kanser Arasındaki Bağlantının Değerlendirilmesi

Tütün kullanımı, tüm kanser ölümlerinin en az %30, ve akciğer kanseri ölümlerinin %87'sinden sorumludur. Bu çalışmanın amacı, sigara içenlerde Fosfataz ve Tensin homolog (PTEN) gen mutasyonlarının taranması ve Suudi populasyonda tütün karsinojenisitesine yönelik farkındalığın değerlendirilmesidir. Bu, Suudi Arabistan Krallığı'nın kuzeyinde yaşayan 2099 kişinin katıldığı kesitsel bir araştırmadır. Bukkal hücrelerden DNA ekstrakte edilen 100 tütün kullanıcısında PTEN gen mutasyonu moleküler tespiti yapıldı. 2099 katılımcıdan 669 (%31.8)'u sigara içenlerden ve 1430 (%68.2)'u sigara içmeyenlerden oluşmaktaydı. Sigara içme tipi ve durumu ile ilgili bilgi bulunan ve 399 kişinin 229 (%57.4)'u halen sigara içmekteydi, 36 (%9.2)'sı nargile içmekteydi ve 134 (%33.6)'ü sigarayı bırakmıştı. PTEN gen mutasyonları, halen sigara içmekte olan iki kişide tespit edildi. Sigara kullanımı Suudi Arabistan'da önemli halk sağlığı sorunudur ve kontrol stratejileri gerektirmektedir. PTEN gen mutasyonu ve sigara kullanımı arasında anlamlı bir korelasyon saptanmamıştır.

Anahtar Kelimeler: PTEN gen mutasyonu, Sigara içenler, Kanser, Suudi populasyonu

INTRODUCTION

Cigarette smoking is a global health concern causing diverse diseases including cardiovascular disease and various cancers comprising those of the respiratory, digestive, and genitourinary systems, etc.[1,2] Tobacco use is globally influenced socioeconomic factors.[3,4] In the developed world, a significant opposite association between socioeconomic status and smoking habit occurs. The poorest and minimally educated individuals are more likely to smoke than others.[5]

Worldwide, the prevalence rates of cancer greatly varies, according to the presence of a certain risk factors, such as environmental exposure to carcinogens and the life style adopted. It is well-known that, more than 30% of cancer deaths could be prevented by modifying or escaping key risk factors, comprising; tobacco use, obesity, unhealthy diet, lack of physical activity, alcoholic abuse, urban air pollution, and indoor smoke from household use of solid fuels.[6,2]

Raise of cancer awareness has prospective impacts on cancer control. Knowledge of potential carcinogenic has great contribution in reduction or prevention of exposure to carcinogens including tobacco use.[7]

Phosphatase and Tensin homolog (PTEN) gene found on chromosome TEN, is a tumor suppressor gene mutated in a variety of human cancers including oral cancer.[8] PTEN expression has been down regulated in many malignancies including oral carcinoma.[9] Loss of PTEN expression is significantly correlated with oral cancer staging, suggesting its significant role in oral cancer development and progression.[8] Therefore, in this study beside the assessment of awareness levels and cigarette smoking habits among Saudi population, the current study investigated the presence of PTEN gene mutation, as cigarette smoking is one of the risk factors for the development of oral precancerous and cancerous lesions.[10]

MATERIALS AND METHODS

In this community-based, cross-sectional study, data were collected during cancer awareness campaign which was conducted in Hai'l Region, Kingdom of Saudi Arabi (KSA), during the period from October 2014 to January 2015. People were assembled in general public entities (University colleges, Health centers, Community gatherings), then were invited to fill a questionnaire about tobacco habits and other information regarding their attitudes towards the relationship between tobacco use and cancer.

Buccal cells were collected by scraping buccal mucosa for subsequence molecular analysis of PTEN gene mutation. PTEN gene mutation was demonstrated using Polymerase Chain Reaction (PCR).

DNA Extraction

Cell's DNA Extraction Steps

Cell lysis: Buccal scraped cells were mixed in preservation solution and placed in a water bath for one hour at 50C. Then 4ml of a mixture (2 ml cells plus 2 ml storage solution) was placed in a 15 ml centrifuge tube. One ml of lysis buffer was added, as well as 10 ul RNaseA solution (10mg/ml). Then incubated at room temperature for 10 minutes then exposed for high-speed vortexing for 10 seconds. Then 1.7 ml was added to the above solution (impurity precipitation lysis mixture). Vortex at high speed 25 seconds. Centrifuged at 8,000 xg for 5 minutes. Impurities and protein precipitation in the bottom of the tube formed a dense precipitate. Less dense protein precipitation was placed on ice for 5 minutes, then step 4 was repeat.

DNA Precipitation

Carefully the supernatant (containing DNA) was transferred to a new 15 ml centrifuge tube. Careful was paid not to touch the bottom of the tube precipitation. Five ml of isopropanol was added and mixed gently inverted 50 times (sometimes the precipitation were seen). Centrifugation at 2.500 RPM was performed for 3 minutes, this time in the bottom of the tube the white precipitate DNA was seen. Supernatant was discarded and up sided down on absorbent paper to dry as possible. Adding 5 ml 70% ethanol, the DNA pellet was rinsed several times upside down. Then was centrifuged at 2.500 RPM for 1 minute, the supernatant was carefully decanted. After tapping on absorbent paper upside down a few times in order to control dry residual ethanol.

DNA Dissolved Hydration

Adding 250 μl-400 μl DNA lysate rehydrated dissolved DNA precipitation, then mixed and placed at 65^{0}C and incubated for 30-60 minutes, then at room temperature or overnight at 4^{0}C to be rehydrated DNA, the intermediate wall from time to time to help flick rehydrated DNA. DNA for storage of at 2-8^{0}C.

The program used for amplification at the thermal cycler (Eppendorf - Master cycler) was: a one-step cycle of pre-denaturation at 94^{0}C for five minutes; 30 three-step cycles – denaturation at 94^{0}C for 30 seconds, annealing at 55^{0}C for 30 seconds and elongation at 72^{0}C for a minute; and an additional 5-minute cycle of elongation at 72^{0}C. The purification success, a total of 5 ul of the purified samples were visualized in 2% agarose gel.

DNA Quantification

To evaluate the DNA quantification after DNA extraction, we had analyzed DNA quantity using a NanoDrop spectrophotometer.

PTEN was detected by PCR using: forward: ACCTGTTAAGTTTGTATGCAAC; Reverse: CTTTCCAGCTTTACAGTGAA

Statistical Analysis

Statistical analysis was performed by proportion. The SPSS software (version 16) was used for statistical analysis.

Ethical consent: A written informed consent was acquired from each respondent, ensuring strict anonymity. This in addition to the fact that, the author followed the tenants of the Declaration of Helsinki.

RESULTS

This study investigated 2099 participants of whom 669/2099(31.8%) were smokers and 1430/2099 (68.2%) were non-smokers, but living with smoker (passive smokers). Of the 2099, 1408/2099(67%) were males and 691/2099(33%) were females, giving males' females' ratio of 2.04: 1.00. Data regarding smoking type and status were available for 399 cases of whom 363/399 (90.8%) were cigarette smokers and the remaining 36/399 (9.2%) were shisha smokers. Of the 363 cigarette smokers, 229/636 (63.1%) were current smokers and 134/363 (36.9%) were ex-cigarette smokers, as indicated in Figure 1. Of the 229 current smokers, 104/229 (45.4%) were males and 125/229 (54.6%) were females.

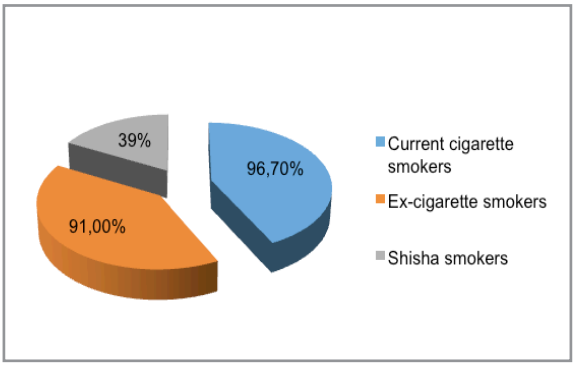


Figure 1. Description of the study subjects by smoking type and status

Out of the 669 smokers, 647/669 (96.7%) were males and 22/669 (3.3%) were females. Of the 134 persons who quit smoking, 122/134 (91%) were males and 12/134 (9%) were females. Of the 1430 individuals living with smokers, 761/1430 (53.2%) were males and 669/1430 (46.8%) were females. Of the 36 shish smokers, 14/36 (38.9%) were males and 22/36 (61.1%) were females, as indicated in Table 1, Figure 2.

Regarding the duration of the smoking, data were available for 415 individuals. The great majority of smokers smoked for duration of less than 5 years, constituting 207/415 (49.9%), followed by 6-15 years, 16-30 years and more than 31, representing 156/415 (37.6%), 49/415 (11.8%) and 3/415 (0.7%), as indicated in Figure 3.

When asking them whether cigarette smoking can cause cancer, 322 of the smokers have answered this question of whom, 249/322 (77.3%) believe that smoking can cause cancer and the remaining 73/322 (22.7%) have declared that there is no relation between smoking and cancer.

Of the 100 cigarette smokers investigated for PTEN gene, PTEN gene mutations were identified among two (2%) current smokers.

Table 1. Distribution of the study population by smoking status and type.

Variable	Males	Females	Total
Smokers	647	22	669
Current cigarette smokers	104	125	229
Ex-smokers	122	12	134
Shish smokers	14	22	36
Passive smokers	761	669	1430

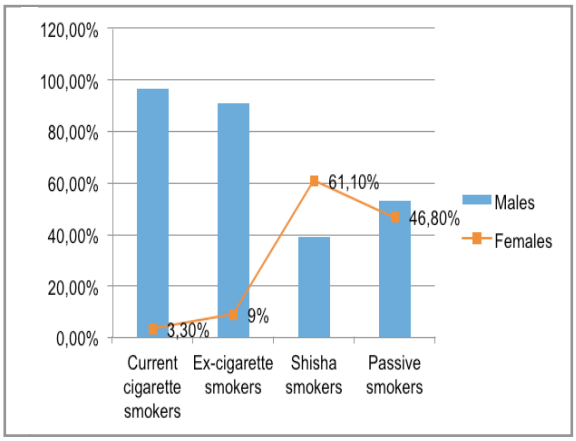


Figure 2. Description of the study population by smoking status and type.

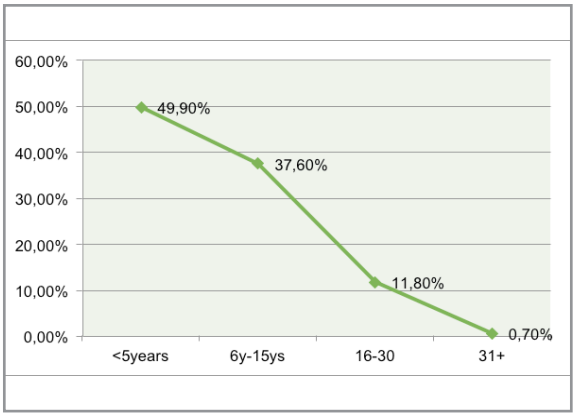


Figure 3. Description of the smokers by duration of smoking in years.

DISCUSSION

There are sufficient evidences for the strong association between cigarette smoking and various cancers, including those of the nasal cavities and paranasal sinuses, nasopharynx and oral.[11,12] Assessment of cancer awareness level among a particular community setting is important step toward cancer prevention and control. Such initiatives give the policy makers and health care providers a chance to understand the importance of the interventions and do what is possible to get the desired outcomes. However, this study has three dimensions; firstly, assessed the burden of tobacco smoking among Northern Saudi population; secondly evaluated the level of awareness toward tobacco carcinogens; thirdly investigated the possibility of PTEN gene mutation as an indicator for early carcinogenesis particularly in oral.

Regarding the issue of tobacco use, 31.8% were found smokers with males more than females. However, similar findings have been previously reported from different part of Saudi Arabia. A review in this context has collected a useful literature on the epidemiology, consumption, trade, control, prevention, and treatment of tobacco smoking in Saudi Arabia. The prevalence of smoking in Saudi Arabia was found ranging between 2.4 and 52.3% (median= 17.5%). Among school students, the prevalence rates ranged from 12 to 29.8% (median= 16.5%), among university students from 2.4 to 37% (median= 13.5%), and among adults between 11.6 and 52.3% (median= 22.6%) and 25% in elderly individuals. The prevalence of smoking in males ranged from 13 to 38% (median= 26.5%), whereas, in females it ranged from 1 to 16% (median= 9%).[13] However, some of the reported prevalence rates were relatively lower than the findings in this study. In a study investigated the status of tobacco use among middle schools students in Jeddah, Saudi Arabia and the factors leading to tobacco use, the prevalence of tobacco use among students was 9.72% with a significant difference between sexes (12.43% for boys and 6.65% for girls).[14] These studies indicate that tobacco smoking is prevalent in different parts of KSA and among different age groups the Saudi population. Moreover, the prevalence of tobacco smoking is greatly higher among males than in females at different ages.

In regard to the evaluation of the level of awareness toward tobacco carcinogens and when ask-

ing smokers whether cigarette smoking can cause cancer or not, 77.3% of the smokers believed that smoking can cause cancer and the remaining 22.7% have declared that there is no association between smoking and cancer. In a recent study from the same area, measured the perception of medical student toward tobacco alcohol consumption and its association with cancer, when asking participants about the relationship between cigarette smoking and cancer, 8.2% disagreed and the remaining 91.8% have agreed that smoking can cause cancer.[15] This relatively higher level of awareness compared to the present study might be attributed to the fact that all of the participants in the later study[15], were medical students.

However, around 30% of cancer cases could be prevented by amending lifestyle and avoiding the well-known risk factors. Approximately 1/3rd of cancer cases could be reduced if there is high level of awareness towards the common risk factor and if patients are detected at an early stage of cancer.[16] Cancer prevalence rates greatly differ worldwide, according to the existence of a particular risk factors, such as environmental exposure to carcinogens and the life style adopted. Moreover, it was found that pictorial health warnings on cigarette packages were significantly associated with increased awareness of smoking-related health hazards and behavior amendment.[17] Exposure to tobacco advertising, promotion, and sponsorship (TAPS) is found to be linked to the initiation and continuation of smoking among young individuals. The World Health Organization (WHO) Framework Convention on Tobacco Control (FCTC) recommends countries to prohibit all forms of TAPS. A number of countries have implemented partial bans covering direct advertising in media. Nevertheless, a small number of countries have adopted inclusive bans on all types of direct and indirect marketing. To evaluate improvement concerning elimination of TAPS and the level of awareness of TAPS among individuals aged ≥15 years, CDC used data from the Global Adult Tobacco Survey (GATS) collected in 14 countries during 2008-2010. Awareness of any TAPS ranged from 12.4% in Turkey to 70.4% in the Philippines. In the four countries where awareness of TAPs was ≤15%, three of the countries had comprehensive bans covering all nine channels measured by GATS, and the fourth country banned seven of the nine channels. In 12 countries, more peoples were aware of advertising in stores than advertising via any other channel. Decreasing exposure to TAPS is essential for preventing initiation of tobacco habit by young adults and helps smokers to quit.[18]

The third point in this study is the investigation of PTEN gene mutation. Oral cells were used to test for this mutation, since a number of studies have clarified the strong causal effects of tobacco in etiology of oral cancerous and precancerous lesions.[12] This in addition to the fact that the relationship between PTEN gene mutation and the development of oral cancer is well established.[8]

Although there are numerous studies on the etiology of oral cancer but the exact pathogenesis still unclear. The major etiologic factors in the development of oral cancer is tobacco use and alcohol consumption.[10,19] The identification of prognostic and predictive markers is clinically essential, since oral cancer is a group of heterogenous diseases with different biological and clinical features. The current study point out the utility of PTEN in the genesis of carcinoma of oral. Loss of PTEN function occur frequently in oral cancer and other cancers.[20,8] However, to the best of my knowledge, this is the first study to report that cigarette smoking can be associated PTEN loss in oral cells. Previous studies have showed that loss of PTEN increased remarkably according to disease stage and lymph node metastasis [8]. This study suggests that PTEN may play a vital role in the regulation of tumor development during the various stages of the disease. Nonetheless the exact role of PTEN in the genesis of oral neoplastic changes requires further investigations.

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