Nicotine content in tobacco used in hubble-bubble smoking

Kamal A. Hadidi, PhD, Faisal I. Mohammed, MD, PhD.

ABSTRACT

Objective: To determine the nicotine content of commonly used tobacco in hubble-bubble (HB) and compare it with that found in cigarettes.

Methods: Analysis of nicotine content of 13 commercial brands of HB tobacco was carried out using gas chromatography over an 8 month period; November 2002 through to June 2003 at the Toxicology Laboratory, University of Jordan.

Results: A wide variation in nicotine content in all brands was noticed with an average of 8.32 mg/g tobacco, range (1.8-41.3 mg/g). The average nicotine content in each HB head (20 gm) of unflavored tobacco was (713mg/head) and flavored tobacco has (67mg/head). One head of unflavored tobacco has nicotine equivalent to 70 regular cigarettes. The addition of adulterations; honey, glycerin and other flavors in the process of preparing the flavored (Mua'sel) tobacco contributes to lowering the nicotine content in each gram of flavored tobacco. Smoking one head of flavored (Mua'sel) tobacco which contains on average one third of nicotine presented in 20 cigarettes (204mg/pack) usually resulted in a higher plasma nicotine level by 20%.

Conclusion: Hubble-bubble smokers are not at a lesser risk from smoking than cigarette smokers in relation to nicotine dependence. The inability of water to trap significant amounts of chemical substances present in tobacco (especially nicotine) and the danger which might result from the combustion of additives like; glycerin, honey and other flavors could be a very important factor to extrapolate the damage resulting from HB smoking.

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Tobacco smoking is one of the leading preventable causes of death in the world. A recent estimate of smoking initiation indicates that worldwide between 82,000 and 99,000 young people begin smoking each year.[1] In the United States of America (USA), 3000 teenagers begin smoking daily; nearly one million a year.[2] The World Health Organization reports more than 4,000,000 deaths a year due to tobacco. They project a death total of more than 10,000,000 a year by 2030, with 70% of these deaths in the developing countries.[3] Smoking is a major risk factor for cancers of the lung and oral tract.[4,5] Cigarette smoking has a higher risk of developing cancer at many areas of the body and even their metastatic rates.[6,7] Hubble-bubble (HB) smoking is a traditional type of smoking traced back to ancient India,[8] known under different names such as oriental pipe, water pipe, Arghileh, Narghileh, Narguila, Hookah, Sheesha, Chicha, Gozah, is widely used in Jordan and in other countries. The tobacco used, called in Arab countries Mua'sel, is usually a local preparation of tobacco leaves with glycerin. Most Mua'sel is highly flavored including honey, mint, strawberry, apple, and other fruits, although some are not flavored. Flavoring additives may attract young HB smokers, especially female teenagers. It is worth mentioning that Mua'sel is an Arabic word

From the Forensic Medicine and Toxicology Division (Hadidi), Department of Physiology and Biochemistry (Mohammed), Faculty of Medicine, University of Jordan, Amman, *Jordan*.

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Address correspondence and reprint request to: Dr. Kamal A. Hadidi, Forensic Medicine and Toxicology Division, Faculty of Medicine, University of Jordan, Amman 11942, *Jordan*. Tel. +962 (6) 5355000 Ext. 2390. Fax. +962 (6) 5356746. E-mail: khadidi@ju.edu.jo

derived from the word "honey". In HB smoking everyone can participate including women and teenagers. For instance, in Egypt HB may be smoked in groups in contrast to cigarette smoking. Hubble-bubble smoking gained great acceptance among all age groups of both sexes and became a very common habit in most middle eastern countries. Few studies dealt with HB smoking. In fact, only for the last few years data is accumulating regarding the health damage induced by HB smoking. Hubble-bubble prevalence is being increased, especially among women and teenagers of both sexes, but public awareness of its risk factors is negligible or still premature. Health officials are not taking any concrete measures or great efforts to reduce HB smoking. It is worth mentioning that health statistics already indicate that among women lung cancer is more prevalent than breast cancer, reflecting the increase in female smoking rates.[9,10]

In the early 90's, Jordan witnessed the opening of a lot of coffee shops, especially inhabited by young teenagers of both sexes that offer HB. Even though there are no epidemiological studies regarding its prevalence in Jordan, it is however observed that HB popularity is being increased. In contrast to cigarette smoking, HB smoking became a trendy social behavior and public toleration to this habit is becoming wider despite all the facts regarding the health problem related to tobacco smoking. People think of HB as an innocent habit and believe that HB smoking is a safe alternative to cigarette smoking; therefore, its consequent increase in Jordan is approaching epidemic proportions. Since quitting smoking is a major aim of the majority of cigarette smokers, shifting to a less harmful smoking pattern is a more achievable goal. To our knowledge, the first scientific report addressing smoking in Jordan was presented by Salhab.[11] Recently, we have reported nicotine and cotinine levels in different biological fluids in HB smokers.[12] Till the present time, no data is available regarding the content of nicotine in the (Mua'sel) tobacco used in HB. In the present study, the nicotine content of 13 popular brands of flavored (Mua'sel) and unflavored tobacco used in HB is determined and compared with the nicotine content in cigarettes and cigars.

Methods. Commercial flavored (Mua'sel) and unflavored tobacco were purchased from local shops. One gram sample from each brand was used for nicotine extraction. Nicotine from tobacco was extracted and determined using a method described elsewhere[1,11] with minor modification. Briefly, 8 ml of HCl(1N) were added to one gram of tobacco and left for 10 min. Then 5 ml of NaOH (36%) were added to alkalize the aqueous solution followed by 25 ml of (60% chloroform: 40% hexane), then the tube was vortexed on rotary mixer for 30 minutes. The sample was centrifuged at 8000 rpm for 15 minutes. The aqueous layer was discarded, and the organic layer (supernatant) was filtered with sodium sulfate then collected in volumetric flask. Lidocaine was used as internal standard. Two µl of the organic layer were injected into the gas chromatography (GC) for assay of nicotine content. In the case of measuring nicotine concentration in the HB water reservoir, the same procedure was used. The nicotine standard used in our method was purchased from (Aldrich Chemical Company, Milwaukee, Wisconsin, USA), reagent grade chemicals and HPLC grade solvents were purchased from BDH (Poole, United Kingdom). The concentration of nicotine was determined using Hewlett Packard 5890 plus II gas chromatography equipped with nitrogen phosphorous detector. The column used was HP-35 (15M x 0.32mm I.D x 0.25µm), oven temperature was programmed at: 110°C for one minute then increased at 10°C/min to 250°C and 20°C/min to 300°C . The injector was set at 200°C on splitless mode using 2µl sample volume. The nitrogen phosphorous detector was set at 280°C. The flow rate of carrier gas (nitrogen) was set at 2.0ml/min. Hewelett Packard-chemistation was used in data acquisition and integration. Samples extraction and chromatographic analysis were performed at the Toxicology Laboratory, Forensic Medicine and Toxicology Division, Faculty of Medicine, University of Jordan. The regular HB apparatus which is commonly used by smoker whether at home or coffee shops consists of a clay basin called (head) which holds on average 20 (range 15-25 g) of tobacco covered with a piece of fenestrated aluminum foil topped with 3-5 pieces of burning charcoal. The mainstream smoke is drawn through the burning tobacco with each puff and passes into a water reservoir via a metal stem of 40cm length and 1 cm internal diameter (standard reservoir has a capacity of 400 ml and large reservoir has a capacity of 600 ml), the smoke usually bubbles in water before being introduced to the mouth via a rubber tube with 1.5 meter length and 1.0 cm internal diameter. The HB smoker usually takes a puff every 30 (15-60 seconds) resulting in an average of 90 (45-180 puffs) per head over the 45 minutes smoking session with an average of puff duration of 3.5 (2-5 seconds), the smoke is usually held for 2-3 seconds before being exhaled. Factors affecting the deepness and number of puffs in each session are the depth of the metal stem in the water reservoir (the amount of water, as the amount increases the more difficult it is to take a deep puff), the number of holes made on aluminum foil, the number of burning charcoal pieces, the quantity of glycerin mixed with tobacco, and the experience of HB smoker. A young regular HB

smoker tends to consume 1-2 heads of Mua'sel per day which is equivalent to 40 g of tobacco. A diagram showing the full detailed description of the apparatus can be seen in reference.[12] The pH of tobacco; flavored (Mua'sel) and unflavored tobacco used in our study was determined as described by Henningfield.[13] Two grams of each of tobacco brand were suspended in 10 ml water, mixed, and then tested at fixed intervals zero, 15, 30, 45 and 60 minutes.

Results. The analytical method offered an excellent detection limit of 0.5 ng/ml and a limit of quantitation 2.0 ng/ml. The method was linear over the entire concentration range (5.0 µg/ml-650µg/ml) and within day and day to day precision of 5.4% and of 7.8%. In case of unflavored tobacco analysis, dilution of the extract is required to obtain a result within the linear range of the method. The retention time of nicotine was 5.31 minutes and 11.27 minutes for the internal standard. **Table 1** shows the nicotine content of 11 brands of flavored (Mua'sel) and 2 brands of unflavored (plain and dry tobacco). The average nicotine content in flavored tobacco was 3.35 (range 1.8-6.3mg/g) while in unflavored tobacco the average nicotine content was 35.65 (range 30-41.3 mg/g). The results showed that nicotine content in each gram of unflavored tobacco is 10, 2.57, and 2.96 times higher than those present in each gram of flavored HB tobacco, cigarettes and cigars, **Table 2**. Monitoring the pH of water used in HB during the course smoking at; 0, 15, 30 and 45 minutes showed an increase by 1.3-1.5 units at the end of the smoking session, regardless the pH of water at zero time. The pH of HB tobacco was acidic in the 13 different samples with no significant difference between the 11 flavored (Mua'sel) 4.78 and 2 unflavored (Plain and dry) tobacco 4.75.

Table 1 - Nicotine concentration (mg/gm) in commercial tobacco used in hubble-bubble smoking purchased from local shops.

Product name	Nicotine concentration (mg/gm)	Nicotine concentration (mg/ Narghileh head)
Two apple nakhleh	3.7	74
Fakhfackeina apple	3.15	63
Fackfackeina fruit	3.0	60
Fackfackeina strawberry	3.2	64
Zaghlool	5.75	115
Salloom	6.3	126
Alsonboleh	1.8	36
Apple - Egypt	2.3	46
Al-Nakhleh	2.25	45
Apple - Jeddah	2.6	52
Al-Noman	2.8	56
Flavored		
Average	3.35	67
Range	(1.8-6.3)	(36-126)
Asfahani*	30	600
Ajami*	41.3	826
Unflavored		
Average	35.65	713
Range	(30-41.3)	(600-826)
Both flavored and unflavored		
Average	8.32	166.4
Range	(1.8-41.3)	(36-826)

*Unflavored (plain and dry tobacco)

Discussion. The observed difference in nicotine content between the flavored and unflavored tobacco could be attributed to many factors such as; the amount of additive added during the course of processing the flavored brands (for each kg of tobacco the following materials are added; 250-350ml glycerin, honey 125-250ml and 50ml flavors solution) mixed together before being mixed by hand for at least 30 minutes with one kg of unprocessed tobacco. The mixture is usually left for 3 weeks before being used; this process is called fermentation. The amount of glycerin, honey and flavor usually depend on the customer smoking taste and habit; the more glycerin present in the mixture the more smoke will be produced with easy puffing during the smoking session. The above mentioned materials added to the tobacco in the process of producing the flavored tobacco (Mua'sel) will contribute in increasing the weight of one kg of unprocessed tobacco by 74-124%. Another contributing factor in increasing the tobacco weight and as a result, decreasing the amount of nicotine present in each gram of the flavored tobacco is the ability of glycerin to absorb moisture during the fermentation process. An observation worth to be addressed to explain the low nicotine content in flavored tobacco is the presence of substantial amounts of stems when the texture of flavored tobacco was compared with unflavored tobacco or regular cigarettes, it was found that the stems that form the tobacco plant were present in large quantities on all types of tobacco used in preparing the flavored tobacco, while unflavored tobacco contains mainly leaves of the tobacco plant. This could be another contributing factor, since high nicotine concentration is expected to be found in leaves. In our study we did not analyze the nicotine content in unprocessed tobacco used in preparing the flavored brands as our main concern was to investigate the nicotine content on the finished product of flavored and unflavored tobacco. Even with such low nicotine content of one gram of flavored tobacco, the single head of HB contains on average of 67mg nicotine which is equivalent to 6.5 regular cigarettes.[14] Smoking one head of flavored

Table 2 - Comparison of nicotine content in tobacco used in hubble-bubble with cigarettes and cigar (Average nicotine percent and content mg/gm).

Type	Average tobacco weight (g)	Average concentration (mg)	Average concentration (mg/g)	Average concentration/ pack mg
***Cigarettes**				
Cigarette	0.738	10.2	13.82	204
Range	(071-1.064)	(7.2-13.4)	(9.75-18.15)	(144-268)
†Cigars				
Cigar	10.27	134.4	12.02	-
Range	(0.77-22.0)	(10.08-444.0)	(4.7-22.0)	-
Hubble-bubble (flavored)				
Head	20		3.35	67/Head
Range	(15-25)		(1.8-6.3)	(36-126)
Hubble-bubble (unflavored)				
Head	20		35.65	713/Head
Range	(15-25)		(30-41.3)	(600-826)
Hubble-bubble (flavored and unflavored)				
Head	20	166.4	8.32	-
Range	(15-25)	(36-826)	(1.8-41.3)	-

Nicotine concentration in *Cigarettes (Krozlowski)[14] was an average of 32 American brands
†Cigars (Henningfield)[13] represents 10 brands, and the hubble-bubble represent 13 brands.

tobacco (67mg/head) showed a steep rise in plasma nicotine concentration over the 45 minutes smoking session and reaches a peak, at the last puff, a level of 60.3 ± 7.58 ng/ml;[12] while when smoking 21 cigarettes (214 mg nicotine) in the following manner; 3 cigarettes per hour for a total of 7 hours, the plasma nicotine concentration reached a steady state after 3 hours with an average concentration of 49 ng/ml.[15] When we compare the 2 cases, the amount of nicotine present in the 21 cigarettes (214mg) is 3 times more than those present in one head of HB (67mg), while plasma nicotine level resulting from smoking one head of HB tobacco is higher by 20% than plasma nicotine concentration resulting from smoking 21 cigarettes. Therefore, the addictive effect which might result for HB tobacco will be as significant as cigarettes smoking, regardless of the variables affecting HB and cigarette smoking especially; dose, time, depth and duration of inhalation. More studies are needed to address the presence of other materials (adulteration) which might be present in the flavor tobacco resulting in low nicotine content and the possible harmful effect of chemical additives like glycerin, honey and flavored is also worth to be studied. The analysis of unflavored tobacco revealed the presence of very high nicotine content; average of 35.65mg/g equivalent to 713mg/head, such high nicotine content represents around 10.6 heads of flavored tobacco and 3.5 packs of cigarettes (70 cigarettes). Therefore, the amount of nicotine intake during one session of HB smoking of unflavored tobacco is expected to be 10 times more than that of flavored, with a more prominent effect of nicotine as an addictive substance and a profound effect on cardiovascular system in terms of increasing the heart rate and blood pressure. On the other hand, the added materials to flavored tobacco work as diluents that constitute around 50% of the 20 g used in each HB run. The amount of alkaloids and other chemicals present are expected to be far lower than those present in unflavored tobacco, but the danger which might result from the combustion of glycerin, honey, flavors and tobacco could be more than that of unflavored tobacco (Plain and dry tobacco leaves) but these combustion products are not measured. The high nicotine levels in the HB tobacco smoke, and the need for forceful inhalation, may lead to high nicotine levels in their plasma[16] that may lead to nicotine dependence. Therefore, HB smoking can be considered as a risk factor. Hence, cigarette smoking related illnesses such as lung cancer, emphysema, and coronary heart disease are to be considered. Unfortunately, cigarette smoking and HB smoking are frequently co-occurring, and cigarette smokers may not benefit when they switch to HB; on the contrary they might be at higher risk since they may have to inhale deeply after switching to HB to maintain the same level of plasma nicotine. This may result in as great

or even greater intake of combustion products. In addition, the flavoring additives may reduce the harshness of nicotine in Mua'sel of HB and encourage deeper inhalation while smoking. Hubble-bubble smokers believe that water may act as a filter. It was found that the nicotine trapped in the HB water was less than 5% of the available tobacco nicotine. The increase in pH of water in the HB reservoir by 1.3-1.5 units at the end of the smoking session regardless of the pH of water at zero time indicates that the slightly alkaline compounds including nicotine and other toxic constituents of smoke have been trapped in water when they are bubbled through the weak acid water resulting in an increase in the pH of water. The amount of alkaloids and chemicals trapped in water are expected to be high in the early stage of HB smoking. This will raise the pH of water that leads to decreased amount of alkaloids trapped resulting in an increase of alkaloids in an un ionized form (less water solubility), resulting in an increase in alkaloids including nicotine that passes through the water and delivered to the mouth of HB smoker. This means that the absorption via the buccal membrane will be increased at the late stage of the HB smoking session accompanied with harshness smoke. A similar observation was noticed in cigar, as the method of preparing cigar usually results in alkaline smoke with a pH closer to or more than the pH of nicotine, where a rapid absorption of nicotine is usually noticed in cigars due to the increase in tobacco pH during the course of smoking.[17] Despite all the above mentioned observations regarding the changes in the pH of both water and tobacco during the course of HB smoking, insignificant amounts of nicotine trapped in water was noticed. But, the question regarding the ability of water and the changes in its pH in trapping other toxic substances (especially carcinogens) is beyond the scope of our study and will be addressed and investigated in the near future. Another contributing factor regarding the expected harmful effect of HB smoking is the tobacco pH. Herning et al[16] reported the need of deep inhalation of acidic smoke to permit effective absorption since nicotine will be in its ionized form which will decrease the absorption through the buccal mucus. In our study, the HB tobacco was acidic or even more acidic than cigarette tobacco; this could explain the need for deep inhalation of a large quantity of HB smoke in the early stage of HB smoking to permit effective absorption which might result in more damage to the lungs. The same observation was reported by Henningfield et al[16] in mildly acid cigarettes with a pH ranged from 5.6-6.5, while rapid absorption of nicotine (pH 8.0) through the buccal mucus was noticed when delivered from alkaline smoke like cigar as nicotine will be in un-ionized form.[17] Finally, how people smoke HB is a strong determination of toxin exposure and probably of the risk of dependence and other diseases. In cigarette smoking most smokers inhale the smoke of a pack or more of cigarettes per day, taking an average of 10 puffs per cigarette over 6 minutes,[13,18] while cigar smokers might spend an hour to one day smoking a single large cigar and these cigars may contain as much as a pack of tobacco cigarettes.[16] The regular HB smoker usually takes 1-2 heads of flavored Mua'sel and the amount of nicotine intake is expected to be more than 2-3 packs of cigarettes but over a shorter period of time when compared to smoking one pack of cigarettes. Such a short period spent in smoking HB, the large quantity of smoke produced and the inability of water to trap significant amounts of chemical substances (especially nicotine) could be a very important factor to extrapolate the damage resulting from HB smoking.

In conclusion, HB tobacco has comparable nicotine content like the tobacco of cigarettes. It has the potential to initiate and sustain tobacco dependence. This refutes the misconception that HB is innocent. It is essential to emphasize the possibility of the non-innocent nature of HB smoking during anti-smoking campaigns. There is no reason to believe that Mua'sel smoke has less toxic chemicals like phenol, hydrogen cyanide, benzopyrene or nitrosamine. Surprisingly, HB smokers are willing to believe that as smoke goes through water, many of the harmful substances are filtered out. However, there is no evidence to support such an assumption; in fact, less than 5% of the nicotine content is trapped in the water of the Narghileh.

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