Levels of urinary cotinine and exhaled carbon monoxide after shisha smoking

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Abstract: Shisha smoking has been prevalent in Indonesia because of the assumption that shisha is safe and harmless compared with cigarettes. Urinary cotinine and exhaled air carbon monoxide (CO) are indicators of cigarette smoke exposure. The aim of this study was to measure the level of urinary cotinine and exhaled air CO levels. We performed cross-sectional study on shisha smokers after using shisha. Respondents were grouped into groups of shisha smokers and non-smokers based on smoking status and shisha use status. Data obtained from questionnaires, urine samples, and exhaled air CO before and 30 min after using shisha. The urinary cotinine levels were measured by enzyme-linked immunosorbent assay (ELISA) and exhaled air CO levels were measured by smokelyzer test. Ninety-six respondents were enrolled in this study, consisted of 48 shisha smokers and 48 non-smokers. The level of urinary cotinine and exhaled air CO level after shisha smoking in shisha smokers were higher than non-smokers (median 162.7 vs. 6.5 ng/mL; p < 0.05 and 85 ppm vs. 1 ppm, p < 0.05). There was an increase in urinary cotinine level and exhaled air CO level after shisha smoking with percentage increase of 3% and 270%. Smoking status affects urinary cotinine levels in shisha smokers, and the type of inhalation affects exhaled air CO levels after shisha smoking.

1. Introduction
Tobacco is currently used by 1.3 billion people, 80% of whom live in developing countries. Today, tobacco is the second leading cause of death worldwide and is estimated to be responsible for the deaths of 1 out of every 10 adults worldwide [1]. The use of tobacco remains high among the youth and young adults, especially students [2].

Tobacco waterpipe devices are called narghile, arghile (Jordan, Lebanon, Syria, and Israel), hookah (Indian subcontinent and African continent), shisha, sheesha, borry, goza (Egypt and Saudi Arabia), shui yan dai (China), or hubble-bubble and have been used for smoking for over four centuries, especially in Asian countries, the Middle East, and North Africa [3,4].

The waterpipe was first created in India by a physician during the reign of Emperor Akbar (1556–1605), who claimed that the waterpipe was a harmless method for tobacco intake. These beliefs continue to be held by many shisha users today [3].
Like other tobacco products, the use of waterpipes is also associated with lung cancer, respiratory diseases, and heart disease. Waterpipe or shisha users are exposed to large concentrations of carbon monoxide (CO), nicotine, tobacco-specific nitrosamines, carcinogenic polycyclic aromatic hydrocarbons, and volatile aldehydes [5].

Shisha cafés are widely accessible in major cities throughout Indonesia, including Jakarta, Bogor, Bandung, Surabaya, Solo, Bali, and Yogyakarta. In Jakarta, shisha cafés spread throughout Kemang, Menteng, Kelapa Gading, and other areas. Currently, shisha use is limited; however, it may become more prevalent if there are no preventive efforts to control its spread [6].

An Indonesian study by Adhalia and Wiratmoko focused on 60 smokers and shisha users found that exhaled CO concentrations were 8.62 ppm in smokers and 20.67 ppm in shisha users [7]. Until now, there has not been much research in Indonesia focusing on levels of urinary cotinine and exhaled air CO in shisha users.

2. Methods
This was a cross-sectional study of shisha users that took place at the Sungaiiat-Bangka shisha café from September 2016 to October 2016. The study protocol had been approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital. The target population of this study included shisha users who met the study criteria. The population was divided into groups of shisha users and non-smokers as a control group. Participants were recruited via consecutive sampling.

Participant inclusion criteria included males and females > 18 years old; shisha users and non-smokers (control group); those willing to not smoke for up to 30 min after using shisha (for smokers); those willing to provide informed consent for interview, questionnaire completion, measurement of exhaled air CO, and urine sampling. This study excluded participants who were unable to urinate and unable to perform the exhaled air CO test; those who were pregnant; and those who used rifampicin, dexamethasone, phenobarbital, or oral contraceptives.

3. Results
A previous statistical calculation obtained a sample size of 48 participants for each group of shisha users and non-smokers (control group), so the total number of respondents are 96 participants. One session of smoking shisha lasted approximately 20–30 min; all smokers used the same waterpipe size (medium, with a height of 50 cm and length of 150 cm), and all used the same brand of tobacco with a nicotine content of 0.05%. As much as 5 g of tobacco were consumed per smoking session, and as many as two pieces of charcoal were used. Shisha users were asked not to use other tobacco products until urine was obtained at 30 min after using shisha.

Table 1 presents the level of urinary cotinine and exhaled air CO before and after using shisha in 48 smokers, revealing significant increases in both variables.

| Variable                | Before shisha | After shisha | p       |
|-------------------------|---------------|--------------|---------|
|                         | Median        | range        | Median  | range     |         |
| Urinary cotinine (ng/ml)| 157.9         | 2.3–167.2    | 162.65  | 0.10–167.3| 0.000*  |
| Exhaled air CO (ppm)    | 25.5          | 0.0–72.0     | 85      | 26.0–150.0| 0.000*  |

*p<0.05 (significant difference)
Tables 2 and 3 present the comparison of urinary cotinine and exhaled air CO levels before and after shisha use in all shisha users \((n = 48)\), smoking shisha users \((n = 41)\), non-smoking shisha users \((n = 7)\), new shisha users \((n = 16)\), and non-smokers/control group \((n = 48)\).

**Table 2.** Comparison of urinary cotinine levels before and after using shisha in smoking shisha users, non-smoking shisha users, new shisha users, and non-smokers/control group

| Characteristics          | Shisha users \((n = 48)\) | Smoking shisha users \((n = 41)\) | Non-smoking shisha users \((n = 7)\) | New shisha users \((n = 16)\) | Non-smokers \((n = 48)\) |
|--------------------------|---------------------------|-----------------------------------|-------------------------------------|-------------------------------|--------------------------|
| Cotinine before shisha (ng/ml) | 157.9 (2.3–167.2) | 159 (7.9–167.2) | 5.5 (2.3–50.5) | 136 (2–163) | 6.5 (0–29) |
| Cotinine after shisha (ng/ml) | 162.65 (8.1–167.3) | 163.2 (13.5–167.3) | 99 (8.1–159) | 162.65 (8.1–165.5) | - |
| Cotinine increase (ng/ml) | 4.15 (0–154) | 3.6 (0–154.6) | 6.2 (4.4–122.1) | 4.45 (1.5–154.6) | - |
| Percentage of cotinine increase (%) | 3 (0–1957) | 2 (0–1957) | 200 (90–914) | (91–1957) | - |

There were 48 non-smoking control group participants who underwent urinary cotinine and exhaled air CO level testing. Their results were compared with those of smokers. (Table 4).

**Table 3.** Comparison of exhaled air CO levels before and after using shisha on smoking shisha users, non-smoking shisha users, new shisha users, and non-smokers/control group

| Characteristics          | Shisha users \((n = 48)\) | Smoking shisha users \((n = 41)\) | Non-smoking shisha users \((n = 7)\) | New shisha users \((n = 16)\) | Non-smokers \((n = 48)\) |
|--------------------------|---------------------------|-----------------------------------|-------------------------------------|-------------------------------|--------------------------|
| CO before shisha (ppm)   | 25.5 (0–72) | 29 (2–72) | 3 (0–9) | 25 (0–51) | 1 (0–5) |
| CO after shisha (ppm)    | 85 (26–150) | 92 (26–150) | 43 (26–150) | 59 (26–150) | - |
| CO increase (ppm)        | 59 (14–149) | 68 (14–148) | 43 (27–149) | 38 (14–149) | - |
| Percentage of CO increase (%) | 270 (50–14900) | 230 (50–3400) | 1150 (178–14900) | 189 (50–14900) | - |

**Table 4.** Comparison of urinary cotinine and exhaled air CO level on shisha users and non-smokers

| Variable                        | Shisha users      | Non-smokers | \(p\)   |
|---------------------------------|-------------------|-------------|--------|
| Urinary cotinine level (ng/ml)  | 162.7 (8.1–167.3) | 6.5 (0–29) | 0.000* |
| Exhaled air CO level (ppm)      | 85 (26–150)       | 1 (0–5)    | 0.000* |

\*\(p<0.05\) (significant difference)
4. Discussion
This study examined respiratory and other complaints after using shisha. Respiratory symptoms were assessed using the American Thoracic Society Division of Lung Disease questionnaire (ATS-DLD-78) questionnaire and consisted of cough (n = 14, 29.2%) and shortness of breath (n = 6, 12.5%). Participants also complained of other symptoms including dizziness (n = 4, 8.3%). Two (4.2%) complained of shortness of breath and dizziness, and two (4.2%) complained of cough and dizziness. There were 20 (41.76%) participants without symptom complaints after using shisha.

4.1. Urinary Cotinine Levels in Shisha Users
The median cotinine level before using shisha was 157.9 ng/mL, whereas the median value of urinary cotinine level after using shisha was 163.65 ng/mL. There was an increase in urinary cotinine level after using shisha, with median elevated urinary cotinine level of 4.15 ng/mL. This represented significance at 3% (p = 0.000).

Shisha users were exposed to nicotine just like regular smokers. The urinary cotinine level of shisha users was significantly higher than that of non-smokers. The median urinary cotinine level in the control group was 6.5 ng/mL. Our results were like those obtained by Suryatama et al. in their study of adult women who were not exposed to cigarette smoke (median urinary cotinine of 7.30 ng/mL) [8].

Shafagoj et al. examined nicotine and cotinine levels in 14 shisha users and found that the levels were increased for 45 min after using shisha. The average plasma nicotine concentration increased by 1.11% to 60.31 ng/mL, and the plasma cotinine increased from 0.79 to 51.95 ng/mL. Salivary nicotine concentrations increased from 0.79 to 283.49 ng/mL. The mean level of nicotine and cotinine excreted in urine, during the 24 h following shisha use was 73.59 and 249 μg [9]. In contrast to our study, Shafagoj et al. carried out an experimental study. Respondents were asked not to smoke for 3 days before the day of research. Additionally, the researchers used as much as 20 g per session. In contrast, our research was cross-sectional, we only examined urinary cotinine, we did not check the smoking status of shisha user participants, and only used a maximum of 5 g of tobacco per session.

There were 7 non-smokers, out of the 48 shisha user participants. The median urinary cotinine level before using shisha was 5.5 ng/mL (2.3–50.5 ng/mL), increasing to 9.9 ng/mL (8.1–159 ng/mL) after shisha use, for a median percentage increase of 200%. One session of shisha use increased urinary cotinine by a median value of 6.2 ng/mL. Rabiei et al. measured salivary cotinine levels and found they were as high as 106.24 ± 135.23 ng/mL in shisha users, 223.74 ± 181.56 ng/mL in smokers, and 0.73 ± 1.24 ng/mL in the control group. Increases in salivary cotinine following one session of shisha use per week were approximately 4.57 ng/mL [10].

4.2. Exhaled Air CO Levels in Shisha Users
Shisha users are also exposed to nicotine and CO just like smoking. There was an increase in exhaled air CO levels after using shisha, with median exhaled air CO increase of 59 ppm (14–149 ppm), and a median 270% increase in exhaled air CO. Exhaled air CO increased significantly before and after using shisha. Exhaled air CO concentrations of shisha users, after using shisha, were higher, compared with those of the control group (85 ppm vs. 1 ppm).

Pascale et al. found a significant difference in exhaled air CO levels before and after using shisha. They concluded a one-time use of shisha was equivalent to smoking seven cigarettes in terms of increases in exhaled air CO [11]. We found that shisha users had high levels of exhaled air CO after using shisha, believed to be due to the burning charcoal used to burn the tobacco. Although the main smoke from the waterpipe causes bubbles to pass through water in the waterpipe bowl, the CO gas is not dissolved or filtered because CO gas has a low solubility in water.
4.3. Respiratory and Other Complaints after Using Shisha

The most frequent respiratory symptoms that arose after using shisha were cough (29.2%), followed by shortness of breath (12.5%), dizziness (8.3%), symptoms of shortness of breath and dizziness (4.2%), and symptoms of cough and dizziness (4.2%). Martinasek et al. found that shisha users reported dizziness (66%), fatigue (57.4%), headache (46.8%), palpitations (21.3%), nausea (17%), dry throat (17%), and shortness of breath (7%) [12].

Large amounts of CO gas are produced from charcoal when smoking with shisha. Barnett et al. found that inhaling 20 ppm or more CO produced symptoms of CO toxicity like headache, fatigue, and vomiting [13]. Some research suggests that shisha users inhale about 50–80 L of smoke per one shisha session, whereas smokers inhale about 0.5–0.8 L of smoke from a cigarette [14].

This study had some limitations. We used questionnaires to obtain sociodemographic and shisha use data in smokers or non-smokers, so there is the possibility of recall bias, although we also conducted direct interviews. Another limitation was the difficulty associated with finding shisha users who only used shisha. In Indonesia, most shisha users are also smokers, and the use of shisha in Indonesia is currently only a trend among young people who are usually found in cafés or restaurants. Rarely do users only use shisha every day or 2–3 times per week. Another limitation was our inability to objectively quantify inhalation volumes. Finally, we were limited by our use of the smokerlyzer tool, which can only detect maximum exhaled air CO levels of 150 ppm.

5. Conclusion

Smoking status affects urinary cotinine levels in shisha smokers, and the type of inhalation affects exhaled air CO levels after shisha smoking.

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