Vitamin B_6 Intake and B_6 Vitamer Concentrations and Excretion in Blood and Urine of Cigarette Smokers, Water-pipe Smokers and Non-smokers

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ABSTRACT: The aim of this study was to evaluate vitamin B_6 and B_6 vitamer concentrations [pyridoxal phosphate (PLP) in plasma and red blood cells and urinary excretion of 4-pyridoxic acid (4-PA)] among adult Saudi cigarettes smokers (n=85), water pipe smokers (n=82) and non-smokers (n=90). The study also investigated dietary intake of vitamin B_6, protein, B_6: protein ratio and % of dietary vitamin B_6 from animal sources. Other biochemical parameters that affect vitamin B_6 status including plasma albumin concentration and plasma alkaline phosphatase activity were also determined. Mean dietary intakes of vitamin B_6 for cigarettes smokers group (n=85), water pipe smokers group (n=82) and non-smokers group (n=90) were 2.13±0.72, 2.47±0.77 and 2.09±0.69 mg/day, respectively. Mean plasma PLP concentrations for the three groups were 26.2±3.2, 44.7±5.1 and 35.0±2.1 nmol/L, respectively. Mean urinary 4-PA excretions for the three groups were 3.8±1.8, 7.2±3.6 and 4.8±2.2 µmol/day, respectively. Dietary and biochemical indices indicated an adequate status of vitamin B_6. However, water pipe smokers had significantly higher means of vitamin B_6 intake and plasma PLP concentration as well as urinary 4-PA excretion compared to the other two groups. Bioavailability of dietary vitamin B_6 was less in water pipe smokers group compared to the other two groups as determined by % of the vitamin from animal source. Cigarettes smokers group had significantly lower mean plasma PLP concentration compared to non-smokers groups. Results of this study suggested that water pipe smoking had no effect on vitamin B_6 indices in blood and urine as effect of cigarettes smoking. Water in the water pipe smoking system may trap chemical components that are generated from tobacco smoking and affect vitamin B_6 vitamers in blood and urine. Further investigations are needed to evaluate influence of water pipe smoking on vitamin B_6 indices, with fixed vitamin B_6 intake..

key words: Cigarettes smoking, water pipe smoking, vitamin B_6 intake, protein intake, plasma albumin, plasma alkaline phosphatase, body mass index, plasma pyridoxal phosphate (PLP), urinary 4-pyridoxic acid (4-PA).

INTRODUCTION

Tobacco consumption, both active and passive, have been related to the development of several diseases and considered as one of the worlds leading causes of death (Ezzati and Lopez, 2003). In the country of Saudi Arabia, smoking is considered prevalent as several studies showed that smoking is a major health problem in the country. Jarallah et al. (1999) reported that in a sample of 8310 subjects from both sexes from different regions of Saudi Arabia, 21.1% and 0.9% of males and females respectively were cigarettes smokers. Similar results were reported in another study by Siddiqui et al. (2001), which investigated a sample of 634 Saudi males and found that 34.4%, 16.4% and 49.2% were current smokers, ex-smokers and non-smokers, respectively. Similar findings were reported by Al-

Assaf (2007) who found that 39.4% of a sample of 132 Saudi males in Riyadh city was cigarettes smokers. Water pipe is a common type of smoking in Saudi Arabia as well as the Middle East countries. A national survey among Saudi male students reported that water pipe smoking is prevalent (Almutairi, 2004). Another national survey by Al-Nozha et al. (1996) found that among Saudi smokers, 78.2% were cigarettes smokers and 20.4% were water pipe smokers. Water pipe smoking used tobacco leaves that are mixed with fermented fruits, honey or mint to add flavors. The tobacco is covered with aluminum paper, placed in a bowel and toped with a piece of burning charcoal that keeps the tobacco burning. Smokers draw air from the burning tobacco; the air passes through a small container of water and then enters a rubber tube and finally delivered to mouth. This type of smoking use water instead of filters that are usually used with cigarettes. There is a misconception among water pipe smokers that this type of smoking is a safe alternative to cigarettes smoking. In the contrary, studies showed that health hazards associated with water pipe smoking were similar to those associated with cigarettes smoking. Zahran et al. (1985) found higher blood concentrations of carboxy hemoglobin among water pipe smokers compared to cigarettes smokers. The study suggested that water pipe smokers probably absorb more carbon monoxide than cigarettes smokers since more tobacco smoke is delivered to lungs. Shafagoj et al. (2002) found significant elevation in levels of nicotine and cotinine in saliva, plasma and urine of water pipe smokers after 45 minutes of smoking compared to baseline (48 hours after avoiding water pipe smoking). Shihadeh and Saleh (2005) reported that large quantities of carbon monoxide are delivered to water pipe smokers due to the high burning of tobacco in water pipe smoking compared to cigarettes smoking. A study by Al-Numair et al. (2007) found that serum concentrations of HDL-cholesterol, apo A1, total antioxidant capacity and vitamin C were lower whereas LDL-cholesterol, apo B, triglycerides and malondialdehyde were higher in water pipe smokers compared to non-smokers. The adverse effect of cigarettes smoking on vitamin B_6 is well established. Several studies showed that cigarettes smoking lower urinary excretion of 4-PA as well as plasma and erythrocyte pyridoxal-5-phosphate (PLP), which is the active form of vitamin B_6 and best single indicator of the vitamin status (Serfontein et al., 1986; Serfontein and Ubbink, 1988; Giraud et al., 1995; Vermaak et al., 1990). However, effect of water pipe smoking on vitamin B_6 is not known yet. Thus, the aim of this study was to evaluate vitamin B_6 vitamer concentrations in blood and urinary 4-PA excretion among cigarettes smokers, water pipe smokers and non-smokers. The study also evaluated other parameters that effect B_6 vitamers in blood and urine including dietary intake of vitamin B_6 and protein, plasma albumin concentration and plasma alkaline phosphatase activity.

MATERIAL AND METHODS

This study recruited its sample from volunteers from Dammam city (east Saudi Arabia). All volunteers received a questionnaire asked for age, weight, height, health status, exercise habits and use of mediations or dietary supplements. Only healthy and non-users

of medications or dietary supplements were eligible to participate in the study. The study protocol was explained to each participant and informed consent was obtained. All participants received written instructions about keeping three consecutive days of food records and urine collection. For each subject, energy requirement (based on weight, height, age and exercise level) and energy intake (based on food record intake), were calculated by the Food Processor (2001). Participant(s) for whom his (their) difference between energy intake and energy requirement exceeded 15% was (were) excluded because this difference indicated invalid food records. The sample size after exclusion was 257 healthy Saudi adult males. Of which, 85 were cigarettes smokers, 82 were water pipe smokers and 90 were non-smokers. The three day food records were collected during October – November, 2008; and analyzed by the Food Processor (2001) to determine dietary intake of vitamin B_6, % of vitamin B_6 from animal sources and protein. On the fourth day, weight and height were measured and body mass index (BMI) was calculated for each participant. Blood samples were collected in heparinized tubes (Becton Dickins, NJ, USA) by trained personal. Blood samples were centrifuged at 1800×g at 5°C for 20 minutes and plasma samples were separated and stored at -70 (±5°C) until analysis. Red blood cells were washed three times by cold 0.9% saline solution and centrifuged at 1800×g for 20 minutes at room temperature in each time (Miale, 1977), then stored at -70 (±5°C) until analysis. The urine samples were collected in brown opaque polyethylene bottles containing 15ml of toluene as a preservative. Total volume of urine for each day was measured and a sample of 10ml was obtained and stored at -25°C until analysis. Concentrations of B_6 vitamers, pyridoxal (PL) and PLP in plasma as well as PLP in red blood cells were analyzed by HPLC with fluorometric detection as described by Kimura et al. (1996). Urinary 4-PA was analyzed by HPLC method as described by Gregory and Kirk (1979). Plasma albumin concentration and alkaline phosphatase activity were determined by colorimetric methods as described by Slater et al. (1975) and Roy (1970), respectively. All samples were analyzed in duplicate and the average was reported if the difference did not exceed 8%. Results are reported as mean ± standard deviation (SD). Comparison between cigarettes smokers, water pipe smokers and non-smokers was performed by one way analysis of variance (ANOVA)-Tukey Kramer procedure. A partial person's correlation analysis was used to determine correlation between measures. Significance of difference was set at P-value ≤ 0.05. All statistical analysis was performed by Statistical Package for Social Sciences (SSPS), Version 10.

RESULTS AND DISCUSSION

Characteristics of participants are shown in Table 1. No significant differences were observed between the three groups in any of the characteristics. Results of BMI as an indicator of weight status showed prevalence of overweight and obesity among all participants. This is in agreement with several previous studies (Al-Nuaim et al, 1997; El-Hazmi and Warsy, 1997; Madani et al., 2000; Al-Assaf and Al-Numair, 2007). The aim of

this study was not to evaluate weight status; however, BMI data is important to indicate that participants were in the state of well or over nutrition. Thus, deficiency of vitamin B_6 due to poor dietary intakes is not expected (Dakshinamurti and Dakshinamurti, 2007; Leklem, 2001). Results of dietary intake are shown in Table 2. Water pipe smokers compared to cigarettes smokers and non-smokers had significantly higher mean intake of vitamin B_6 with no significant difference in protein intake. Thus, mean of vitamin B_6: protein ratio was significantly higher in water pipe smokers compared to the other two groups. contribution of animal foods to vitamin B_6 was significantly lower in the water pipe smokers group compared to the other two groups. Thus, bioavailability of vitamin B_6 to water pipe smokers was less due to source of the vitamin (Hansen et al., 1996). Based on the DRI recommendation of vitamin B_6 for adult male (1.3 mg/day) (DRI, 1998), all of participants in the three groups had an adequate intake. Also, intakes of protein for all participants were within the Acceptable Macronutrient Distribution Ranges (AMDRs) of dietary reference intakes for adult males (10-35% of energy) (DRI, 2005). The adequate intake of vitamin B_6 and protein found in this study is in consistent with previous data although there was region variation (Al-Assaf and Al-Numair, 2007). Mean of vitamin B_6: protein ration was significantly higher in water pipe smokers group compared to the other two groups due to the higher intake of vitamin B_6. However, the ratios of the three groups indicated an adequate status of the vitamin based on the suggested ratio reported by Leklem (1990) ($\geq$0.020). Other biochemical parameters that affect vitamin B_6 indices are shown in Table 3. Plasma albumin (carrier of PLP in blood) concentrations for all participants in the three groups were within normal range (38-55 g/L for adult males) as reported by Gibson (2005). Similarly, results of plasma alkaline phosphatase activity, which is essential in formation of PLP from other B_6 vitamers, were within normal range (13-39 UL for adult males) according to Lee and Nieman (2003). Results of the both biochemical parameters are in agreement with normal ranges of adult Saudis reported by El-Hazmi et al. (1982) and Scott (1982). The means of plasma PLP, PL, RBC PLP and urinary 4-PA excretion are shown in Table 4. Plasma PLP concentrations and urinary 4-PA excretions for all participants in the three groups indicated an adequate status of vitamin B_6 based on the suggested values ($\geq$20 nmol/L) for plasma PLP (Lui et al., 1985) and ($\geq$3.0 μmol/day) for urinary 4-PA (Leklem, 2001). Plasma PLP concentrations and urinary 4-PA excretions were significantly correlated with vitamin B_6 intake (r=0.42). Means of B_6 vitamer concentrations in blood and urinary 4-PA excretion were significantly higher in water pipe smoking group compared to the other two groups. Comparison of these results with previous data is not possible since this is the first study investigated vitamin B_6 indices in blood and urine for water pipe smokers. Comparison between cigarettes smokers and non-smokers in vitamin B_6 indices (Table 4) indicated significant reduction in plasma PLP concentration in cigarettes smoking group compared to the non-smoking group. This result was expected and in agreement with the previous studies mentioned in the introduction section indicated that cigarettes smoking reduce the concentration of plasma PLP. Same trend was found for the mean urinary 4-PA excretion which was lower in cigarettes smokers group compared to the non-smokers group, but the difference was not significant. This is in agreement with previous findings (Giraud et al, 1995). Major

chemical components generated by cigarettes smoking affect B_6 vitamers are carbon monoxide, cyanide, nicotine and aldehydes (Cross et al., 1993). Cyanide reacts with PLP and form a cyanohydrine complex, which may cause rapid depletion of plasma PLP (Keniston et al., 1987). This study found same effect of cigarettes smoking on vitamin B_6 indices that was reported in previous study. However, effect of water pipe smoking on the vitamin B_6 indices was not the same. Mean concentrations of B_6 indices in blood and urinary 4-PA excretion (Table 4) were higher in water pipe smoking compare to non-smokers as well as to cigarettes smokers. There are two possible explanations for these results. First, intake of vitamin B_6 and its ratio to dietary protein were higher in water pipe smoking group as compared to the other two groups. However, this explanation is weak due to the lower bioavailability of vitamin B_6 for water pipe smokers as mentioned earlier. The second possible explanation is that the water in the water pipe smoking system filters out the chemical components that affect vitamin B_6 indices, especially the cyanide. Further studies are needed to determine the chemical components generated by tobacco smoking in water of the water pipe smoking system and in blood as well as vitamin B_6 indices in blood and urine with fixed intake of the vitamin.

Table 1. Mean (±SD) of age, height, weight and BMI for cigarettes smokers, water pipe smokers and non-smokers.

Characteristics	Cigarettes smokers (n=85)	Water pipe smokers (n=82)	Non-smokers (n=90)
Age (yr)	28±7.0	27±5.0	30±7.0
Height (cm)	169±8.0	170±6.0	168±6.0
Weight (kg)	79.6±20.2	77.3±17.2	83.4±19.7
BMI (kg/m^2)	27.1±7.4	25.7±6.8	27.9±8.0

Table 2. Mean* (±SD) intake of vitamin B_6 and protein; vitamin B_6: protein ratio and % of vitamin B_6 from animal source for cigarettes smokers, water pipe smokers and non-smokers.

Nutrients and source	Cigarettes smokers (n=85)	Water pipe smokers (n=82)	Non-smokers (n-90)
Vitamin B_6 (mg)	2.13 ± 0.72^a	2.47 ± 0.77^b	2.09 ± 0.69^a
Protein (g)	102.7 ± 28.3^a	102.2 ± 27.6^a	104.1 ± 30.3^a
B_6: protein ratio	0.020 ± 0.008^a	0.024 ± 0.006^b	0.20 ± 0.07^a
% of B_6 from animal sources	66.2 ± 6.9^a	55.3 ± 10.4^b	67.3 ± 8.7^a

*Means are the average intake of three consecutive days.
Different letters in a given row denote a significant difference $P\leq0.05$.

Table 3. Mean (±SD) plasma albumin concentration and alkaline phosphatase activity for cigarettes smokers, water pipe smokers and non-smokers.

Biochemical measurements	Cigarettes smokers (n=85)	Water pipe smokers (n=82)	Non-smokers (n-90)
Plasma albumin (g/L)	47.6±1.6	48.2±2.0	46.7±1.9
Plasma alkaline phosphatase (U/L)	29.2±3.8	26.9±3.3	28.6±4.5

Table 4. Mean (±SD) concentrations of plasma PLP, PL, red blood cells (RBC) PLP and urinary 4-PA excretion for cigarettes smokers, water pipe smokers and non-smokers.

B_6 vitamer	Cigarettes smokers (n=85)	Water pipe smokers (n=82)	Non-smokers (n-90)
Plasma PLP (nmol/L)	26.2 ± 3.2^{a}	44.7 ± 5.1^{c}	35.0 ± 2.1^{b}
Plasma PL (nmol/L)	9.4 ± 4.6^{a}	16.8 ± 5.0^{b}	11.3 ± 4.0^{a}
RBC PLP (nmol/L)	$24.9\pm+7.2^{a}$	34.5 ± 9.1^{b}	26.2 ± 6.6^{a}
Urinary 4-PA (μmol/day)*	3.8 ± 1.8^{a}	7.2 ± 3.6^{b}	4.8 ± 2.2^{a}

* Average of three consecutive days.
Different letters in a given row denote a significant difference $P\leq0.05$.

CONCLUSIONS

Results of this study showed adequate status of vitamin B_6 based on its dietary intake, vitamer concentrations in blood and urinary 4-PA excretion among cigarettes smokers, water pipe smokers and non-smokers. Mean plasma PLP concentration in cigarettes smoking group compared to non-smokers group was lower with no difference in vitamin B_6 intake. No significant differences were found between the last two mentioned groups in other vitamin B_6 indices in blood and urine. Water pipe smoking group compared to cigarettes smoking and non-smoking groups had significantly higher concentrations of vitamin B_6 indices in blood and urine combined with higher intake of vitamin B_6 with low bioavailability. This study suggests that water in the water pipe smoking system may filter out the chemical components generated by tobacco smoking that affect vitamin B_6 indices. Further investigations are needed to evaluate the influence of water pipe smoking on vitamin B_6 indices in blood and urine.

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المتناول من فيتامين ب$_6$ وتركيز مكوناته والمطروح منه في الدم والبول لمدخني السجائر، ومدخني الشيشة وغير المدخنين

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الملخص: استهدفت هذه الدراسة تقييم المتناول من فيتامين ب $_6$ وتركيز مكوناته [البيرودكسال فوسفات (PLP) في البلازما وكرات الدم الحمراء والمطروح في البول من 4- حامض البيرودكسك (4-PA)] لعينة من السعوديين البالغين مدخني السجائر (85 فرد)، مدخني الشيشة (82 فرد) وغير المدخنين (90 فرد). قيمت الدراسة كذلك المتناول من فيتامين ب$_6$، البروتين، نسبة ب $_6$ إلى البروتين و% لفيتامين ب $_6$ من المصادر الحيوانية. قيمت الدراسة كذلك بعض المؤشرات الكيموحيوية المؤثرة على حالة فيتامين ب $_6$ وشملت تركيز الألبيومين في البلازما وتقدير نشاط إنزيم الفوسفاتيز القاعدي في البلازما. دلت النتائج على أن متوسط المتناول من فيتامين ب $_6$ لمجموعة مدخني السجائر (85 فرد)، مدخني الشيشة (82 فرد) وغير المدخنين (90 فرد) كان 2.13±0.72، 2.47±0.77 و 2.09±0.69 ملجم/يوم على التوالي. متوسط تركيزات PLP في البلازما للمجاميع الثلاثة كان 26.2±3.2، 44.7±5.1 و 35.0±2.1 نانومول/لتر على التوالي. بينما كان متوسط المطروح في البول من 4-PA كان للمجاميع الثلاثة 3.8±1.8، 7.2±3.6 و 4.8±2,2 ميكرومول/يوم على التوالي. دلت المؤشرات التغذوية والكيموحيوية على كفاية الحالة لفيتامين ب$_6$ ومع ذلك وجد لدى مدخني الشيشة متوسط أعلى معنوياً للمتناول من فيتامين ب $_6$، تركيز PLP في البلازما والمطروح من 4-PA مقارنة بالمجموعتين الأخرى. الوفرة الحيوية لفيتامين ب$_6$ المتناول كانت أقل لدى مجموعة مدخني الشيشة مقارنة بالمجموعتين الأخرى كمقياس % المتناول من الفيتامين من المصادر الحيوانية في حين أن متوسط تركيز PLP في البلازما كان أقل بدرجة معنوية لدى مدخني السجائر مقارنة بغير المدخنين. تشير نتائج هذه الدراسة تشير إلى احتمالية عدم تأثير تدخين الشيشة على أشكال فيتامين ب $_6$ في الدم والبول كالتأثير الذي يسببه تدخين السجائر. من المحتمل أن الماء في الشيشة يقوم بحجز المواد الكيماوية الناتجة عن تدخين التبغ والمعروف أنها مؤثرة على أشكال فيتامين ب $_6$ في الدم والبول. تقترح الدراسة كذلك إجراء المزيد من البحث لمعرفة تأثير تدخين الشيشة على أشكال فيتامين ب$_6$ في الدم والبول مع تثبيت المتناول من الفيتامين.