Relationship Between Nicotine Dependence Scores and Nicotine, Cotinine, 3'-Hydroxycotinine and Nicotine Metabolite Ratio in Chinese Male Smokers *

by

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SUMMARY

Smoking is mainly sustained by nicotine dependence (ND), which varies across ethnic groups principally due to genetic as well as environmental factors. The Fagerström Test for Nicotine Dependence (FTND) and biomarkers of tobacco exposure are two important approaches to assess ND. However, the relationship between ND and FTND of Chinese smokers has not been studied. The aim of this study was to assess the relationship between FTND scores and nicotine, cotinine, 3'-hydroxycotinine (3HC) and nicotine metabolite ratio (NMR, the concentration ratio of 3HC to cotinine) in Chinese smokers. FTND was carried out and general characteristics were collected using a self-administered smoking questionnaire with 289 smokers. Nicotine, cotinine and 3HC in urine were simultaneously determined by liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). The concentrations of nicotine, cotinine and 3HC in the urine of smokers with a high FTND score were higher than in the urine of those with a low FTND score. There were significant correlations between urinary biomarker and FTND scores. Except for FTND item 2 (difficulty to refrain), the other items showed significant associations with the urinary biomarkers. No relationship was found between the nicotine metabolite ratio (NMR, 3'-hydroxycotinine/cotinine) and FTND scores or general characteristics of the participants. In conclusion,

biomarkers of tobacco exposure levels are significantly associated with FTND scores. However, FTND Item 2 and NMR were not found to be associated with nicotine dependence in Chinese smokers. [Beitr. Tabakforsch. Int. 29 (2020) 136–144]

KEY WORDS

Nicotine; cotinine; 3'-hydroxycotinine; nicotine-metabolite ratio; nicotine dependence

ZUSAMMENFASSUNG

Fortgesetztes Rauchen wird vor allem durch die Nikotinabhängigkeit (NA) begünstigt. Diese variiert je nach ethnischer Gruppe hauptsächlich aufgrund von genetischen sowie umweltbedingten Faktoren. Zwei wichtige Methoden zur Bestimmung der NA sind der Fagerström-Test für Nikotinabhängigkeit (FTNA) sowie Biomarker für Tabakexposition. In chinesischen Raucher*innen wurde die Beziehung zwischen NA und FTNA jedoch noch nicht untersucht. Das Ziel der vorliegenden Studie war die Bestimmung der Beziehung zwischen FTNA-Scores und Nikotin, Kotinin, 3'-Hydroxycotin (3HC) und dem Nikotin-Metaboliten-Verhältnis (NMR, Konzentrationsverhältnis von

3HC zu Kotinin) in chinesischen Raucher*innen. Mithilfe eines selbst auszufüllenden Rauchfragebogens wurde bei 289 Raucher*innen der FTNA durchgeführt und es wurden allgemeine Merkmale erhoben. Parallel wurde die Konzentration an Nikotin, Kotinin und 3HC im Urin mittels Flüssigkeitschromatographie in Verbindung mit Tandem-Massenspektrometrie (LC-MS/MS) bestimmt. Im Urin von Raucher*innen mit einem hohen FTNA-Score waren die Konzentrationen an Nikotin, Kotinin und 3HC höher als im Urin derjenigen mit einem niedrigen FTNA-Score. Es gab signifikante Korrelationen zwischen den Biomarkern im Urin und den FTNA-Scores. Mit Ausnahme von Frage 2 (Schwierigkeit aufs Rauchen zu verzichten) ergaben sich signifikante Unterschiede bei den Ergebnissen für die Biomarker im Urin und die Scores für die anderen FTNA-Fragen. Abgesehen von Frage 2 (Schwierigkeit auf das Rauchen zu verzichten) wurden signifikante Zusammenhänge zwischen den übrigen Fragen und den Biomarkern im Urin gezeigt. Es wurde keine Beziehung zwischen dem Nikotin-Metaboliten-Verhältnis (NMR, 3'-Hydroxycotin/ Kotinin) und den FTNA-Scores bzw. den allgemeinen Merkmalen der Teilnehmer*innen festgestellt. Zusammenfassend lässt sich sagen, dass die Konzentrationen an Biomarkern für Tabakexposition und die FTNA-Scores signifikant assoziiert sind. Jedoch konnte bei Frage 2 des FTNA sowie beim NMR kein Zusammenhang mit der Nikotinabhängigkeit in chinesischen Raucher*innen gezeigt werden. [Beitr. Tabakforsch. Int. 29 (2020) 136–144]

RESUME

Le tabagisme est principalement entretenu par la dépendance à la nicotine (DN), qui varie selon les groupes ethniques en raison surtout de facteurs génétiques ainsi qu'environnementaux. Le test de Fagerström quantifiant la dépendance à la nicotine (FTND) et les biomarqueurs de l'exposition à la nicotine constituent deux approches importantes en vue de l'évaluation de la DN. Toutefois, le lien entre la DN et le FTND des fumeur.euse.s chinois.es n'avait, à ce jour, pas été analysé. L'objet de la présente étude fut d'évaluer le lien entre les scores du FTND et la nicotine, la cotinine, la 3'-hydroxycotinine (3HC) et le ratio des métabolites de nicotine (NMR, à savoir le ratio de concentration de la 3HC par rapport à la cotinine) parmi les fumeur.euse.s chinois.es. Le FTND fut réalisé et des caractéristiques générales furent relevées à l'aide d'un questionnaire de fumage auto-administré auprès d'un échantillon de 289 fumeur.euse.s. La présence de nicotine, de cotinine et de 3HC dans l'urine fut déterminée simultanément par une chromatographie en phase liquide couplée à une spectrométrie de masse en tandem (LC-MS/MS). Les concentrations de nicotine, de cotinine et de 3HC dans l'urine des fumeur.euse.s présentant un score FTND élevé furent supérieures aux concentrations chez les fumeur.euse.s présentant un faible score FTND. Des corrélations significatives existent entre les biomarqueurs urinaires et les scores FTND. À l'exception de la question 2 (difficulté d'abstinence), la présente étude épingla des résultats significativement différents pour les marqueurs urinaires et les scores obtenus sur les autres questions du FTND. À l'exception de la question 2 (difficulté d'abstinence), les

autres questions mirent en lumière des associations significatives avec les biomarqueurs urinaires. Aucun lien ne fut trouvé entre le ratio des métabolites de nicotine (NMR ou 3'-hydroxycotinine/cotinine) et les scores du FTND ou les caractéristiques générales des participant.e.s. En conclusion, les niveaux de biomarqueurs de l'exposition au tabac sont significativement associés aux scores du FTND. Cependant, aucun lien ne fut observé entre la question 2 du test de Fagerström et le NMR, d'une part et la dépendance à la nicotine chez les fumeur.euse.s chinois.es, d'autre part. [Beitr. Tabakforsch. Int. 29 (2020) 136–144]

1. INTRODUCTION

Nicotine dependence (ND) is an extremely complex phenomenon with nicotine intake, genetic variability, personality traits, social pressure and environmental determinants (11, 12, 14, 25). The complex mechanisms of ND make comprehensive and accurate assessment of smokers' dependence challenging. From different perspectives, multiple approaches have been used to measure ND (11), such as the smokers' self-administered questionnaire method (standardized questionnaires), the objective method (tests the smokers' actual exposure to nicotine directly). The best-known measures are those developed by Fagerström (7). The Fagerström Test for Nicotine Dependence (FTND), which derives from the Fagerström Tolerance Questionnaire (FTQ), is a six-item self-reporting questionnaire reflecting a continuum of dependence. It is widely used to assess ND (21, 16, 17, 20). Derived from this questionnaire, the 'Heaviness of Smoking Index' (HSI) is a shorter validated version (7). Measurement of tobacco exposure biomarkers in plasma, urine and saliva is an objective approach by which ND can be assessed (14, 32, 19). Nicotine is mostly metabolised to cotinine and about 15% cotinine is excreted from the body in the urine (6). Nicotine is metabolised to cotinine by the major nicotine-metabolising CYP2A6 enzyme, and 3'-hydroxycotinine (3HC) is the main metabolite of cotinine, which accounts for about 50% of cotinine excretion (6, 18). NMR is a biomarker of CYP2A6 activity (31). As a genetically influenced biomarker of nicotine clearance, lower NMR appears to increase likelihood of successful cessation (22). Previous studies already found that NMR is related to cigarette consumption (5), quitting the smoking habit (18), and smoking behaviours (34). There is no generally accepted relationship between NMR and ND (29, 28, 32, 34). However, ND may depend on the ethnicity of various smoking populations (1). The value of NMR may be different in African, Northern European, and East Asian populations (18, 36). Some studies examined ND among Chinese smokers by use of only a self-reporting questionnaire (8, 11, 26, 35, 37, 38). To our knowledge, there are limited data available regarding NMR determined in urine and its relation to ND in Chinese male smokers. The aims of the current study were (a) to investigate the relationship between ND as measured by the FTND and biomarkers of tobacco exposure of daily smokers and (b) to determine whether the NMR was associated with ND in Chinese smokers.

2. MATERIALS AND METHODS

2.1 Participants

Daily smokers ($n = 289$) were recruited from Chengdu, Sichuan Province, China. All participants were adult male smokers, older than 18 years, who were in stable and generally good health and not taking medications. Because about 2% of Chinese women smoke (23) and nicotine metabolism is gender-dependent (4), this study only focussed on male smokers. Due to the chronic and stable condition of ND, eligible adult smokers have smoked at least five cigarettes per day for the past six months. All participants completed the informed consent form. The research and ethical committee of the West China School of Public Health, Sichuan University, approved the study protocol before data collection commenced.

2.2 Samples and data collection

A urine sample from each participant was collected, in collaboration with occupational medicine physicians. Subjects were provided with containers for cigarette butts, and 24-h urine was collected in polyethylene urine containers on ice prior to delivery to the lab. Urine samples were refrigerated at -20°C until sample analyses were carried out for nicotine, cotinine, 3HC and creatinine. The number of cigarettes smoked per day was documented by collecting cigarette butts and self-reported number of cigarettes. There was close agreement between self-reported CPD (number of cigarettes smoked per day) and butt collection. About 95% of the volunteers reported the same number of cigarettes. For about 5% of inconsistent volunteers (the number of cigarettes varied by about 1–3 cigarettes), we contacted the volunteers to confirm the final number of cigarettes. All the volunteers were asked to complete a self-reporting questionnaire. Information covered basic participant demographics, including sex, age, height, weight and education as well as smoking history information, such as the age when the volunteers first started smoking and the number of cigarettes smoked per day (CPD). FTND was used to assess tobacco dependence. The FTND score ranged from 0 to 10, with higher scores indicating a more marked ND.

2.3 Analysis of urine samples

Urine samples were thawed at 4°C and thoroughly mixed. Total nicotine, cotinine and 3HC in human urine were simultaneously determined using a method previously developed (40) by liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). This method made a simultaneous quantification of both the free and glucuronide-conjugated metabolites possible. Values were normalised by creatinine levels being adjusted for possible variability in the urine.

2.4 Data analysis/statistical analysis

Descriptive statistics were generated for the general characteristics of the participants and for urinary biomarker data. We used standard categories of BMI in analyses: underweight (< 18.5), normal ($18.5 - 23.9$), overweight ($24.00 - 27.9$), and obese (≥ 28). We calculated mean and

standard deviations of FTND scores, NMR and nicotine, cotinine and 3HC concentrations by individual characteristics: age, BMI, smoking years and CPD, taken from the questionnaires. Data was analysed using analysis of variance (ANOVA) for the comparison of means among different groups. If the detected data was not normally distributed, the Wilcoxon test was used to analyse biomarker data among different groups. We also assessed differences in each group of nicotine, cotinine, and 3HC concentrations according to each single FTND item. Potential correlations between FTND, nicotine, cotinine, 3HC, NMR, CPD, age and years of smoking were explored with the Spearman's rank correlation. $P < 0.05$ was considered statistically significant. All analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

Table 1 presents the general characteristics of participants and biomarkers of exposure levels. Of the total participants ($N = 289$), the mean score of the FTND, nicotine, cotinine, 3HC concentrations and NMR were 3.2, 1282.7 ng/mg creatinine, 1549.4 ng/mg creatinine, 3859.1 ng/mg creatinine, and 2.8, respectively. In terms of three age groups, 86 participants were younger than 35 (29.8%), 106 participants were aged from 35 years to 50 years (36.7%), and 97 participants were older than 50 years (33.6%). Each age group accounted for approximately one third of the participants. Among the three age groups, participants had a mean FTND score of 2.4, 3.3 and 3.6, respectively. Nicotine concentrations were 913.9, 1374.0 and 1510.1 ng/mg creatinine, respectively. FTND scores and nicotine concentrations were substantially different among the three age groups. However, none of the cotinine, 3HC and NMR showed statistical significance among the three age groups. We observed significant differences in nicotine and cotinine concentrations connected to different BMIs, with the lowest value in the obese group. No statistically significant differences in FTND scores, 3HC or NMR were found between the different BMI groups. In terms of groups by number of smoking years, both the FTND scores and the nicotine concentration levels showed significant differences, with the highest FTND scores and nicotine concentration levels in the group of smoking years ≥ 31 . No statistically significant differences in cotinine and 3HC concentration by smoking years were found. FTND scores and nicotine, cotinine and 3HC levels showed statistical significance among different CPD groups ($P < 0.05$). Participants who smoked more cigarettes per day had higher FTND scores and higher nicotine, cotinine and 3HC levels. No statistical significance in the NMRs was obtained among the four groups.

Correlation coefficients between measured biomarkers and variables obtained from questionnaires are shown in Table 2. There were significant correlations between FTND scores and nicotine, cotinine and 3HC concentrations. Each of the three biomarkers was considerably correlated with CPD, age and smoking years. Significant correlations were also observed between NMR and nicotine, but no correlations between NMR and other variables were found.

Table 1 . FTND score, nicotine, cotinine, 3HC concentration (ng/mg creatinine), and NMR according to general characteristics of the study population.

		N (%)	FTND mean (SD)	P value	Nicotine (ng/mg) mean (SD)	P value	Cotinine (ng/mg) mean (SD)	P value	3HC (ng/mg) mean (SD)	P value	NMR mean (SD)	P value
Total		289 (100)	3.2 (2.4)		1282.7 (1344.4)		1549.4 (1420.7)		3859.1 (4174.3)		2.8 (1.8)	
Age				0.002*		0.007*		0.385		0.245		0.736
	18–34	86 (29.8)	2.4 (2.1)		913.9 (993.8)		1382.9 (1230.9)		3379.4 (3449.1)		2.7 (1.8)	
	35–49	106 (36.7)	3.3 (2.4)		1374.0 (1544.5)		1573.7 (1673.9)		3754.6 (3544.6)		2.9 (2.1)	
	≥ 50	97 (33.6)	3.6 (2.5)		1510.1 (1324.9)		1670.5 (1268.5)		4398.6 (5242.7)		2.7 (1.6)	
BMI				0.988		0.048*		0.040*		0.209		0.214
	<18.5	13 (4.5)	3.2 (2.0)		1394.4 (1665.7)		1669.1 (1191.3)		3779.0 (2829.8)		3.1 (2.4)	
	18.5–23.9	144 (49.8)	3.2 (2.3)		1308.2 (1314.5)		1623.8 (1398.2)		4237.3 (4913.5)		2.7 (1.8)	
	24.00–27.9	99 (34.3)	3.1 (2.5)		1430.3 (1482.7)		1648.1 (1583.1)		3759.6 (3528.3)		2.6 (1.6)	
	≥28	33 (11.4)	3.2 (2.4)		685.6 (573.4)		881.5 (838.2)		2538.7 (2309.5)		3.3 (2.5)	
Smoking years				0.000*		0.005*		0.059		0.123		0.579
	≤15	91 (31.5)	2.2 (2.1)		908.8 (1244.2)		1270.1 (1273.3)		3118.1 (3651.7)		2.6 (1.7)	
	16–30	113 (39.1)	3.3 (2.3)		1469.2 (1340.6)		1740.9 (1614.3)		4195.9 (3605.5)		2.8 (1.8)	
	>31	85 (29.4)	4.1 (2.5)		1435.1 (1385.6)		1593.7 (1254.1)		4204.6 (5220.4)		2.9 (2.1)	
CPD				0.000*		0.002*		0.001*		0.000*		0.103
	≤10	95 (32.9)	1.2 (1.6)		890.6 (904.9)		1090.5 (1069.6)		2435.0 (2741.7)		2.6 (1.9)	
	11–20	160 (55.4)	3.9 (2.0)		1490.6 (1470.4)		1781.7 (1541.9)		4394.7 (3836.0)		2.7 (1.5)	
	≥21	34 (11.8)	5.3 (2.3)		1400.2 (1535.5)		1738.2 (1413.2)		5317.6 (7094.9)		3.4 (2.7)	

* Significant at $P < 0.05$; FTND: Fagerström Test for Nicotine Dependence; 3HC: 3'-Hydroxycotinine; NMR: Nicotine metabolite ratio;

BMI: Body Mass Index; CPD: Cigarettes smoked per day, SD: Standard deviation.

Table 2. Spearman correlation coefficients among measured biomarkers and variables obtained from questionnaires.

	FTND	Nicotine	Cotinine	3HC	CPD	Age	Smoking years	NMR
FTND	1.000	0.301**	0.371**	0.365**	0.615**	0.238**	0.317**	0.057
Nicotine	0.301**	1.000	0.702**	0.482**	0.163**	0.245**	0.264**	-0.204**
Cotinine	0.371**	0.702**	1.000	0.762**	0.251**	0.155**	0.197**	-0.153**
3HC	0.365**	0.482**	0.762**	1.000	0.293**	0.131*	0.186**	0.431**
CPD	0.615**	0.163**	0.251**	0.293**	1.000	0.228**	0.278**	0.075
Age	0.238**	0.245**	0.155**	0.131*	0.228**	1.000	0.897**	0.014
Smoking years	0.317**	0.264**	0.197**	0.186**	0.278**	0.897**	1.000	0.037
NMR	0.057	-0.204**	-0.153**	0.431**	0.075	0.014	0.037	1.000

*Significant at $P < 0.05$, **Significant at $P < 0.01$; FTND: Fagerström Test for Nicotine Dependence; 3HC: 3'-Hydroxycotinine; NMR: Nicotine metabolite ratio.

The geometric means (GMs) of nicotine, cotinine and 3HC concentrations, according to the single FTND items, are shown in Table 3. The lowest concentrations were found in the zero-score groups of the six items. The lowest concentrations were observed in smokers whose time to their first cigarette of the day was longer than 60 min after rising, who had no difficulty refraining from smoking, who least hated to give up the first one in the morning, who smoked fewer than 10 cigarettes per day, who smoked less in the first hours of the day and who had no difficulty refraining from smoking when ill or forbidden, although some of these differences were not statistically significant. Except for item 2 (difficulty refraining), which showed no

differences in nicotine, cotinine or 3HC concentrations, most of the FTND items showed higher concentrations at higher single scores.

We sought to compare the relationship between the FTND scores and cotinine, nicotine and 3HC levels in human urine. The FTND scores were categorised as low (score: 0–4) or high (score: 5–10) based on previously published studies (10). Of the 289 subjects, 197 (68.2%) were classified in the low-nicotine dependence group, and 101 (34.9%) in the high-nicotine dependence groups. Cotinine, nicotine and 3HC concentrations in the urine of participants with high FTND scores were significantly higher than in those with low FTND scores ($P < 0.05$) (Figure 1 and Table 4).

Table 4. Relationship between the FTND scores and cotinine, nicotine and 3HC levels in human urine.

Cotinine	low	1314.0 ± 1115.1 ng/mg creatinine
	high	1813.61 ± 1262.2 ng/mg creatinine
3HC	low	3086.9 ± 2899.6 ng/mg creatinine
	high	4930.3 ± 4033.8 ng/mg creatinine
Nicotine	low	1062.9 ± 1072.6 ng/mg creatinine
	high	1670.2 ± 1545.9 ng/mg creatinine

Figure 1. Comparison of cotinine, nicotine and 3HC levels in human urine by the FTND scores. Nicotine dependence groups (low, FTND score = 0 – 4; high, FTND score = 5 – 10); FTND: Fagerström Test for Nicotine Dependence; 3HC: 3'-Hydroxycotinine; * Significant at $P < 0.05$.

4. DISCUSSION

We found that the FTND scores differed significantly among different age, CPD and smoking-years groups. The higher the age/CPD/smoking years, the higher the FTND scores in this study population of daily smokers. We also found significant correlations between FTND scores and urinary biomarker concentrations (nicotine, cotinine, 3HC). The concentrations of nicotine, cotinine and 3HC in the urine of smokers with high FTND scores were higher than those with low FTND scores. All items except item 2 (difficulty refraining) showed differences in nicotine, cotinine and 3HC concentrations, with higher concentrations at higher single scores. And there was no association between the NMR and either the FTND or general characteristics such as basic information of participants, e.g., age, BMI, smoking years, number of cigarettes smoked per day, etc. These findings indicated that there were relationships between ND scores and nicotine, cotinine and 3HC. NMR possibly has no impact on ND in Chinese male smokers.

The current study shows differences in FTND scores based on age and years of smoking. This study suggests that those who started smoking at a younger age and had smoked for more years were more dependent on nicotine. One study in China demonstrated that FTND scores largely increased with age among daily smokers and also earlier smoking commencement was associated with higher ND (38). We also found that FTND scores showed statistical significance among different CPD groups. This conclusion is consistent with the results of BECONA *et al.* (2) who reported that Fagerström scores increased significantly with a rise in age and cigarette consumption in a Spanish population. The result of the FTND scores of smokers across different BMI groups showed that there was no statistically significant difference, which agrees with the conclusions of other studies (33). The mean FTND score in our study – 3.2 – is similar to that observed in other studies (32, 39). We also found that the NMR was not related to age, BMI, years of smoking and CPD, same with other studies (29, 34). Among the three age groups, nicotine concentrations were substantially different, whereas cotinine and 3HC showed no statistical significance. Previous studies (13, 30) found that the role of age in cotinine concentrations was still not clear, indicating no association between cotinine concentrations and age, whereas others found cotinine levels increased with increasing age (9, 24).

In the correlation analysis, correlation coefficients between the FTND scores and nicotine, cotinine and 3HC concentrations showed positive relationships; the higher the FTND scores, the higher the nicotine/cotinine/3HC concentrations. The correlation coefficients between the FTND scores and nicotine, cotinine and 3HC were 0.301, 0.371, 0.365, respectively, suggesting that FTND scores displayed a positive association with the levels of nicotine/cotinine/3HC. These results were consistent with previous studies (27, 32). NMR, as a reliable measure of CYP2A6 activity, has a role in ND (5, 18, 29). However, in our study, we did not find a significant correlation between FTND scores and NMRs, just in accordance with results reported in a previous study (34).

Table 3. Nicotine, cotinine, 3HC concentration (ng/mg creatinine) and NMR according to FTND.

FTND items:	N (%)	Nicotine		Cotinine		3HC		NMR	
		Mean (SD)	P	Mean (SD)	P	Mean (SD)	P	Mean (SD)	P
(1) Time to first cigarette		0.000*		0.000*		0.000*		0.763	
After 60 min (0 points)	107 (37.0)	850.2 (875.0)		1029.4 (935.2)		2457.2 (2579.9)		2.7 (1.8)	
31–60min (1 point)	61 (21.1)	1298.2 (1490.4)		1476.6 (1137.5)		3771.0 (3390.6)		2.9 (1.8)	
6–30min (2 points)	53 (18.3)	1487.7 (1299.1)		2002.0 (1754.2)		5329.7 (6596.0)		2.6 (1.7)	
Within 5 min (3 points)	68 (23.5)	1789.8 (1632.7)		2078.0 (1691.6)		4997.8 (3760.0)		2.9 (2.0)	
(2) Difficult to refrain from smoking		0.08		0.297		0.800		0.721	
No (0 points)	223 (77.2)	1207.5 (1228.1)		1501.9 (1284.1)		3893.1 (4374.4)		2.8 (1.7)	
Yes (1 point)	66 (22.8)	1536.9 (1665.0)		1710.0 (1810.1)		3744.2 (3440.0)		2.7 (2.2)	
(3) Hating most to give up smoking		0.002*		0.000*		0.010*		0.729	
All others (0 points)	200 (69.2)	1121.4 (1303.9)		1316.3 (1225.0)		3440.0 (4370.7)		2.8 (1.9)	
The first one in the morning (1 point)	89 (30.8)	1645.3 (1370.6)		2073.2 (1676.8)		4800.9 (3540.0)		2.7 (1.7)	
(4) Cigarettes per day		0.005*		0.002*		0.000*		0.034	
10 or fewer (0 points)	97 (33.6)	893.3 (902.0)		1098.0 (1065.4)		2415.6 (2721.3)		2.6 (1.9)	
11–20 (1 point)	153 (52.9)	1508.3 (1490.3)		1800.5 (1577.4)		4443.1 (3911.3)		2.7 (1.6)	
21–30 (2 points)	28 (9.7)	1403.1 (1655.7)		1690.8 (1249.3)		5319.7 (7599.2)		2.9 (1.7)	
31 or more (3 points)	11 (3.8)	1273.0 (889.6)		1677.3 (1478.8)		4747.4 (3164.9)		4.3 (3.7)	
(5) Smoking more in the first hours		0.033*		0.038*		0.131		0.158	
No (0 points)	244 (84.4)	1210.5 (1311.6)		1475.1 (1362.5)		3700.0 (4278.5)		2.7 (1.6)	
Yes (1 point)	45 (15.6)	1674.2 (1464.0)		1952.1 (1662.2)		4722.8 (3471.3)		3.1 (2.7)	
(6) Smoking when ill		0.006*		0.005*		0.000*		0.353	
No (0 points)	189 (65.4)	1125.7 (1188.8)		1377.9 (1302.0)		3176.5 (2832.8)		2.7 (1.8)	
Yes (1 point)	100 (34.6)	1579.6 (1561.5)		1873.6 (1578.2)		5149.2 (5734.1)		2.8 (2.0)	

*Significant at $P < 0.05$; FTND: Fagerström Test for Nicotine Dependence; 3HC: 3'-Hydroxycotinine; NMR: Nicotine metabolite ratio;

The data shows that the time between rising and smoking the first cigarette of the day, hating most to give up smoking, number of cigarettes smoked per day, smoking more in the first hours of the day and smoking when ill are associated with nicotine, cotinine and 3HC concentrations. The shorter the time between waking up and smoking the first cigarette of the day, the higher the concentrations of nicotine, cotinine and 3HC in the urine. These results agree with the findings described by FU *et al.* (15).

After adjustment for the number of cigarettes smoked in the previous 24 h, the time to the first cigarette of the day is associated with cotinine concentration (15). The higher the number of cigarettes smoked, the higher the cotinine concentration. One likely reason is that smokers regulate their smoking behaviour to compensate for variations of nicotine levels in the body (3). Thus, smokers in the highly dependent group may smoke more cigarettes per day and during the first minutes of the day, may hate most to give up the first cigarette in the morning and smoke even when ill, resulting in higher nicotine, cotinine and 3HC concentrations compared with low-dependent smokers. The concentrations of nicotine, cotinine and 3HC in the urine of smokers of high FTND scores were higher than in those with low FTND scores.

In conclusion, our data confirm the association between FTND scores and levels of nicotine, cotinine and 3HC in Chinese male smokers. Smokers' ND may not be affected by the NMR. FTND item 2 (difficulty refraining) was not found to be related to ND in Chinese smokers. Further studies are necessary to investigate the potential role of NMR and FTND item 2 (difficulty refraining) in large numbers of Chinese smokers.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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