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Original Research Article

The interplay between nicotine metabolites and reproductive hormones in women beedi rollers occupationally exposed to tobacco dust

Spandhana Racharla, Shehnaz Sultana, P. P. Reddy*

Department of Genetics, Bhagwan Mahavir Medical Research Centre, Hyderabad, Telangana, India

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***Correspondence:**
Dr. P. P. Reddy,
E-mail: pardhananda.reddy@gmail.com

ABSTRACT

Background: Although beedi-rolling is a convenient, home-based occupation for many women from lower socio-economic backgrounds, the strenuous and time-consuming process exposes beedi-rollers to tobacco dust for prolonged periods. This study aimed to investigate the effects of long-term exposure to tobacco dust on beedi-rollers and to examine the impact of nicotine metabolites on hormonal levels.
Methods: The study included 320 beedi-rollers (BR) and 280 control subjects (NBR). Signed consent forms were collected from all participants before conducting face-to-face interviews and blood collection. Serum was isolated from the blood samples to estimate reproductive hormones and nicotine metabolites using ELISA and LC-MS, respectively.
Results: Significant differences in hormone and nicotine metabolite levels were observed between the BR and NBR groups. The BR group was further divided into two subgroups based on years of service. Anabasine and 4-Methylnitrosamino-1-3- pyridyl-1 butanone (NNK), levels were significantly higher in beedi-rollers with more than 10 years of service. Increased anabasine and Cotinine levels were significantly correlated with reduced reproductive hormone levels.
Conclusions: Long-term exposure to tobacco dust leads to elevated nicotine metabolite levels, which may disrupt hormonal function and levels. Further research with a larger sample size is needed to better understand the impact of nicotine metabolites on the reproductive health of beedi-rollers.

Keywords: Tobacco dust, Reproductive hormones, Nicotine metabolites

INTRODUCTION

In India, smoking is not the primary route of tobacco exposure for women; instead, occupational exposure and the use of unburnt tobacco are the main sources. Occupational exposure occurs primarily through beedi-making, one of India's largest agro-based industries, which provides home-based employment for rural women from low socio-economic backgrounds.[1] Beedi rolling is a strenuous, time-consuming process that exposes workers to unburnt tobacco dust for extended periods. While the health effects of tobacco smoke are well-studied, the impact of unburnt tobacco dust from occupational exposure remains less understood.[2]

Nicotine, the main alkaloid in tobacco, is well known for its addictive properties and adverse health effects.[3] However, nicotine's influence extends beyond its addictive nature, affecting multiple physiological processes, including the endocrine system.[4] Nicotine is rapidly metabolised in the body, yielding several metabolites, some of which have been shown to interact with hormone pathways.[5] Hormones can modulate the metabolism and effects of nicotine. Both nicotine and its metabolites have been implicated in various physiological processes, including hormone regulation. Understanding the interplay between nicotine metabolites and hormones is crucial for comprehensively assessing the health consequences of

nicotine exposure, particularly in vulnerable populations such as women engaged in beedi-rolling occupations.[6]

In the present study, we aimed to examine the influence of nicotine metabolites on hormonal fluctuations in women beedi rollers. We also investigated the effects of long-term exposure to tobacco dust on hormonal and nicotine metabolite levels in beedi rollers compared to control subjects.

METHODS

Study subjects

The present study was carried out at the Genetics Department of Bhagwan Mahavir Medical Research Centre from July 2021 to August 2024. The study involved a cohort of 320 women employed as beedi rollers and 280 women without occupational exposure to chemicals for comparison. The study encompassed individuals aged between 15 and 50 years. Following the acquisition of informed consent from each participant, a standard questionnaire was utilized to collect information on various aspects, including age, gender, marital status, living conditions, habits, work duration, socioeconomic status, daily working hours, tobacco usage, and both present and past health history. Exclusions from the study involved individuals who consumed pan, gutka, or tobacco, and those with chronic diseases. For comparison, women residing in the same areas with similar socio-economic status, but without occupational exposure to any chemical, including tobacco dust, were selected (control group). This study was approved by the Institutional Ethics Committee (IEC) of Bhagwan Mahavir Medical Research Centre. All the participants provided their consent.

Sample collection

8 ml of venous blood was collected from all the participants in the early morning after overnight fasting. The blood samples were transferred to a plain vacutainer to separate the serum. The isolated serum samples were stored at -20º C before use.

Estimation of hormonal levels

Serum samples were brought to room temperature before analysis. FineTest USA ELISA kits were used to estimate hormonal levels. Competitive ELISA was employed to estimate estrogen, progesterone, and testosterone, while Sandwich ELISA was used for LH and FSH analysis.

Estimation of nicotine metabolites

The study involved the assessment of five nicotine metabolites Cotinine, Anabasine, Nornicotine, Nitrosonornicotine (NNN), and 4-(methylnitrosamino)-1-(3-pyridyl)-1 butanone (NNK) in 320 samples from beedi rollers and 280 samples from individuals not engaged in beedi rolling. The nicotine metabolites in beedi rollers were quantified through Liquid Chromatography Coupled with Mass Spectrometry (LCMS). All serum samples from both the study and control groups underwent Nicotine metabolite analysis using the Quattro premier XE system (Waters Systems, USA) and the Acquity UPLC system (Waters Systems, USA) as a front-end (LC). Nicotine metabolites were quantified using LC-MS/MS with the Positive ESI Method.

Sample preparation

Samples were prepared by weighing and transferring 1.0 mg of nicotine metabolites in 1.0 mL of DMSO to achieve a concentration of 2 mg/ml. The standard was dissolved in 1.000 ml of methanol, resulting in a final concentration of 1.00 mg/ml. Individual metabolites were combined to form a mixed standard. Adequate separation was achieved using an Agilent Zordax-XDB column (C18 2.1 × 50 mm, 3.5 µm). Mobile phase A consisted of 100% acetonitrile (approximately 1000 ml), while mobile phase B was comprised of approximately 500 mL of HPLC-grade water. To this, 126 mg of ammonium formate and 2 mL of formic acid were added, and the volume was adjusted to 1000 ml with water.

Sample analysis

Stored serum samples were allowed to reach room temperature. The mixed nicotine metabolite spiking solutions were added to the samples, followed by vortexing to ensure complete mixing. A 20 µl solution of 50% methanol in water was added to a ria vial and labelled as blank. Subsequently, 20 µl of ISTD (Verapamil-2 µg/ml) was added to all pre-labelled ria vials (except blank), and 100 µl of respective CC and QC samples, and study samples were added and vortexed. Further, 0.250 mL of acetonitrile was added to all samples, followed by vortexing and centrifugation at 4000 rpm, at 20°C for 10 min. The supernatant layer (0.150 ml) was separated and loaded into auto-injector vials. Finally, 10 µl was injected into the LC-MS/MS system, and the samples were processed for the quantification of metabolites. The data produced in the study underwent analysis utilizing SPSS software along with other statistical tools available online.

Statistical analysis

In this study, statistical analysis was performed to examine the differences in hormonal levels and nicotine metabolite levels between beedi rollers and control subjects. The beedi rollers were divided into two groups based on the duration of service, and these groups were compared with each other and with control subjects to understand the impact of exposure to tobacco at different time intervals on hormonal levels and nicotine metabolites. Data for hormonal levels and nicotine metabolites are presented as mean with standard error of the mean (SEM). A two-tailed t-test was conducted using GraphPad to calculate p-values, assessing the significance of group differences. Correlation analysis was performed in SPSS version 30 to

investigate the relationships between hormone levels and nicotine metabolites, with results reported as beta (β) values and p-values, providing insight into the strength and significance of these associations.

RESULTS

In this present study, we attempted to understand the effect of duration of service and nicotine metabolites on the reproductive hormone levels in beedi rollers compared to control subjects.

Table 1 presents the demographic data of the participants. The majority of the beedi-rollers (BR) and non-beedi rollers (NBR) belonged to the 21-30 age group and were married and non-vegetarians.

Table 2 shows various nicotine metabolites and hormone levels in the BR and NBR groups, and the results revealed significant differences in nicotine metabolites and hormonal levels between the two groups. The BR group had notably higher levels of anabasine, N-Nitrosonornicotine (NNN), 4- Methylnitrosamino-1-3-pyridyl-1 butanone (NNK), nornicotine, cotinine, and estrogen compared to the NBR group. In contrast, progesterone levels were lower in the BR group than in the NBR group. However, there was no significant difference in testosterone levels between the two groups. For LH and FSH, the BR group exhibited higher levels than the NBR group.

Table 1: Demographic details of the study population.

Variable	NBR, n=280 (%)	BR, n=320 (%)	P value
Age group (years)			
15-20	28 (10)	5 (1.56)	<0.001**
21-30	142 (50.71)	169 (52.8)	
31-40	65 (23.2)	94 (29.37)	
41-49	33 (11.78)	50 (15.62)	
50	12 (4.258)	2 (0.62)	
Marital status			
Married	246 (87.86)	308 (96.25)	<0.001**
Un-married	34 (12.14)	12 (3.75)	
Food habits			
Vegetarian	19 (6.8)	10 (3.1)	0.05*
Non-vegetarian	261 (93.2)	310 (96.9)	

**Highly significant; *Significant

Table 2: Distribution of nicotine metabolites and hormones in BR and NBR groups.

Parameters	BR(n=320) (Mean±SEM) (Range)	NBR(n=280) (Mean±SEM) (Range)	95% CI	P value
Anabasine (ng/ml)	7.29±0.44	1.72±0.15	3.61, 5.53	<0.001**
N-Nitrosonornicotine (NNN) (ng/ml)	2.13±0.17	0.12±0.04	1.64, 2.37	<0.001**
4- Methylnitrosamino-1-3- pyridyl-1 butanone (NNK) (ng/ml)	0.35±0.10	0.03±0.01	0.11, 0.53	0.003**
Nornicotine (ng/ml)	0.87±0.10	0.004±0.002	0.66, 1.08	<0.001**
Cotinine (ng/ml)	122.17±13.89	6.34±1.10	86.58, 145.08	<0.001**
Estrogen (pg/ml)	101.73±15.87	2.96±0.32	65.43, 132.11	<0.001**
Progesterone (ng/ml)	2.49±0.23	7.42±0.11	-5.43, -4.41	<0.001**
Testosterone (ng/ml)	4.91±0.55	4.70±0.51	-1.28, 1.70	0.78ns
LH (mIU/ml)	6.83±0.40	5.26±0.22	0.64, 2.50	0.001**
FSH (mIU/ml)	25.25±3.84	2.48±0.10	14.70, 30.84	<0.001**

**Highly significant; *Significant; nsNot Significant

We segregated the BR group into two subgroups based on the years of service; <10 years of service and >10 years of service. It allows us to understand the effect of long-term exposure on hormonal and nicotine metabolite levels (Table 3). The results showed a significant increase in Anabasine levels in beedi rollers with more than 10 years of service. In contrast, no significant difference was noted for NNN, Nornicotine, Cotinine, and Estrogen levels

between the two-time intervals. However, NNK levels were significantly higher in the beedi rollers with >10 years of service. Progesterone levels showed a near-significant decrease in individuals with more than 10 years of service. There were no significant differences in Testosterone, LH, and FSH levels between the two-time intervals.

Table 3: Distribution of nicotine metabolites and hormone levels in the BR group with duration of exposure.

Parameters	<10 Years of service (n=210)	>10 Years of service (n=110)	P value
Anabasine (ng/ml) Mean ± SEM (95% CI of mean)	6.26±0.51 (5.26, 7.26)	9.25±0.81 (7.64, 10.87)	0.001**
NNN (ng/ml) Mean ± SEM (95% CI of mean)	1.98±0.22 (1.56, 2.41)	2.42±0.29 (1.84, 3.00)	0.23^{ns}
NNK (ng/ml) Mean ± SEM (95% CI of mean)	0.16±0.06 (0.05, 0.28)	0.71±0.26 (0.21, 1.22)	0.01*
Nornicotine (ng/ml) Mean ± SEM (95% CI of mean)	0.86±0.13 (0.61, 1.11)	0.88±0.16 (0.56, 1.21)	0.92^{ns}
Cotinine (ng/ml) Mean ± SEM (95% CI of mean)	115.30±17.47 (80.85, 149.74)	135.29±22.87 (89.97, 180.61)	0.50^{ns}
Estrogen (pg/ml) Mean ± SEM (95% CI of mean)	113.58±23.64 (66.98, 160.18)	79.11±9.61 (60.07, 98.16)	0.30^{ns}
Progesterone (ng/ml) Mean ± SEM (95% CI of mean)	2.79±0.31 (2.18, 3.41)	1.91±0.28 (1.36, 2.46)	0.06^{ns}
Testosterone (ng/ml) Mean ± SEM (95% CI of mean)	4.98±0.63 (3.74, 6.22)	4.76±1.05 (2.68, 6.85)	0.85^{ns}
LH (mIU/ml) Mean ± SEM (95% CI of mean)	6.46±0.47 (5.52, 7.39)	7.54±0.75 (6.06, 9.03)	0.20^{ns}
FSH (mIU/ml) Mean ± SEM (95% CI of mean)	25.01±4.56 (16.02, 34.00)	25.71±7.01 (11.81, 39.60)	0.93^{ns}

** Highly significant; * Significant; ^{ns}Not Significant

Table 4: Association between hormones and nicotine metabolites in the BR group.

Estrogen (pg/ml)					
Variable	**β**	**S.E**	**Standardized β**	**t**	**P value**
Anabasineng/ml	0.00	0.00	-0.14	-2.58	0.01*
NNN ng/ml	0.00	0.00	0.00	-0.07	0.94^{ns}
NNK ng/ml	0.00	0.00	-0.02	-0.31	0.76^{ns}
Nornicotineng/ml	0.00	0.00	0.04	0.77	0.44^{ns}
Cotinine ng/ml	0.10	0.05	0.12	2.13	0.03*
Progesterone (ng/ml)					
Variable	**β**	**S.E**	**Standardized β**	**t**	**P value**
Anabasineng/ml	-0.25	0.10	-0.13	-2.47	0.01*
NNN ng/ml	0.03	0.04	0.04	0.65	0.52^{ns}
NNK ng/ml	-0.03	0.02	-0.06	-1.02	0.31^{ns}
Nornicotineng/ml	0.00	0.03	0.00	-0.08	0.94^{ns}
Cotinine ng/ml	6.70	3.40	0.11	1.97	0.05*

Continued.

Estrogen (pg/ml)					
Testosterone (ng/ml)					
Variable	**β**	**S.E**	**Standardized β**	**t**	**P value**
Anabasineng/ml	0.08	0.04	0.10	1.92	0.06^{ns}
NNN ng/ml	0.03	0.02	0.11	1.91	0.05*
NNK ng/ml	0.00	0.01	-0.01	-0.11	0.91^{ns}
Nornicotineng/ml	0.01	0.01	0.07	1.27	0.21^{ns}
Cotinine ng/ml	0.78	1.41	0.03	0.55	0.58^{ns}
LH (mIU/ml)					
Variable	**β**	**S.E**	**Standardized β**	**t**	**P value**
Anabasineng/ml	-0.18	0.06	-0.16	-2.98	0.00**
NNN ng/ml	0.03	0.03	0.08	1.30	0.20^{ns}
NNK ng/ml	-0.01	0.01	-0.03	-0.47	0.64^{ns}
Nornicotineng/ml	0.01	0.01	0.05	0.84	0.40^{ns}
Cotinine ng/ml	3.87	1.95	0.11	1.98	0.05*
FSH (mIU/ml)					
Variable	**β**	**S.E**	**Standardized β**	**t**	**P value**
Anabasineng/ml	-0.02	0.01	-0.19	-3.54	0.00**
NNN ng/ml	0.00	0.00	-0.01	-0.23	0.82^{ns}
NNK ng/ml	-0.03	0.02	-0.06	-1.02	0.31^{ns}
Nornicotineng/ml	0.00	0.00	-0.08	-1.32	0.19^{ns}
Cotinine ng/ml	-0.27	0.20	-0.08	-1.34	0.18^{ns}

** Highly significant; * Significant; ^{ns}Not Significant

Our investigation focused on understanding how nicotine metabolites influence hormone levels in the body. Our findings revealed significant associations, particularly with anabasine. We observed that higher levels of anabasine were linked to decreased levels of key reproductive hormones, including Estrogen (β = -0.14, p = 0.01), Progesterone (β = -0.13, p = 0.01), Luteinizing Hormone (LH) (β = -0.16, p = 0.00), and Follicle-Stimulating Hormone (FSH) (β = -0.19, p = 0.00). Cotinine levels showed a significant positive association with Estrogen (β = 0.12, p = 0.03), Progesterone (β = 0.11, p = 0.05), and LH (β = 0.11, p = 0.05), suggesting a potential stimulatory effect on these hormone levels. We also observed a significant positive association between NNN and Testosterone (β = 0.11, p = 0.06), indicating a possible influence on androgenic pathways. However, we did not find significant associations between Nornicotine, NNK, and hormone levels.

DISCUSSION

Our study provides a comprehensive analysis of the relationship between tobacco exposure, nicotine metabolites, and hormone levels among the study population. To our knowledge, this is the first study conducted to understand the relationship between nicotine metabolites and hormones in beedi-rollers. In the analysis, the distribution of nicotine metabolites and hormone levels was compared between beedi-rollers (BR) and non-beedi-rollers (NBR). We observed significant differences in hormonal levels and also observed significantly increased levels of nicotine metabolites in the BR group compared to NBR.

Similarly, Srinivasan et al examined the reproductive hormone levels among female workers in tobacco-processing facilities and observed disturbances in estrogen and progesterone levels in female workers with prolonged exposure to tobacco, indicating potential reproductive health implications associated with occupational tobacco exposure.[7] In another study, Lee et al investigated urinary levels of nicotine metabolites among workers in a tobacco-processing factory and showed elevated concentrations of nicotine metabolites in workers compared to non-smoking controls.[8]

The present investigation further focused on understanding how the duration of service impacts hormonal and nicotine metabolite levels. We observed increased levels of anabasine and NNK in beedi-rollers who have been in service for more than 10 years. Similar to our observations, Mendes et al examined nicotine metabolites in hair samples of workers exposed to tobacco smoke in hospitality venues. The study found significantly increased levels of nicotine metabolites in workers with longer duration of service, suggesting a potential biomarker for assessing long-term exposure.[9]

We also investigated how nicotine metabolites affect hormone levels in female workers. Significant associations were found, particularly with anabasine. Cotinine levels showed a significant positive association with progesterone, estrogen, and LH, suggesting a possible stimulatory effect on these hormones. However, no significant associations were found between nornicotine, NNN, NNK, and hormone levels. Rehan et al. (2020) described how nicotine and cotinine contribute to

hormonal imbalances in smokers. They observed that nicotine and cotinine can form hydrophobic interactions with amino acid residues in the ligand-binding pockets of estrogen, progesterone, and androgen receptors. This interaction disrupts steroid hormone signalling, ultimately affecting hormonal regulation.[10]

Our findings highlight the complexity of nicotine's effects on endocrine function, emphasizing the need for further investigation into the underlying mechanisms driving these associations. Such insights are crucial to take up public health initiatives and clinical interventions aimed at mitigating the adverse effects of nicotine exposure on hormonal balance and reproductive health.

CONCLUSION

This study examines the effects of nicotine metabolites on hormonal regulation in women working as beedi rollers. Our findings show significant differences in nicotine metabolite and reproductive hormone levels between beedi rollers and control subjects. Higher levels of anabasine and cotinine were linked to alterations in progesterone, estrogen, and LH levels. The duration of exposure to tobacco dust influenced nicotine metabolite levels but did not significantly affect all hormone levels. These results suggest that exposure to nicotine metabolites may disrupt hormonal balance, highlighting the need for further research on the health impacts of tobacco dust in occupational settings.

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