

Research Article

Occupational Exposure to Unburnt Tobacco Dust and Thyroid Function: A Case-Control Study

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Introduction

Beedi, a popular tobacco product in India, consists of tobacco wrapped in a dried tendu leaf and secured with a thread. It is a significant source of employment for rural and illiterate women, who often roll beedis at home [1]. These workers, typically exposed to unburnt tobacco dust for 5-6 hours daily, face significant health risks due to toxic components such as nicotine, cotinine, and formaldehyde [2,3]. Studies show that beedi rollers suffer from a range of health issues including chronic bronchitis, tobacco-related cancers, and respiratory problems [4,5,6]. Additionally, they are at higher risk for infertility, tuberculosis, and gynaecological problems [7,8].

Nicotine in tobacco converts largely to cotinine, a stable metabolite with a long half-life, affecting thyroid function by binding to Thyroid-Binding Globulin (TBG) and disrupting thyroid hormone levels [9]. Thyroid hormones (T3 and T4) are regulated by Thyroid-Stimulating Hormone (TSH), and alterations in these hormones can lead to various health issues, including thyroid disorders and systemic conditions like diabetes and cancer [10,11]. While extensive research has explored the impact of smoking on thyroid function, there is a lack of studies spe-

Abstract

Beedi rolling is one of the most common home-based occupations used to meet daily expenses. The process of making beedis is mainly done by women, especially in rural areas. Due to a lack of awareness regarding safety measures, beedi-rolling women are exposed to toxic components present in unburnt tobacco dust. This exposure leads to many health complications among women beedi-rollers. In this study, we investigated how unburnt tobacco dust disrupts thyroid gland homeostasis by altering levels of thyroid hormone and TSH. We included 421 beedi rolling women (BR) and 426 control subjects (NBR) who were not exposed occupationally to any chemical agents. Levels of thyroid hormones (T3, T4) were analysed using competitive ELISA, and TSH levels were analysed using sandwich ELISA. Serum nicotine metabolites were analysed using LC-MS. We observed a statistically significant increase in the levels of T3, T4, anabasine, nornicotine, and cotinine in BR compared to the NBR group. Additionally, significantly lower TSH levels were observed in the BR group. However, the Correlation between nicotine metabolites, and hormones did not show a significant difference in the BR group. We conclude that prolonged exposure to unburnt tobacco dust must have disrupted thyroid function by altering the thyroid hormone, and TSH levels.

Keywords: Tobacco dust; Nicotine metabolites; Thyroid function

cifically addressing the effects of unburnt tobacco exposure on thyroid health in women beedi rollers.

Materials and Methods

Study Population

The present study comprises 847 participants, including 421 beedi rolling women (BR) exposed to unburnt tobacco dust and 426 control subjects (NBR). We have carried out studies in women beedi rollers from different villages in Jagityal district, Demographic data and blood samples were collected from women beedi rollers and control subjects after taking their written consent forms. A questionnaire was prepared; to collect information on demographic data such as age, gender, educational status, marital status, income, health problems etc.

Inclusion Criteria

Women aged 15-50 years were enrolled for participation in the present study. Women who have not been exposed occupationally to other chemicals including agriculture chemicals and radiation were included for comparison (control group).

Exclusion Criteria

The present study excluded women aged above 50 years, as well as those currently under hormone treatment. Pregnant women were also excluded. Those not consent to participate were also excluded from the study.

Sample Collection

Blood collection was done in the early morning after overnight fasting. 8ml of blood was collected into EDTA-coated vacutainer and plain vacutainer. Serum was isolated from the plain vacutainer and stored at -80°C in fresh centrifuge tubes.

Measurement of Thyroid Hormones and TSH

Total T3, total T4, and TSH in serum were measured using Enzyme-linked immunosorbent assay (ELISA) (Fine test, USA) kits. Competitive ELISA was used to assess TT3 and TT4 levels, while sandwich ELISA was used for TSH.

Measurement of Serum Cotinine, Anabasine, and Nicotine Levels

Serum cotinine, anabasine, and nornicotine levels were measured using liquid chromatography-mass spectrometry (LC-MS) with a positive ESI method. Quattro Premier XE (Waters Systems, USA) and Acquity UPLC (Waters Systems, USA) system as a Front End (LC) used for the analysis of nicotine metabolites.

Preparation of Calibration Curve Standards and Quality Control Sample

For standard samples, we added 1mg of nicotine metabolites to 1 mL of methanol to obtain the final concentration of 1mg/mL. All the individual standards were mixed to form mixed standards. For the stock solution, 2mg of verapamil was added to 1 mL of Dimethyl Sulfoxide (DMSO) to obtain the concentration of 2mg/mL. These standards were further serial diluted to get concentrations ranging from 10 ng/mL to 10000 ng/mL and labelled as CC8 to CC1 (Table 1).

Sample Preparation

Retrieved serum blank and Study serum samples, from the deep freezer and allowed them to reach room temperature. 20 µL of 50% Methanol was added in water to a ria vial and labelled as blank. 20 µL of ISTD (Verapamil-2µg/mL) was added to all the pre-labelled ria vials (except blank), then transferred 100 µL of respective CC samples, study samples and vortexed.

Added 0.250 mL of Acetonitrile to all samples and Vortexed, centrifuged at 4000 rpm, 20°C for 10 min. Separated supernatant layer of 0.15 ml and loaded into auto-injector vials and Injected 10 µL onto LC-MS/MS system and processed the samples for quantification of metabolites.

Table 1: Column curve standard dilution.

Preparation of spiked CC's in human serum					
Mix stock conc. (µg/mL)	The volume of stock mL	The volume of Human serum	Final volume (mL)	Mix final conc. (ng/mL)	Label
100	0.02	0.18	0.2	10000	CC8
80	0.02	0.18	0.2	8000	CC7
50	0.02	0.18	0.2	5000	CC6
10	0.02	0.18	0.2	1000	CC5
5	0.02	0.18	0.2	500	CC4
1	0.02	0.18	0.2	100	CC3
0.2	0.02	0.18	0.2	20	CC2
0.1	0.02	0.18	0.2	10	CC1

CC-Column Curve

Statistical Analysis

Beedi rollers were categorised into 2 groups based on years of exposure, Group 1: exposed for less than 10 years and Group 2: exposed for more than 10 years. Thyroid hormone levels (TT3 and TT4), TSH, and nicotine metabolite levels were shown as mean with standard error of the mean. Multivariate linear regression analysis for the controls and two groups of beedi rollers was done using SPSS-IBM version 20 to understand the association between years of exposure and thyroid hormone, TSH, and nicotine metabolite levels were adjusted for age. *P*-value was calculated using graph pad t-test calculator version 10.2.3.

Results

In the present study, we investigated the levels of thyroid hormones (TT3 and TT4), TSH Beedi Rollers (BR) exposed to unburnt tobacco at different time intervals and control subjects (NBR). As shown in Table 2, we classified BR and NBR into three groups based on age (<25 years, 25-45 years, and >45 years). The mean (\pm SEM) age of the BR group is 36.20 \pm 9.47, and the

Table 2: Demographic data of BR and NBR.

Variable	BR (n=421) (%)	NBR (n=426) (%)	X ²	P value
Age (Mean \pm SD)	36.20 \pm 9.47	28.28 \pm 8.65	NA	
Age Groups (Years)				
<25	109 (25.9)	225(52.8)	64.4	<0.001**
25-45	238(56.5)	156(36.6)		
>45	74(17.6)	45(10.6)		
Marital Status				
Unmarried	36(8.55)	106 (24.8)	40.5	0.01**
Married	385 (91.45)	320(75.2)		
Educational Status				
Illiterate	152 (36.10)	51(11.9)	187.9	<0.001**
Primary (5th Std)	221(52.5)	143 (33.6)		
Secondary (10 th Std)	48 (11.4)	232 (54.5)		
Occupation			847	<0.001**
Beedi Roller	421(100.0)	0(0)		
Other Private Job	0(0)	72 (16.9)		
Housewife / Homemaker	0(0)	283 (66.43)		
Student/Others	0(0)	71 (16.6)		
Income/Month				
<1000	14 (3.3)	0(0)	347.2	0.000**
1000-5000	294(69.8)	47 (11)		
>5000	21 (5)	25 (5.9)		
Not reported	92 (21.9)	354(83.1)		
Years of exposure				
<10 years	183(43.5)	NA		0.000**
>10 years	238(56.5)			
Hours of exposure				
<5 hours	172(40.85)	NA		0.000**
>5 hours	249(59.15)			

**-Highly Significant; NA-Not Applicable

mean(\pm SEM) age of the NBR group is 28.28 \pm 8.65. The majority of BR belongs to the 25-45 years age group (56.5%), whereas NBR (52.8%) belongs to the <25 years age group. 91.45% of BR and 75.2% of NBR were married. Most of the BR completed only primary education (52.5%) while a high percentage of NBR had secondary education (54.5%). 69.8% of the beedi rollers earn between Rs. 1000-5000/- per month, while the majority of the NBR (66.43%) are settled as housewives. None of the NBR were occupationally exposed to tobacco. Women beedi rollers were categorized into two groups based on duration of service. (< 10 years of service and > 10 years of service) and hours of handling (<5 hours of exposure and >5 hours of exposure per day) of tobacco. The majority of BR (56.5%) had more than 10 years of service, and 59.15% worked more than 5 hours per day.

Table 3 presents BR and NBR's thyroid hormone, TSH, and nicotine metabolite levels. TT3 was significantly elevated in BR (129.91 \pm 1.04 ng/dL) compared to NBR (119.23 \pm 1.48 ng/dL) (P <0.001). Similarly, TT4 levels were also significantly increased (P <0.001) in BR (8.52 \pm 0.09 ug/dL) compared to NBR (7.51 \pm 0.11 ug/dL). However, TSH levels significantly decreased in BR (1.90 \pm 0.05 uIU/mL) compared to NBR (2.17 \pm 0.07 uIU/mL) (P =0.002).

Three nicotine metabolites i.e., anabasine, nornicotine, and cotinine were estimated in serum samples from both BR and NBR groups. The estimated serum anabasine, nornicotine, and cotinine levels were significantly higher in BR (8.88 \pm 0.39 ng/mL, 0.81 \pm 0.08 ng/mL, and 108.84 \pm 11.40 ng/mL respectively) compared to serum anabasine, nornicotine, and cotinine levels in NBR (1.72 \pm 0.12 ng/mL, 0.002 \pm 0.001 ng/mL, and 5.67 \pm 0.82 ng/mL).

We categorised the BR group into two subgroups based on the duration of their service as beedi rollers (<10 years of exposure (BR1); >10 years of exposure (BR2)) and assessed the correlation between the duration of exposure and levels of thyroid hormones, TSH, and nicotine metabolites.

TT3 levels were significantly higher in BR at both time intervals (BR1: 126.41 \pm 1.50, P <0.001), and (BR2: 132.61 \pm 1.41, P =0.004) compared to NBR (119.23 \pm 1.48). TT4 levels were also significantly increased in the BR group (BR1: 7.95 \pm 0.13, P =0.02) and (BR2: 8.95 \pm 0.11, P <0.001) compared to the NBR group (7.51 \pm 0.11). Declined TSH levels were observed in BR (BR1: 1.94 \pm 0.09, P =0.06) and (BR2: 1.87 \pm 0.06, P =0.004) groups compared to the NBR group (2.17 \pm 0.07). Anabasine levels were higher in (BR2 group: 9.03 \pm 0.48, P <0.001) compared to (BR1:

Table 3: Thyroid hormones, TSH, and nicotine metabolite levels in BR and NBR.

Parameter	NBR (n=426) (Mean \pm SEM)	BR (n=421) (Mean \pm SEM)	P-value
Tri-iodothyronine Total (TT3) (ng/dL)	119.23 \pm 1.48	129.91 \pm 1.04	<0.001**
Thyroxine – Total (TT4) (ug/dL)	7.51 \pm 0.11	8.52 \pm 0.09	<0.001**
Thyroid Stimulating Hormone (TSH) (uIU/mL)	2.17 \pm 0.07	1.90 \pm 0.05	0.002**
Anabasine (ng/mL)	1.72 \pm 0.12	8.88 \pm 0.39	<0.001**
Nornicotine (ng/mL)	0.002 \pm 0.001	0.81 \pm 0.08	<0.001**
Cotinine (ng/mL)	5.67 \pm 0.82	108.84 \pm 11.40	<0.001**

**-Highly Significant

Table 4: Thyroid hormones, TSH, and nicotine metabolite levels in BR at different time intervals.

Parameter	BR (n=421)		NBR (n=426) (Mean \pm SEM)	P1	P2
	With <10 years of service (BR1) (n=183) (Mean \pm SEM)	With >10 years of service (BR2) (n=238) (Mean \pm SEM)			
Tri-iodothyronine Total (TT3) (ng/dL)	126.41 \pm 1.50	132.61 \pm 1.41	119.23 \pm 1.48	0.004**	<0.001**
Thyroxine – Total (TT4) (ug/dL)	7.95 \pm 0.13	8.95 \pm 0.11	7.51 \pm 0.11	0.02*	<0.001**
Thyroid Stimulating Hormone (TSH) (uIU/mL)	1.94 \pm 0.09	1.87 \pm 0.06	2.17 \pm 0.07	0.06 ^{ns}	0.004**
Anabasine (ng/mL)	8.68 \pm 0.63	9.03 \pm 0.48	1.72 \pm 0.12	<0.001**	<0.001**
Nornicotine (ng/mL)	0.77 \pm 0.11	0.84 \pm 0.11	0.002 \pm 0.001	<0.001**	<0.001**
Cotinine (ng/mL)	80.16 \pm 16.30	130.89 \pm 15.68	5.67 \pm 0.82	<0.001**	<0.001**

P1: <10 years of exposure vs NBR; P2: >10 years of exposure vs NBR **-Highly Significant; *- Significant; ns- Not significant

Table 5: Multivariable linear regression analysis demonstrating association between years of exposure and thyroid hormone, TSH, and nicotine metabolite levels.

Nicotine metabolites	Parameters	<10 years of service		>10 years of service	
		Beta coefficient (95% CI)	P-Value	Beta coefficient (95% CI)	P-Value
Anabasine	Tri-iodothyronine Total (TT3) (ng/dL)	0.11 (-0.01, 0.11)	0.12	-0.02 (-0.52, 0.04)	0.73
	Thyroxine – Total (TT4) (ug/dL)	0.05 (-0.47, 0.93)	0.52	-0.01 (-0.58, 0.51)	0.90
	Thyroid Stimulating Hormone (TSH) (uIU/mL)	-0.16 (-2.24, -0.13)	0.03	-0.10 (-1.96, 0.22)	0.116
Nornicotine	Tri-iodothyronine Total (TT3) (ng/dL)	0.04 (-0.01, 0.01)	0.57	0.07 (-0.005, 0.02)	0.31
	Thyroxine – Total (TT4) (ug/dL)	0.07 (-0.07, 0.19)	0.36	-0.02 (-0.15, 0.11)	0.73
	Thyroid Stimulating Hormone (TSH) (uIU/mL)	-0.03 (-0.23, 0.16)	0.70	-0.06 (-0.14, 0.37)	0.38
Cotinine	Tri-iodothyronine Total (TT3) (ng/dL)	0.06 (-0.92, 2.27)	0.40	0.11 (-0.17, 2.72)	0.08
	Thyroxine – Total (TT4) (ug/dL)	0.05 (-24.78, 12.11)	0.50	-0.11 (-32.99, 2.39)	0.09
	Thyroid Stimulating Hormone (TSH) (uIU/mL)	-0.07 (-41.8, 13.96)	0.33	-0.03 (-43.56, 26.93)	0.64

CI: Confidence Interval

8.68±0.63, $P<0.001$) and NBR group (1.72±0.12). Similarly, nornicotine and cotinine levels were also significantly elevated in (BR1: 0.77±0.11, $P<0.001$; 80.16±16.30, $P<0.001$) and (BR2: 0.84±0.11, $P<0.001$; 130.89±15.68, $P<0.001$) groups respectively compared to NBR group (0.002±0.001; 5.67±0.82) (Table 4).

Table 5 explains the correlation between nicotine metabolites, thyroid hormones, TSH and years of service in beedi rollers. As shown in Table 5 a significant negative correlation was observed between anabasine and TSH in beedi rollers with 10 years of service ($\beta = -0.16$, $P=0.03$, CI: -2.24, -0.13). Cotinine was positively correlated ($\beta = 0.40$, $P=0.08$, CI: -0.17, 2.72) with T3 in beedi rollers with more than 10 years of service. However, this association was not statistically significant.

Discussion

In the present study, we found that exposure to unburnt tobacco dust is associated with lower serum TSH and increased thyroid hormone levels in rural women beedi workers when compared to unexposed non-beedi rollers. The results are by the studies of Zhang et al. (2019), King et al. (2022), and Horton et al. (2015) who stated that thiocyanate (SCN-) present in cigarette smoke, interferes with thyroid hormone binding to thyroid binding globule (TBG) which leads to an increase in the levels of free thyroid hormone levels [11,12,13]. Another proposed mechanism by Rehan et al. (2022) stated that nicotine metabolites such as cotinine, trans-3'-hydroxycotinine, and 5'-hydroxycotinine compete with thyroid hormones and bind to TBG resulting in increased thyroid hormone levels and decreased TSH in the blood [9].

Earlier studies carried out established an association between smoking and thyroid hormone levels, including TSH levels [9,10,11,14-18]. However, no study has examined how occupational exposure to unburnt tobacco dust disrupts thyroid gland homeostasis. This study has identified that tobacco is associated with decreased serum concentrations of TSH and increased thyroid hormone levels. Similar observations have been reported in previous studies [9,11,13,14,15,20,22]. In contrast, Meral et al. (2015) reported that the ft3, ft4, tT3, tT4, and TSH levels were not statistically different between smokers and non-smokers, concluding that no association between smoking and thyroid function [21].

In the present study, higher levels of anabasine, nornicotine, and cotinine were observed in the BR groups. Multivariate and univariate analysis revealed the increase in levels of anabasine, nornicotine, and cotinine in both subgroups of BR was statistically significant compared to NBR.

To identify the association of nicotine metabolites with levels of thyroid hormones and TSH, we performed multivariate linear regression analysis, with nicotine metabolites as dependent variables and a negative correlation was observed between TSH and serum levels of anabasine, nornicotine, and cotinine levels in both the BR groups. Regarding T3 and T4 associations with nicotine metabolites, a positive correlation was observed between T3, cotinine and nornicotine metabolites in both the BR groups. A positive correlation was observed between T4 and all three nicotine metabolites in BR with less than 10 years of service. However, the relationship between hormones and serum nicotine metabolites showed no statistical significance. Soldin et al. (2009) also studied the correlation between serum cotinine levels, TSH, and thyroid hormone levels and did not find any statistically significant differences [15]. Kim et al. (2019),

and Gauthier et al. (2020) studied the association between urinary cotinine and thyroid function and observed a significant negative correlation between TSH and urinary cotinine levels [17,22].

Conclusion

The present study comprises a total of 847 participants, classified into three groups based on exposure to unburnt tobacco dust. Thyroid hormone levels, TSH, and nicotine metabolite levels were analysed using serum samples. Multivariate linear regression analysis was performed to investigate the association between hormones and nicotine metabolites in women beedi rollers at different time intervals. Significantly lower TSH levels and increased thyroid hormonal levels were observed in the BR group when compared to NBR. Additionally, all three nicotine metabolites were elevated in the BR group compared to non-exposed healthy controls. A negative correlation was observed between TSH and all three nicotine metabolites. However, no significant correlation was observed between hormones and nicotine metabolites.

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