Original Article

# Exploring the Hormonal Profiles of Beedi-Rolling Women and Unravelling Health Implications

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**Abstract**

**Background:** In the rural regions of Telangana State, many women are actively involved in the occupation of beedi rolling. Cigarette smoke either active or passive exerts deleterious effects on reproductive organs and function by means of both direct toxic influences, termed ovotoxicity, and the reduction of hormone secretion.

**Methods:** The study involved a cohort of 320 women employed as beedi rollers and 280 women with no occupational exposure to chemicals for comparison. Serum samples were used to estimate the levels of Estrogen, Progesterone, Testosterone, Follicle Stimulating Hormone, luteinizing hormone, T3, T4 and TSH using Sandwich and competitive ELISA methods.

**Conclusion:** The findings of the current study unveiled compelling statistical significance in the levels of estrogen, progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), T3 and T4.In conclusion, our study reveals a noteworthy correlation between beedi rolling and alterations in hormonal profiles of rural women in Telangana.

**Keywords:** Beedi rolling, Estrogen, Progesteron, Follicle stimulating hormone (FSH), Testesterone

## Introduction

In the rural regions of Telangana State, many women are actively involved in the occupation of beedi rolling. While pursuing this livelihood, these women face potential exposure to tobacco through skin contact, inhalation, or ingestion of food and water

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contaminated with tobacco dust. Cigarette smoking exerts deleterious effects on reproductive organs and function by means of direct toxic influences, termed ovotoxicity, and the reduction of hormone secretion[1]. Despite substantial advancements in raising awareness regarding the association of cigarettes with cardiovascular and pulmonary diseases, there exists a notable gap in knowledge concerning the endocrine repercussions of nicotine in beedi rolling women. Estrogen, Progesterone, Testosterone, T3, T4, Luteinizing Hormone, Follicle-Stimulating Hormone, and Thyroid-Stimulating Hormone are pivotal female hormones crucial for the development and regulation of the reproductive system. Consequently, it becomes imperative to comprehensively comprehend the health challenges, reproductive well-being, and hormonal profiles related to the reproductive system of beedi rolling women. The present study aims to utilize the ELISA method to analyze the levels of Estrogen, Progesterone, Luteinizing Hormone, Follicle-Stimulating Hormone, Testosterone, T3, T4, and TSH hormones in women involved in beedi rolling within rural communities of Telangana.Top of Form

This understanding is crucial for implementing proactive measures and welfare programs aimed at the prevention and enhancement of their overall well-being.

## Materials and Methods

### Study Subjects

The study involved a cohort of 320 women employed as beedi rollers and 280 women with no 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, socio-economic status, daily working hours, tobacco usage, and both present and past health history. The study was conducted among women employed as beedi rollers in various villages, namely Ibrahimpatnam, Varshakonda, Sattakkapally, Mularampur, Vellulla, Bandalingapur, Chinthalpet, Yousufnagar, Korutla, Athmakur, Aarapet, Athmanagar, Godhur, Kanapur, Jaggasagar, Kathlapur, Metpally, Mogilpet, Thimmapur, and Vemulakurthi in the Jagityal District, as well as Kammarpally and Bhemghal in the Nizamabad District of Telangana State, India. Exclusions from the study involved individuals who consumed pan, gutka, or tobacco, and those with chronic diseases. As a comparison group, women residing in the same areas with similar socio-economic status, but without exposure to any chemical, including tobacco dust, were selected as the control group. Approval from the Institutional Ethics Committee at Bhagwan Mahavir Hospital and Research Centre in Hyderabad, Telangana, was obtained for this study. All the participants provided their consent. The present study was carried out at Genetics Department of Bhagwan Mahavir Hospital and Research Centre during July 2021 to November 2023.

### Sample Collection

Blood samples were obtained from both women engaged in beedi rolling and the control group. These samples were then placed into plain vacutainers, and the serum was separated. Serum samples were used to estimate the hormone levels using Sandwich and competitive ELISA methods.

### Estimation of Estrogen, Progesterone, Testosterone, Follicle Stimulating Hormone, Luteinizing hormone, T3, T4 and TSH by ELISA

### Sandwich ELISA Procedure:

Add 100 µl of samples to each well, seal with a plate sealer, and incubate for 90 minutes at 37°C.After incubation, discard the sample solution, wash the wells twice, and tap them on a tissue bed to remove all remaining solution, ensuring no air bubbles are present. Introduce 100 µl of biotin-labeled antibody solution into each well. Shake the plate to thoroughly mix the components, cover with a plate sealer, and incubate for 60 minutes at 37°C.Discard the solution after incubation, wash the wells three times with wash buffer, and tap the plate on a tissue. Add 100 µl of SABC to each well, tap the plate to mix, and close with a plate sealer. Incubate for 30 minutes at 37°C.After incubation, discard the solution, wash the wells five times with wash buffer, and add 90 µl of TMB substrate to each well. Incubate the plate for 10 minutes at 37°C. Upon color development, add 50 µl

of stop solution. The color will change from blue to yellow. Read the OD values at 450 nm.

**Competitive ELISA Procedure:**

Add 50 µl of biotin-labelled antibody to each well. Shake the plate well, cover it with a plate sealer, and incubate for 45 minutes at 37°C.After incubation, discard the solution from the wells, wash them three times with wash solution, and tap the wells on tissue paper. Add 100 µl of SABC solution (diluted with SABC dilution buffer) to each well and incubate at 37°C for 30 minutes. Discard the solution, wash the microplate five times with wash buffer, and add 90 µl of TMB substrate solution in the dark. Incubate for 10 minutes. Add 50 µl of stop solution. Read the plate at 450 nm and analyse the data. The data obtained from the study was analysed using SPSS software and various other online statistical tools.

## Results and Discussion

The demographic data of the study subjects is given in Table 1.

**Table 1. Demographic data of Beedi Rollers and Control Subjects**

| Variable | Beedi Rollers (n=320) | Non-Beedi Rollers (n=280) | X2 | P value |
|---|---|---|---|---|
| Age (Mean ±SD) | 37.14±10.30 | 35.57±9.23 | NA | |
| **Age Groups(Years)** | | | | |
| <25 | 101 (31.56) | 107 (22.67) | 4.338 | 0.1143 |
| 25-45 | 158(49.38) | 134(49.85) | | |
| >45 | 61(19.06) | 39(27.48) | | |
| **Marital Status** | | | | |
| Unmarried | 34(10.62) | 42 (15.00) | 2.58 | 0.1080 |
| Married | 286 (89.38) | 238(85.00) | | |
| **Educational Status** | | | | |
| Illiterate | 130 (56.20) | 49(17.50) | 69.76 | <0.0001 |
| Primary (5th Std) | 206 (30.36) | 124 (44.29) | | |
| Secondary (10$^{th}$ Std) | 44 (13.44) | 107 (38.21) | | |
| **Occupation** | | | | |
| Beedi Roller | 380(100.0) | 0(0) | 600.0 | <0.0001 |
| Other Private Job | 0(0) | 51 (18.21) | | |
| House wife / Home maker | 0(0) | 219 (78.21) | | |
| Student/Others | 0(0) | 10 (3.57) | | |
| **Income/Month** | | | | |
| <1000 | 8 (2.11) | 0(0) | 205.0 | <0.0001 |
| 1000-5000 | 229(76.05) | 44 (15.71) | | |
| >5000 | 7 (1.84) | 22 (7.86) | | |
| Not reported | 76 (20.00) | 214 (76.43) | | |

Hormonal levels were assessed in beedi rollers and non-beedi rollers. In non-beedi rollers, the mean Estrogen level was 2.414 pg/mL, while in beedi rollers, it was 100.2 pg/mL. The observed mean difference was 97.77, with a standard error of 14.03, indicating high significance ($P < 0.0001$) between the two groups. For Progesterone, non-beedi rollers had a mean level of 7.421 ng/mL, compared to 2.628 ng/mL in beedi rollers. The mean difference was -4.793, and the standard error was 0.2626, demonstrating high significance ($P < 0.0001$). In Testosterone levels, non-beedi rollers averaged 4.699 ng/mL, while beedi rollers averaged 5.093 ng/mL, resulting in a mean difference of 0.3934. However, the standard error was

0.7560, and the P value was not significant (0.603). For LH, non-beedi rollers had a mean level of 5.257 mIU/mL, whereas beedi rollers had 6.876 mIU/mL. The mean difference was1.619, and the standard error was 0.4772, indicating high significance (P = 0.0007). FSH levels in non-beedi rollers (2.477 mIU/mL) differed significantly from beedi rollers (25.36 mIU/mL), with a mean difference of 22.88 and a standard error of 4.100 (P < 0.0001). Overall, the t-test analysis confirmed the significant increase in Estrogen, Progesterone, LH, and FSH levels (Table 2).

**Table 2. Estimation of Hormonal levels in beedi rollers and non-beedi rollers**

| Hormone | Beedi Rollers (n=320) | Non-Beedi Rollers (n=280) | Mean SD | Difference between means ± SEM | P value |
|---|---|---|---|---|---|
| Estrogen (pg/mL) | 100.2 | 2.414 | 97.77±14.03 | 70.23 to125.3 | <0.0001 |
| Progesterone (ng/mL) | 2.628 | 7.421 | 4.793±0.2626 | 0.5.309 to-4.278 | <0.0001 |
| Testosterone (ng/mL) | 5.093 | 4.699 | 0.3934±0.7560 | (-)1.091 to1.878 | 0.603 |
| LH (mIU/mL) | 6.876 | 5.257 | 1.619±0.4772 | 0.6819 to2.556 | 0.0007 |
| FSH (mIU/mL) | 25.36 | 2.477 | 22.88±4.100 | 14.83-30.93 | <0.0001 |

p< 0.05 indicates significance

The mean values of T3 were124.94 ng/dL in beedi rollers, while in control subjects, it was 51.37 ng/dL. Similarly, the T4 level was 8.69 ug/dL in beedi rollers and 4.10 ug/dL in control subjects, which were statistically significant. (Table 3).

**Table 3. Thyroid profile in beedi rollers and non-beedi rollers**

| Thyroid Profile | BR (n=320) Mean ± SEM | NBR (n=280) Mean ± SEM | p value |
|---|---|---|---|
| T3 (ng/dL) | 124.94 ± 2.34 | 51.37 ± 37.46 | 0.036* |
| T4 ( ug/dL) | 8.69 ± 0.29 | 4.10 ± 2.45 | 0.048* |
| TSH (uIU/mL) | 4.66 ± 0.97 | 5.59 ± 2.97 | 0.753 |

p< 0.05 indicates Significance

Cigarette smoke is recognized for containing reproductive toxicants, and its association with adverse reproductive outcomes in women is well-established. Notable consequences include infertility, subfecundity, an earlier onset of menopause, and menstrual disorders.Active smoking is linked to an unfavourable prognosis in assisted reproduction cycles, impacting ovarian response and pregnancy outcomes. This association implies that the negative effects may, in part, be mediated by an accelerated alteration in ovarian reserve, as evidenced by reduced yet still within normal range serum anti-Müllerian hormone (AMH) concentrations. Moreover, smoking women face an elevated risk of premature ovarian failure compared to nonsmokers[2].

The endocrine system, comprised of various glands, orchestrates the maintenance of bodily homeostasis through the secretion of diverse hormones. Regulation of many of these hormones occurs through intricate regulatory axes, including the hypothalamic–pituitary–adrenal axis (HPA), the hypothalamic–pituitary–gonadal axis (HPG), and the hypothalamic–pituitary–thyroid axis (HPT). Data from animal studies elucidates three potential mechanisms that account for the antiestrogenic effects exerted by cigarette smoking in females: 1. Reduced activity of aromatases in granulosa cells and peripheral tissue, along with diminished C-20,22 desmolase activity, leading to an overall reduction in steroid production, particularly estradiol[1]. 2. Induction of enzymes producing low biopotent estrogens – diluted mainstream cigarette condensates, generated by mixing fresh smoke with filtered air, can bind to and activate the aryl hydrocarbon receptor, thereby inducing transcription of estrogen-catabolizing enzymes such as CYP1A1 and CYP1A2.

Through this mechanism, smoking increases the rate of 2-hydroxylation of E2, diverting available estrogen from the more potent 16a-hydroxylation competing pathway. 3. Competitive inhibition by binding to estrogen receptor – diluted mainstream cigarette condensates binding to the estrogen receptor have been observed to displace tritiated E2 bound to the rat uterine estrogen receptor in a dose-dependent manner[3-6].

Smokers were previously observed to exhibit distinct characteristics in their menstrual cycles compared to nonsmokers. Heavy smoking correlated with shorter and more variable cycle lengths, primarily during the follicular phase. While there were indications of an elevated risk of a short luteal phase (< 11 days) and anovulation, the confidence intervals were wide. The mechanism behind these effects remains unclear, but it is hypothesized that components of tobacco smoke may alter hormone function, potentially exerting antiestrogenic effects[7]. The present study results are consistent with our earlier pilot study results [8].To the best of our knowledge, our research represents the first attempt in India to investigate the influence of tobacco exposure on hormonal levels and health of women involved in beedi rolling in rural Telangana. The findings of the current study unveiled compelling statistical significance in the levels of estrogen, progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), T3 and T4. This notable observation prompts a more detailed and nuanced discussion on the intricate interplay of these hormonal dynamics and their potential implications on the physiological and reproductive health of the study population. The divergent effects of cigarette smoking on hormonal balance may be attributed to the array of compounds present in cigarette smoke, each with varying pro and antiapoptotic influences. The intricate interplay of these compounds, along with the involvement of multiple mechanisms, contributes to the observed contradictions in the impact of smoking on hormonal equilibrium (Lilach Marom-Haham and Adrian Shulman, 2016). Enhancing our comprehension of nicotine's impact on the endocrine system and its consequent implications for the pathogenesis of various endocrine disorders in women engaged in beedi rolling is crucial. This understanding will enable the development of targeted therapies and furnish valuable insights for the formulation of more efficacious preventive measures.

## Conclusion

In conclusion, our study reveals a noteworthy correlation between beedi rolling and alterations in estrogen, progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), T3 and T4 levels. The observed associations emphasize the need for additional research to delve into the mechanisms that underlie the hormonal changes associated with beedi rolling. Further investigations are essential for a comprehensive understanding of the intricate interplay between beedi rolling and hormonal fluctuations in women, shedding light on potential health implications and facilitating targeted interventions.

**Ethical Approval:** This study was approved by Institutional Ethics Committee for Biomedical Research Bhagwan Mahavir Medical Research Centre Dated 21 /10/2013

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**Conflict of Interest:** None to declare.

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