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Original Research Article

Association of GSTM1 and GSTT1 polymorphisms with reproductive health outcomes in women beedi rollers exposed to unburnt tobacco dust

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ABSTRACT

Background: Beedi rolling, a labour-intensive activity mainly done by women in India, exposes workers to tobacco dust, leading to various health issues. This study examines the prevalence of GSTM1 and GSTT1 genotypes and their association with reproductive health in beedi rollers.
Methods: The study comprises 500 women beedi rollers (BR) and 500 non-beedi rollers (NBR). A face-to-face interview was conducted with them to collect information on the reproductive outcome. GSTM1/T1 polymorphisms were studied using polymerase chain reaction. Tobacco exposure was estimated by serum nicotine metabolite levels through LC-MS.
Results: There is a significant increase in reproductive complications in the BR group compared to the NBR. Null genotypes of both genes were significantly high in the BR group. Nicotine metabolite levels were significantly elevated in the BR group compared to NBR. Increased incidences of miscarriages and oligomenorrhea were significantly associated with the null genotypes of both genes.
Conclusions: These findings suggest that genetic predisposition, combined with tobacco exposure, enhances health risks.

Keywords: GSTM1, GSTT1, Nicotine metabolites, Reproductive outcome

INTRODUCTION

Beedi rolling is a home-based, labour-intensive task primarily undertaken by women and girls in India.[1] Beedis, which are inexpensive cigarettes, release significantly higher levels of nicotine, carbon monoxide and tar compared to regular cigarettes. Beedi rollers are exposed to tobacco dust through multiple pathways, including inhalation, skin contact and ingestion, as they breathe in contaminated air and consume tainted food.[2] Prolonged exposure to tobacco and working in a fixed position for extended hours can lead to various health complications. In tobacco, nicotine is the primary pharmacologically active substance. It interacts with the nervous system and other biological systems in the human body.[3] Our previous studies showed a significant increase in health problems such as joint pains, cough, headache and eye burning.[4] Devi et al, observed that continuous tobacco dust exposure in beedi rollers was associated with an increased risk of abortions, neonatal deaths and stillbirths.[5] Yasmin et al, surveyed beedi rollers and observed gastrointestinal problems and decreased haemoglobin.[6]

An epidemiological study by Senthil Kumar et al, also observed an increased risk of respiratory and digestive problems in Beedi rollers.[7] Ancy et al, studied beedi rollers for cancer incidence using the marker sialic acid and observed increased levels of this marker in beedi rollers compared to control subjects.[8] Bhisey et al,

conducted the Ames test in beedi rollers and control subjects to find mutations in the DNA and showed the mutagenic potency of tobacco in beedi rollers.[9] Glutathione-S-transferase µ1 (GSTM1) and Glutathione-S-transferase θ1 (GSTT1) are important phase II enzymes that play a key role in detoxifying reactive electrophiles from tobacco and are classified as oxidative stress-related genes. These GST enzymes are recognized for their capacity to neutralize toxic compounds in tobacco smoke, including polycyclic aromatic hydrocarbons and aromatic amines.

GST enzymes are encoded by polymorphic genes that can have null genotypes due to the deletion of both parental alleles. These null genotypes result in the absence of active GST enzyme function.[10]

Previous studies have shown a higher risk for the development of various types of cancers in people having the null genotype of GST genes when exposed continuously to different forms of tobacco.[11,12] In the present study, we investigated the association between reproductive outcomes and polymorphisms of GST (GSTM1 and GSTT1) genes in women beedi rollers.

METHODS

Study place

The study was carried out studies on women beedi rollers from different villages in the Jagityal district Hyderabad, Telangana, India.

Study duration

The study period was from July 2021 to December 2023.

Study population

The present prospective randomized case-control study comprises 1000 participants, including 500 beedi rollers (BR) exposed to unburnt tobacco dust and 500 control subjects (NBR) not exposed to any chemicals.

Demographic data

Demographic data and blood samples were collected from women beedi rollers and control subjects after obtaining their written consent. A questionnaire was prepared; to collect information on demographic data such as age, gender, educational status, marital status, income, reproductive outcomes, etc.

Inclusion criteria

The study focuses on women beedi rollers and controls aged between 15 and 50 years. All participants provided informed consent prior to inclusion. To maintain consistency, only female participants from both the beedi roller and control groups were considered for the study.

Exclusion criteria

Those who use Pan, Gutka or chew tobacco, as well as individuals suffering from chronic diseases, were excluded.

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.

Genotyping

Whole genomic DNA was isolated using the QIAamp Qiagen DNA Blood Mini Kit (Qiagen, Germany). A three-step Polymerase Chain Reaction (PCR), as described by Movva et al, (2007), was performed to examine the wild or null genotypes using specific primers for Phase II detoxification genes (GSTT1 and GSTM1). For GSTT1 (Exon 5), the forward primer was TGAGGTCATTCTGAAGGCCA and the reverse primer was GGCTGAGCCCAGGTTTATTA. For GSTM1 (Exon 5), the forward primer was GACCTTGAGTTCTGGCCTTATT and the reverse primer was CAACCACTAACAGGAAGGAAGG. The quality and concentration of the DNA and PCR products were measured using a photometer and agarose gel electrophoresis.

Measurement of serum Cotinine, anabasine and nornicotine 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

To prepare the standard samples, 1 mg of nicotine metabolites was dissolved in 1 mL of methanol, yielding a final concentration of 1 mg/mL. The individual standards were then combined to create a mixed-standard solution. For the stock solution, 2 mg of verapamil was dissolved in 1 ml of Dimethyl Sulfoxide (DMSO), resulting in a concentration of 2 mg/ml. These standards were subsequently serially diluted to achieve concentrations ranging from 10 ng/mL to 10,000 ng/ml and were labelled from CC8 to CC1 (Table 1).

Sample preparation

The serum blank and study serum samples were retrieved from the deep freezer and allowed to reach room

temperature. To prepare the blank, 20 µl of 50% methanol in water was added to an RIA vial. Next, 20 µl of the internal standard (ISTD) solution containing Verapamil at 2 µg/ml was added to all pre-labelled RIA vials, except for the blank. Following this, 100 µl of the respective calibration curve (CC) samples and study samples were transferred and vortexed. Then, 0.250 ml of acetonitrile was added to all samples, which were vortexed again and centrifuged at 4000 rpm for 10 minutes at 20°C. The supernatant layer (0.15 ml) was carefully separated and loaded into auto-injector vials and 10 µl was injected into the LC-MS/MS system for the quantification of metabolites.

Statistical analysis

Descriptive statistics, including means, standard deviations and frequencies, were computed to summarise the data and provide insights into the sample characteristics. GraphPad was used to calculate P-values and 95% confidence intervals (95% CI), with a significance threshold of less than 0.05 indicating statistically significant results. Odds ratios (OR) and 95% CI for genotypes were calculated using Vassar Stats to assess the strength of associations between categorical variables.

RESULTS

The results on the demographic information, reproductive outcome, incidence of different genotypes of GST genes and the levels of nicotine metabolites are shown in tables 1-4. Table 1 shows that the mean age (±SD) of the BR group was 36.20 (±9.47), while the mean age of the NBR group was 28.28 (±8.65). Most of the participants were married. The majority of beedi rollers had more than 10 years of experience, as they had started their career in beedi rolling at an early age.

Table 2 shows different reproductive health complications in BR and NBR. A high incidence of oligomenorrhea (8.6% vs. 2.8%), Premature ovarian insufficiency (POI) (14.8% vs. 1%), miscarriage (19.3% vs. 12.8%), stillbirths (4.2% vs. 0.5%) and neonatal deaths (1.1% vs. 0.3%) was observed in BR compared the NBR.

The differences in reproductive complications were significant between the two groups except for neonatal deaths. The tobacco exposure was estimated by quantifying the serum nicotine metabolites. All three nicotine metabolites (Anabasine, nornicotine and cotinine) showed a significant increase in the BR group compared to NBR (Table 3).

There were significant differences in the genotypes and allelic frequencies of GSTM1 and GSTT1 between beedi rollers (BR) and non-beedi rollers (NBR) (Table 3). For the GSTM1 genotype, the wild-type homozygous (W/W) frequency was 65% in the BR group compared to 84% in the NBR group. The null genotype (N/N) was observed in 35% of the BR group and 16% of the NBR group, yielding an odds ratio (OR) of 0.35 (95% CI: 0.26-0.48) with a p value of <0.001. For GSTT1, the W/W genotype was present in 65.4% of the BR group and 90.4% of the NBR group. The N/N genotype was found in 34.6% of the BR group and 9.6% of the NBR group, yielding an OR of 0.201 (95% CI: 0.14-0.28) with a P-value of <0.001 (Table 4).

Table 5 represents the incidence of various reproductive outcomes between the BR and NBR groups for GSTM1 and GSTT1 genotypes. The results have shown a significantly increased frequency of Oligomenorrhea and miscarriage in beedi rollers with the null genotype compared to the wild type.

Table 1: Demographic data of BR and NBR.

Variable	BR (n=500) (%)	NBR (n=500) (%)	P value
Age (Mean±SD)	36.20±9.47	28.28±8.65	0.57^{ns}
Marital status			<0.001**
Unmarried	45 (9)	125 (25)	
Married	455 (91)	375 (75)	
Years of service			0.001**
<10 years	224 (44.8)	NA	
>10 years	276 (55.2)		

** Highly Significant ns-not significant

Table 2: Self-reported reproductive complications in BR and NBR.

Variable	BR n=500 (%)	NBR n=500 (%)	P value
Oligomenorrhea	43 (8.6)	14 (2.8)	<0.001**
Premature ovarian insufficiency (POI)	74 (14.8)	5 (1)	<0.001**
Variable	BR (n=455) (%)	NBR (n=375) (%)	
Miscarriage	88 (19.3)	48 (12.8)	<0.001**
Stillbirths	19 (4.2)	2 (0.5)	<0.001**
Neonatal deaths	5 (1.1)	1 (0.3)	0.16^{ns}

** Highly Significant ns-not significant

Table 3: Nicotine metabolites in BR and NBR.

Parameter	BR n-500 (Mean±SEM)	NBR n-500 (Mean±SEM)	95% CI	P value
Anabasine (ng/ml)	9.07±0.35	1.71±0.11	6.64, 8.08	<0.001**
Nornicotine (ng/ml)	0.74±0.07	0.00±0.00	0.60, 0.88	<0.001**
Cotinine (ng/ml)	105.95±10.21	5.45±0.82	80.38, 120.62	<0.001**

** Highly Significant

Table 4: GSTM1 and GSTT1 genotypes in BR and NBR.

Genotypes	BR (n=500) (%)	NBR (n=500) (%)	OR (95% CI)	P value
GSTM1				
W/W	325 (65)	420 (84)	1	
N/N	175 (35)	80 (16)	0.354 (0.262, 0.478)	<0.001**
GSTT1				
W/W	327 (65.4)	452 (90.4)	1	
N/N	173 (34.6)	48 (9.6)	0.201 (0.142-0.285)	<0.001**

**Highly significant W/W= wild genotype N/N= null genotype

Table 5: Reproductive health complications in BR with wild/null genotypes.

Parameter(GSTM1)	BR (n=500)		NBR (n=500)		P-value
	W/W	**N/N**	**W/W**	**N/N**	
Oligomenorrhea	17 (3.4)	26 (5.2)	12 (2.4)	2 (0.4)	0.01*
POI	31 (6.8)	43 (8.6)	4 (0.8)	1 (0.2)	0.23^{ns}
	BR(N=455)		NBR (N=375)		
Miscarriage	36 (7.9)	52 (11.4)	39 (10.4)	9 (2.4)	<0.001**
Stillbirths	8 (1.8)	11 (2.4)	1 (0.3)	0 (0)	0.92^{ns}
Neonatal deaths	1 (0.2)	4 (0.9)	2 (0.5)	0 (0)	0.28^{ns}
Parameter (GSTT1)	BR (N=500)		NBR (N=500)		
	W/W	N/N	W/W	N/N	
Oligomenorrhea	13 (2.6)	30 (6)	11 (2.2)	3 (0.6)	0.004**
POI	54 (10.8)	20 (4)	5 (1)	0 (0)	0.42^{ns}
	BR (N=455)		NBR (N=375)		
Miscarriage	28 (6.2)	60 (13.2)	44 (11.7)	4 (1.1)	<0.001**
Stillbirths	7 (1.5)	12 (2.6)	2 (0.5)	0 (0)	0.33^{ns}
Neonatal deaths	2 (0.4)	3 (0.7)	1 (0.3)	1 (0.3)	1.00^{ns}

** Highly significant * significant ^{ns} not significant W/W= wild genotype N/N= null genotype

DISCUSSION

In the present study, we investigated the effect of phase II detoxification gene polymorphisms on reproductive outcomes among women beedi-rollers in Telangana. This is the first study conducted on beedi-rollers in the Telangana region. Exposure to unburnt tobacco dust has been shown to have severe ill effects on human health. To assess the effect of tobacco dust on women beedi-rollers reproductive health, we studied the incidence of oligomenorrhea, premature ovarian insufficiency (POI) and reproductive outcome. We found a significantly increased incidence of Oligomenorrhea, POI, abortions and stillbirths in the beedi-roller group compared to the non-beedi-roller (NBR) group and confirmed adverse effects on reproductive health in women beedi-rollers. Similarly, Kulkarni et al, (2017) conducted a study on 1,200 North Indian women and observed that women exposed to tobacco were more likely to have irregular menstrual periods.[13] Skogsdal et al, conducted a cohort study on pregnant women in Sweden and also observed an increased risk of spontaneous abortions in active smokers compared to never-smokers.[14]

The present study estimated tobacco exposure by measuring serum nicotine metabolite levels (anabasine, nornicotine and cotinine). The nicotine metabolite levels were significantly increased in the beedi-roller (BR) group. Shenoy et al, (2020) observed elevated serum cotinine levels in both maternal and cord blood and found a significant association between tobacco exposure, gestational hypertension and fetal growth restriction in pregnant women beedi-rollers.[15] Lindsay et al, (2022) also found higher levels of cotinine in women exposed to second-hand smoke.[16]

GSTM1 and GSTT1 are phase II detoxifying genes that play an important role in inactivating toxic substances

and reducing oxidative stress. In our study of gene polymorphisms, we observed an increased frequency of null genotypes in women beedi-rollers, along with a significant association between GSTM1/T1 null genotypes and oligomenorrhea and miscarriages. Similarly, a study conducted on 115 Japanese women who experienced pregnancy loss and 160 control subjects found a higher number of GSTM1/T1 null genotypes in the patients compared to the controls.[17] Parveen et al, conducted a study on 200 North Indian women who experienced recurrent abortions and found a significant association between the GSTT1 null genotype and abortions.[18]

Contrary to our findings, they did not observe any association between the GSTM1 null genotype and the condition. Mendola et al, (1998) found no interaction between GSTM1 genotypes and spontaneous abortions in female smokers, concluding that while smoking may increase the chance of pregnancy loss, GSTM1 polymorphism is unrelated to the condition.[19] Even though increased risks of POI, neonatal death and stillbirths were observed in the BR group, the difference was not significant for both GSTM1 and GSTT1 polymorphisms. Several studies provided evidence for the association of GSTM1/T1 null genotypes with adverse reproductive outcomes, including infertility, low birth weight, oral cleft and intrauterine growth restriction (IUGR).[20-23] The absence of active detoxifying GST enzymes has been linked to various cancers in smokers.[24,25]

The present study was conducted on 500 beedi rollers from several villages in Telangana, South India, focusing exclusively on phase II detoxification genes. Further research is warranted with larger sample sizes from other districts in Telangana and across South India, extending the investigation to additional genes involved in detoxification.

CONCLUSION

The present study highlights the significant health risks faced by women beedi rollers, particularly in terms of reproductive outcomes, due to prolonged exposure to unburnt tobacco dust. Our findings demonstrate a high prevalence of reproductive complications, such as oligomenorrhea, premature ovarian insufficiency, miscarriages and stillbirths, in beedi rollers compared to non-beedi rollers. Elevated levels of nicotine metabolites in the beedi roller group indicate exposure to tobacco. The study also reveals an association between null genotypes of GSTM1 and GSTT1 genes and adverse reproductive outcomes, particularly miscarriage and oligomenorrhea. These results suggest that women with null GST genotypes may have a reduced capacity to detoxify harmful substances found in tobacco, leading to an increased risk of reproductive health issues.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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