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Association Between ESR1, ESR2, and FSHR Gene Polymorphisms and Infertility in Rural Women Beedi-rollers

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Abstract

Background: Beedi-rolling is a traditional occupation in India that predominantly involves women and exposes them to tobacco dust. Tobacco exposure is associated with various health risks, including disruptions in hormonal pathways critical for female fertility.

Aims: This case-control study aimed to investigate the association between polymorphisms in estrogen receptor genes (ESR1 and ESR2) and the follicle-stimulating hormone receptor gene (FSHR) with female infertility in beedi-rollers.

Methods: A total of 500 beedi-rollers (BR) and 500 non-beedi-rollers (NBR) of reproductive age were recruited. DNA was extracted from blood samples and genotyped for ESR1, ESR2, and FSHR polymorphisms using PCR and Sanger sequencing.

Statistical Analysis: Statistical analysis was conducted using SPSS version 30, including chi-square tests for genotype distribution, odds ratios for assessing the risk of infertility, and logistic regression models to adjust for potential confounders.

Results: Reproductive health problems were more prevalent among BR compared to NBR, with significantly higher incidences of infertility ($p = 0.02$), premature ovarian insufficiency ($p < 0.001$), and miscarriage ($p < 0.001$). Genotypic analysis revealed a significant association between ESR2 (CG/GG) and FSHR (CT/TT) polymorphisms and infertility risk among BR. Combined genotype analysis demonstrated that carriers of ESR2 (CG/GG) and FSHR (CT/TT) exhibited the highest susceptibility ($p = 0.02$).

Conclusions: The findings highlight that prolonged exposure to tobacco dust and specific genetic predispositions contribute to infertility in beedi-rollers. This study underscores the importance of genetic screening to mitigate infertility risks in vulnerable occupational groups.

Keywords: ESR1, ESR2, FSHR, Infertility, Beedi-rollers, Tobacco dust.

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INTRODUCTION

Beedi-rolling is an agroforest-based traditional occupation predominantly undertaken by women in India.[1] Occupational stresses, including prolonged exposure to tobacco, long working hours in a fixed posture, and inadequate nutrition, contribute to significant health risks.[2] Beedi rollers, while working, are usually exposed to fine particles of tobacco that enter the body through the respiratory and digestive tracts. This fine tobacco dust (omit) contains a complex mixture of components such as nicotine, polycyclic aromatic hydrocarbons (PAHs), heavy metals, and pesticide residues.

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Nicotine, a major alkaloid in tobacco, is known to interfere with the endocrine system, including the hypothalamic-pituitary-gonadal (HPG) axis, the hypothalamic-pituitary-thyroid (HPT) axis, and the hypothalamic-pituitary-adrenal (HPA) axis ultimately impacting the women fertility.[3]

Infertility is a complex condition influenced equally by genetic, epigenetic factors and environmental factors. Key contributors to female infertility include hormonal imbalances, oligomenorrhea, polycystic ovary syndrome (PCOS), endometriosis, and premature ovarian insufficiency (POI).[4] Among these factors, estrogen signalling plays a pivotal role in maintaining female reproductive health. This process is mediated by two nuclear hormone receptors, ESR1 (estrogen receptor alpha) and ESR2 (estrogen receptor beta), which regulate the transcription of genes critical for ovarian function, uterine health, and overall fertility. Polymorphisms in ESR1 and ESR2 can alter receptor sensitivity to estrogen, thereby disrupting hormonal signalling pathways resulting in disruptions of follicular development, compromise uterine function, and eventually reduce fecundity.[5] Osiński et al. (2018) observed increased expression of ESR1 and ESR2 in mid-follicular eutopic endometrium of infertile women with endometriosis. They concluded that the ESR1 gene mutations may be related to the abnormal function of estrogen.[6]

The FSHR (follicle-stimulating hormone receptor) gene encodes a receptor critical for follicular maturation and ovulation. Polymorphisms in FSHR can disrupt the receptor's binding efficiency to follicle-stimulating hormone (FSH), affecting ovarian responsiveness and contributing to conditions such as anovulation or diminished ovarian reserve.[7] Falconer et al., (2004) conducted a study on 68 infertile women to observe the association between Ser/Ser variants in FSHR at amino acid position 680 and infertility. They found that Ser/Ser was associated with increased levels of Follicle-stimulating hormone (FSH) and abnormal ovarian reserve.[8]

While several studies have explored health outcomes in beedi rollers, literature on infertility and its association with gene polymorphisms remains limited. In the present study, we investigated the association between ESR1, ESR2, FSHR, and the incidence of infertility in women engaged in beedi rolling.

Few lines on environmental factors as co-variables will add value.

MATERIALS AND METHODS

Study centre? This case-control study was approved by the Institutional Ethics Committee (IEC) of Bhagwan Mahavir Medical Research Centre, Hyderabad. Between July 2021 and November 2024, 500 Beedi-rollers (BR) and 500 non-beedi-rollers (NBR) were recruited. The control subjects had no occupational exposure to any form of tobacco or chemicals. Demographic and clinical information, including age, sex, health problems, and reproductive outcomes, was collected through personal interviews using a structured questionnaire. All participants provided signed informed consent. In the present study, women of reproductive age (mention mean sd) were included. Individuals who consume pan, gutka, or other forms of tobacco, as well as smokers, were excluded. Pregnant women and women with chronic health conditions were also excluded. Any biochemical test to test nicotine in serum?

DNA EXTRACTION AND GENOTYPIC ANALYSIS

5 mL of blood was collected from each participant early in the morning after overnight fasting. 2mL of blood was transferred to an EDTA-coated vacutainer and the vacutainers were stored at -20°C until use. Genomic DNA was isolated using the QIAamp Qiagen DNA Blood Mini Kit (Qiagen, Germany). The quality and quantity of the isolated DNA was assessed using a biophotometer (Eppendorf, USA) and agarose gel electrophoresis. DNA polymorphisms were analyzed using polymerase chain reaction (PCR) and Sanger sequencing. For ESR1 (location: Exon 2), the forward primer sequence was TCTCCCAGAGAGTGCATGTT, and the reverse primer sequence was TCAGTCGCTTTGGCTCTTAGG. For the ESR2 (location: 3'UTR/Exon8), the forward primer sequence was AACAGCTGAGCACACGACTT, and the reverse primer sequence was CCGTGGAGCACATAATCCCA. For FSHR (Location: Exon 10) the forward primer sequence was ATTGGCTGGTAGTTAGGATCAC, and the reverse primer sequence was CTTCTGGCTCCTTGACTGTG. PCR conditions for ESR1 included an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60.6°C for 30 seconds, and extension at 72°C for 30 seconds, with

a final extension at 72°C for 7 minutes. For ESR2, the PCR conditions were identical, except the annealing temperature was 62°C the final extension was 10 minutes, and the annealing temperature for FSHR was 62.5°C the other remaining PCR conditions were similar to those of ESR1. PCR products were quantified using 1.5% agarose gel electrophoresis. The purified PCR products were sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit, following the manufacturer's instructions.

Statistical Analysis: The continuous data for the BR and NBR groups was presented as Mean ± Standard Deviation (SD), while other variables were summarized using counts and frequencies for both groups. The significance of age differences was calculated using the Graph Pad T-test calculator, and significance for other measurements was determined using the VassarStats 2x2 contingency table. Segregation of BR and NBR groups based on reproductive health problems and genotypes, along with the calculation of Odds Ratios (OR) and 95% Confidence Intervals (CI), was performed using IBM SPSS statistic 30 software (IBM Corp., Armonk, NY).

RESULTS

The demographic and genotypic analysis in infertile BR and NBR groups are presented in Table 1-3.

Table 1: Clinicopathological characteristics of BR and NBR groups

Variable	BR n =500 (%)	NBR	X^2	P-value
Age (Mean ±SD)	36.20±9.47	28.28±8.65	NA	<0.001**
Age Groups (Years)				
<25	134 (26.8)	272(54.4)	79.02	<0.001**
25-45	281(56.2)	177(35.4)		
>45	85(17)	51(10.2)		
Years of exposure				
<10 years	224(44.8)	-		NA
>10 years	276(55.2)			
Infertile	50(10)	29(5.8)	5.5	0.02*
Miscarriage	88(19.3)	48(12.8)	11.24	< 0.001**
Premature ovarian insufficiency (POI)	74(14.8)	5(1)	65.44	< 0.001**
Late-onset menopause	4(8)	0(0.0)	4.016	0.045*

SD, Standard Deviation; NA, not applicable; Cell counts less than one; ** Highly Significant; * Significant

Table 1 presents a comparative analysis of demographic and clinical characteristics between the BR and NBR groups. The mean age of the BR group was 36.20 ± 9.47 years, while the NBR group had a mean age of 28.28 ± 8.65 years. The BR group was further classified into two subgroups based on years of exposure. The majority of the BR group had more than 10 years of experience in beedi-rolling. We also enquired about reproductive health problems and outcomes, the BR group was significantly more susceptible to conditions like infertile, POI, miscarriage, oligomenorrhea, and late-onset menopause.

To understand the patterns of ESR1, ESR2, and FSHR genotypes associated with the risk of infertility in women beedi-rollers, we studied the combinations of genotypes. The reference group comprised individuals with homozygous dominant genotypes with a lower risk of the condition, ESR1 AA, ESR2 CC, and FSHR CC. T allele(ESR1 and FSHR) and G allele (ESR2) carriers in homozygosity and heterozygosity were grouped for this analysis due to the low rate of homozygosity (TT and GG).

Table 2: Association between ESR1 and ESR2 genotype combinations and female infertility

ESR1	ESR2	BR (n=500)(%)	NBR (n=500) (%)	OR(95% CI)	X^2	P-Value
0	0	14(2.8)	19(3.8)	1.0		
0	1	11(2.2)	0(0)	NC	8.92	0.02**
1	0	16(3.2)	7(1.4)	3.81(1.19, 12.16)	3.00	0.06%^{ns}
1	1	9(1.8)	3(0.6)	4.29(0.98, 18.72)	2.82	0.09%^{ns}

For ESR1: 0, AA; 1, AT/TT; For ESR2: 0, CC; 1, CG/GG; OR, Odds Ratio; ns, not significant; **, Highly significant;*, significant

Table 2 evaluates the relationship between ESR1 and ESR2 genotype combinations and female infertility among BR and NBR. The 0, 0 combinations (AA and CC) were the reference group. The 0, 1 combination (AA and CG/GG) was observed in 2.2% of BR and absent in NBR, resulting in a highly significant association (p = 0.002) with a χ^2 value of 8.92. The 1, 0 combinations (AT/TT and CC) showed an OR of 3.81 (95% CI: 1.19–12.16). The 1, 1 combination (AT/TT and CG/GG) demonstrated an OR of 4.29 (95% CI: 0.98–18.72), which was not statistically significant (p = 0.09).

Table 3: Association between ESR1 and FSHR genotype combinations and female infertility

ESR1	ESHR	BR (n=500)(%)	NBR (n=500) (%)	OR(95% CI)	X^2	P-Value
0	0	17(3.4)	18(3.6)	1.0		
0	1	8(1.6)	0(0)	NC	5.12	0.02**
1	0	17(3.4)	10(2.0)	2.25(0.77, 6.56)	0.76	0.38^{ns}
1	1	8(1.6)	1(0.2)	8.47(0.96, 75.08)	3.24	0.07^{ns}

For ESR1: 0, AA; 1, AT/TT; For FSHR: 0, CC; 1, CT/TT; OR, Odds Ratio; ns, not significant

Table 3 examines the association between ESR1 and FSHR genotype combinations and female infertility. The 0, 0 combination (AA and CC) was nearly equally distributed between BR (3.4%) and NBR (3.6%) and served as the reference group. The 0, 1 combination (AA and CT/TT) showed a significant association (p = 0.02) and was only observed in BR (1.6%). The 1, 0 combinations (AT/TT and CC) demonstrated an OR of 2.25 (95% CI: 0.77–6.56), which was not significant (p = 0.38). The 1, 1 combination (AT/TT and CT/TT) had the highest OR of 8.47 (95% CI: 0.96–75.08), but the result was not significant (p = 0.07).

Table 4: Association between ESR2 and FSHR genotype combinations and female infertility

ESR2	FSHR	BR (n=500)(%)	NBR (n=500) (%)	OR(95% CI)	x^2	P-Value
0	0	23(4.6)	23(4.6)	1.0		
0	1	7(1.4)	3(0.6)	2.33(0.54, 10.16)	0.64	0.42^{ns}
1	0	11(2.2)	3(0.6)	3.67(0.90, 14.89)	2.5	0.11^{ns}
1	1	9(1.8)	0(0)	NC	5.82	0.02^{ns}

For ESR2: 0, CC; 1, CG/GG; For FSHR: 0, CC; 1, CT/TT; OR, Odds Ratio; NC, Not Calculable; ns, not significant;*, significant

Table 4 explores the relationship between ESR2 and FSHR genotype combinations and female infertility. The 0, 0 combinations (CC and CC) were evenly distributed between BR and NBR (4.6%) and served as the reference group with an OR of 1.0. The 0, 1 combination (CC and CT/TT) showed an OR of 2.33 (95% CI: 0.54–10.16), but the association was not statistically significant (p = 0.42). The 1, 0 combinations (CG/GG and CC) exhibited a non-significant trend with an OR of 3.67 (95% CI: 0.90–14.89, p = 0.11). The 1, 1 combination (CG/GG and CT/TT) was observed only in BR (1.8%) and was significantly associated with infertility (p = 0.02) with a χ^2 value of 5.82.

Table 5: Association between ESR1, ESR2, and FSHR genotype combinations and female infertility.

ESR1	ESR2	FSHR	BR (n=500)(%)	NBR (n=500) (%)	OR(95% CI)	X^2	P-Value
0	0	0	13(2.6)	18(3.6)	1.0		
0	1	0	4(0.8)	0(0)	NC	2.74	0.10^{ns}
0	0	1	1(0.2)	2(0.4)	0.69(0.06, 8.47)	0.11	0.74^{ns}
1	0	0	10(2.0)	5(1.0)	2.77(0.76.10.05)	1.58	0.21^{ns}
1	0	1	6(1.2)	1(0.2)	8.31(0.89, 77.57)	2.8	0.09^{ns}
1	1	0	7(1.4)	3(0.6)	3.23(0.70, 14.91)	1.39	0.24^{ns}
0	1	1	7(1.4)	0(0)	NC	5.57	0.02*
1	1	1	2 (0.4)	0(0)	NC	0.75	0.39^{ns}

For ESRI: 0, AA; 1, AT/TT; For ESR2: 0, CC; 1, CG/GG; For FSHR: 0, CC; 1, CT/TT; OR, Odds Ratio; NC, Not Calculable; ns, not significant; *significant

Table 5 evaluates the combined effect of ESR1, ESR2, and FSHR genotypes on female infertility. The 0, 0, 0 combinations (AA, CC, and CC) were the reference group with an OR of 1.0. The 0, 1, 0 combinations (AA, CG/GG, and CC) were observed in 0.8% of BR and absent in NBR, yielding a non-significant association (p = 0.10). The 0, 0, 1 combination (AA, CC, and CT/TT) had an OR of 0.69 (95% CI: 0.06–8.47), but the result was not significant (p = 0.74).

The 1, 0, 1 combination (AT/TT, CC, and CT/TT) showed a trend toward significance with an OR of 8.31 (95% CI: 0.89–77.57, p = 0.09). The 0, 1, 1 combination (AA, CG/GG, and CT/TT) demonstrated a significant association (p = 0.02). The remaining genotype combinations did not show significant associations.

DISCUSSION

In the present study, we have tested plausible association between ESR1, ESR2, and FSHR polymorphisms and susceptibility to infertility in beedi-rollers. We find statistically significant differences in the mean ages of the BR and NBR groups. An interview was conducted to collect information about reproductive health problems and outcomes and we found a higher prevalence of reproductive health issues among beedi-rollers, such as oligomenorrhea, primary ovarian insufficiency (POI), late-onset menopause, miscarriages, and infertility. A previous epidemiology study interviewed rural beedi-rollers and found they have a higher risk of infertility.[9] Similarly, Rudrama Devi et al., (2012) surveyed 128 beedi-roller families and observed a decrease in the fertility ratio in beedi-rollers who are continuously exposed to tobacco dust.[10] few more references (International or Indian).

In the current investigation, we explored the combined effect of ESR1, ESR2, and FSHR polymorphisms in infertile beedi-rollers compared to infertile non-beedi-rollers. Our results indicate that carriers of ESR2(CG/GG) and FSHR(CT/TT) appear to be more susceptible to infertility and we did not find an association between ESR1(AT/TT) and infertility. We also observed that irrespective of the genotype, beedi-rollers who were exposed to tobacco dust for a longer duration were at a significantly higher risk of infertility.

In support of our findings, Anagnostou et al., (2012) investigated the genotypes of ESR1, ESR2, and FSHR in infertile women who were shown poor response to IVF treatment. They analyzed each locus individually and in combination with others, and observed a significant association between all three gene polymorphisms and poor IVF outcomes. Carriers of Asn/Asn at 680 position of FSHR and CC genotype of ESR gene carriers were 1.5-2.4 times more likely to experience infertility.[11] A meta-analysis by Ya Li et al., (2012) confirmed that ESR1 (T/C) and (A/G) polymorphisms may not be associated with endometriosis-related infertility. Our study also implicate association between ESR1 (A/T) polymorphisms and infertility.[12]

However, in contrast to our findings, Aktaş et al., (2024) investigated the association between ESR1, ESR2, and FSHR polymorphisms and low ovarian reserve. However, they did not find any effect of ESR1, ESR2, and FSHR polymorphisms on low ovarian reserve and FSH-AMH discordance.[13] Paskulin et al., (2013) conducted a study on 98 infertile women compared with 134 fertile women. They found SNP ESR1 rs9340799 was associated with infertility and failed IVF attempts in the affected women.[14]

CONCLUSION

In summary, our study implicate critical roles of ESR2 and FSHR polymorphisms in influencing infertility risk among beedi-rollers, particularly in the context of prolonged exposure to tobacco dust. This highlights the need for targeted early intervention and policies to mitigate occupational hazards faced by beedi-rollers. Future research incorporating larger sample sizes should explore the complex gene-environment interactions contributing to infertility, and diverse populations to validate and replicate the findings.

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