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"ROLE OF RAPID MOLECULAR TEST TO DETECT CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEA IN DIAGNOSING SUBCLINICAL PID"

Obstetrics & Gynaecology

Dr Parul Girotra	Junior Resident, Dept Of Obstetrics And Gynaecology, Netaji Subhash Chandra Bose Subharti Medical College Swami Vivekanand Subharti University
Dr Smriti Gupta	MBBS, DNB, Professor And Hod Obstetrics And Gynaecology, Netaji Subhash Chandra Bose Subharti Medical College Swami Vivekanand Subharti UniversitY
Dr Rekha Gupta	MBBS, MD, Associate Professor, Obstetrics And Gynaecology, Netaji Subhash Chandra Bose Subharti Medical College Swami Vivekanand Subharti University
Dr Ravi Pratap Singh	MBBS, MD, Associate Professor, Dept. Of Biochemistry, Netaji Subhash Chandra Bose Subharti Medical College Swami Vivekanand Subharti University
Anjali Khare	MBBS, MD, Professor And Hod, Dept. Of Pathology, Netaji Subhash Chandra Bose Subharti Medical College Swami Vivekanand Subharti University

ABSTRACT

Background: Pelvic Inflammatory Disease (PID), especially its subclinical form, remains a significant cause of infertility and reproductive morbidity in women. Chlamydia trachomatis and Neisseria gonorrhoeae are key pathogens implicated in PID, often remaining undetected due to asymptomatic infections. **Objectives:** To evaluate the role of rapid molecular testing for Chlamydia trachomatis and Neisseria gonorrhoeae in diagnosing subclinical PID among women attending a gynecology outpatient clinic. **Methods:** A cross-sectional study was conducted on 80 women aged 18–45 years, divided into clinically diagnosed PID cases and controls. Endocervical swabs were analyzed using real-time PCR for Chlamydia trachomatis and Neisseria gonorrhoeae. Bacterial vaginosis was assessed via Nugent scoring and Amsel's criteria. **Results:** PCR positivity for Chlamydia trachomatis and Neisseria gonorrhoeae was significantly higher among clinical PID cases ($p<0.05$). Combined testing improved diagnostic sensitivity and specificity. **Conclusion:** Rapid molecular tests are effective in early detection of subclinical PID, enhancing reproductive health outcomes.

KEYWORDS

Pelvic Inflammatory Disease; Chlamydia trachomatis; Neisseria gonorrhoeae; Polymerase Chain Reaction; Subclinical PID.

INTRODUCTION

Pelvic Inflammatory Disease (PID) remains a major public health concern worldwide, contributing significantly to infertility, ectopic pregnancy, and chronic pelvic pain in women of reproductive age. Subclinical PID, the silent variant of this disease, is particularly worrisome as it often progresses unnoticed due to the absence of classical clinical symptoms, resulting in long-term reproductive morbidity (1,2).

Among the most common causative organisms of PID are Chlamydia trachomatis and Neisseria gonorrhoeae, both sexually transmitted pathogens with the potential to cause severe, asymptomatic infections of the lower and upper genital tract (3). Approximately 80% of Chlamydia trachomatis infections and 40% of Neisseria gonorrhoeae infections in women remain asymptomatic, leading to unrecognized progression to fallopian tube damage, tubal scarring, and infertility (4). Conventional diagnostic methods such as clinical assessment, culture, and serological testing often lack sensitivity, particularly for detecting asymptomatic or subclinical infections (5). Nucleic Acid Amplification Tests (NAATs) have emerged as a gold standard for detecting C. trachomatis and N. gonorrhoeae, offering high sensitivity, specificity, and the advantage of non-invasive sample collection (6,7). Despite their proven utility, the routine application of rapid molecular tests in the diagnosis of subclinical PID remains underexplored, particularly in low-resource settings like India, where the burden of undiagnosed sexually transmitted infections remains high (8,9).

This study aimed to evaluate the role of rapid molecular testing in detecting Chlamydia trachomatis and Neisseria gonorrhoeae in women attending gynecology outpatient clinics. The findings may help in the early identification of subclinical PID, thereby improving reproductive health outcomes and guiding preventive strategies.

MATERIALAND METHODS

This cross-sectional observational study was carried out in the Department of Obstetrics and Gynaecology at Subharti Medical College, Meerut, with the aim of evaluating the role of rapid molecular testing for Chlamydia trachomatis and Neisseria gonorrhoeae in the diagnosis of subclinical Pelvic Inflammatory Disease (PID). The study was conducted over a period of two years, from July 2023 to February 2025. Women aged between 18 and 45 years attending the gynaecology outpatient department were considered for participation. Only those who provided written and informed consent in a language they understood were included in the study. Pregnant women were excluded.

A total of 80 women were recruited through convenient sampling. The study participants were divided into two groups. The first group consisted of 40 women presenting with clinical signs and symptoms of PID, while the second group included 40 women without any clinical features suggestive of PID and served as the control group.

All participants underwent collection of an endocervical swab for detection of Chlamydia trachomatis and Neisseria gonorrhoeae using real-time polymerase chain reaction (PCR) testing. The PCR analysis targeted the cryptic plasmid multicopy sequence and the 16S ribosomal RNA gene specific to each pathogen. The use of fluorescence-labelled probes allowed real-time monitoring of the amplification process, with increased fluorescence indicating the presence of pathogen DNA. To enhance sensitivity and prevent contamination, the diagnostic kit incorporated "hot-start" technology and uracil-DNA glycosylase, ensuring high specificity and reliability. The system was capable of detecting as little as a single genome copy of C. trachomatis or N. gonorrhoeae, making it highly suitable for detecting even low-grade asymptomatic infections.

In addition to the endocervical swab, a high vaginal swab was collected from all participants to assess for bacterial vaginosis (BV). The Nugent scoring system, based on Gram staining and microscopic evaluation of bacterial morphotypes, was used to diagnose BV, with a score of seven or more considered positive. Amsel's clinical criteria were also applied, requiring the presence of at least three of four clinical features, including homogeneous vaginal discharge, clue cells on microscopy, elevated vaginal pH, and a positive whiff test.

Women who tested positive for either Chlamydia trachomatis, Neisseria gonorrhoeae, or BV were classified as PID-positive for the purpose of this study, while those negative for all three were considered PID-negative. All data were systematically recorded and subjected to statistical analysis using Systat 13.2 software to determine the association between the presence of these infections and clinical diagnosis of PID, in accordance with the study objectives.

RESULTS

The present study assessed the role of rapid molecular tests in detecting Chlamydia trachomatis (ChT) and Neisseria gonorrhoeae (NG) for diagnosing subclinical pelvic inflammatory disease (PID) among women. The demographic and baseline characteristics such as age, socioeconomic status, age at coitarche, residence, and parity were comparable between the clinical PID group and controls, with no statistically significant differences, confirming that both groups were well-matched for meaningful comparison (**Table 1**). The diagnostic test positivity revealed a significantly higher prevalence of Chlamydia trachomatis (27.5% vs. 5%, p=0.02) and Neisseria gonorrhoeae (20% vs. 2.5%, p=0.03) among clinical PID cases, highlighting their role as key contributors to subclinical PID, whereas bacterial vaginosis showed no significant association with PID status (p=0.42) (**Table 2**). The diagnostic performance of PCR for Chlamydia trachomatis demonstrated high specificity (95%) and positive predictive value (84.6%), though sensitivity was moderate (27.5%), indicating its reliability in confirming PID diagnosis. Similarly, PCR for Neisseria gonorrhoeae showed excellent specificity (97.5%) and PPV (88.9%), despite low sensitivity (20%), reflecting its importance in detecting PID cases with active infection. Bacterial vaginosis testing showed limited diagnostic value, with low sensitivity (17.5%) and specificity (87.5%), suggesting it plays only a secondary role in PID detection. Importantly, combined testing for any positive result significantly improved diagnostic accuracy, with a sensitivity of 65% and specificity of 80%, supporting the utility of multiplex molecular testing for early identification of subclinical PID (**Table 3**).

Table 1: Distribution of Demographic and Baseline Characteristics Among Study Participants

Characteristics	Parameter	Cases (n=40)	Controls (n=40)	P-value
Age (Years)	18–26	8	7	**0.084**
	27–35	21	22	
	36–44	11	11	
Socioeconomic Status	Lower	17	19	**0.078**
	Lower Middle	10	9	
	Middle	11	10	
	Upper Middle	2	2	
Age at Coitarche (Years)	18–20	25	17	**0.084**
	21–23	11	10	
	24–26	4	10	
	≥27	0	3	
Residence	Residence	12	10	**0.077**
	Residence	28	30	
Parity	Nullipara	4	9	**0.051**
	Primipara	8	5	
	Multipara	28	26	

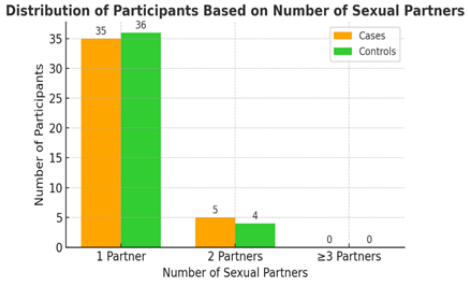

Figure 1: Distribution of Participants Based on number of Sexual Partners

Table 2: Diagnostic Test Positivity Among Study Groups

Test Parameter	Clinical PID Positive (n=40)	Clinical No PID Positive (n=40)	P-value
PCR Chlamydia Positive	11	2	0.02
PCR Gonorrhoea Positive	8	1	0.03
Bacterial Vaginosis Positive	7	5	0.42

Table 3: Diagnostic Performance of PCR for Chlamydia, Gonorrhoea, Bacterial Vaginosis, and Combined Testing in Detecting Subclinical PID

Variables	Cases (%)	Controls (%)	Sensitivity	Specificity	PPV	NPV
PCR Chlamydia Positivity	Positive	11 (27.5%)	2 (5%)	27.5%	95%	84.6%	56.7%
	Negative	29 (72.5%)	38 (95%)				
PCR Gonorrhoea Positivity	Positive	8 (20%)	1 (2.5%)	20%	97.5%	88.9%	54.9%
	Negative	32 (80%)	39 (97.5%)				
Bacterial Vaginosis Positivity	Positive	7 (17.5%)	5 (12.5%)	17.5%	87.5%	58.3%	51.5%
	Negative	33 (82.5%)	35 (87.5%)				
Any Test Positivity (Combined)	Positive	26 (65%)	8 (20%)	65%	80%	76.5%	69.6%
	Negative	14 (35%)	32 (80%)				

DISCUSSION

Our study revealed that the majority of participants belonged to the 27–35 years age group, with no significant difference between cases and controls (p = 0.084). This is consistent with findings from Fuller et al. (2021) and Rackova et al. (2022) who reported a higher burden of ChT and NG infections among women in the reproductive age group, particularly between 18–35 years (9,10). Similarly, studies by Hoenderboom et al. (2019) and Heijer et al. (2019) emphasized that younger women are at increased risk of these infections and PID complications (11,12). Socioeconomic status also did not show a statistically significant association with PID (p = 0.078) in our study. However, previous research by Fuller et al. (2021) and Rowley et al. (2019) has established that women from lower socioeconomic backgrounds are generally more vulnerable to undiagnosed sexually transmitted infections, reinforcing the need for targeted public health screening in these populations (9,13). Regarding sexual behavior, the majority of our participants reported coitarche between 18–20 years and a single sexual partner. Similar trends were observed in Indian studies by Patel V et al. (2006), which associated early sexual debut with increased risk of ChT infection (14). However, multiple sexual partners were not significantly associated with PID in our cohort, possibly due to the conservative sexual behavior of the studied population. Analysis of residence showed a higher number of participants from urban areas, but this was not statistically significant (p = 0.077). Similar observations were made by Munro MG et al. (2018), indicating no conclusive difference in PID prevalence between urban and rural women (15).

In our study, PCR positivity for Chlamydia trachomatis was significantly higher in clinical PID cases (27.5%) compared to controls (5%), with a p-value of 0.02. These findings align with those of Wiesenfeld et al. (2005) and Oakeshott et al. (2010), who reported a significant association between Chlamydia infection and PID, albeit with varying prevalence rates (16,17). PCR detection for Neisseria gonorrhoeae also showed a significant association with clinical PID (20% in cases vs. 2.5% in controls, p = 0.03), consistent with global trends highlighted by Bender et al. (2011) and Peeling et al. (2006) (18,19). Despite moderate sensitivity (27.5% for Chlamydia, 20% for Gonorrhoea), both tests demonstrated excellent specificity (95% and 97.5%, respectively). Bacterial vaginosis showed no significant difference between groups (p = 0.42), aligning with previous research indicating its limited role in PID diagnosis. Importantly, combined test positivity yielded improved sensitivity (65%) and specificity (80%), supporting the role of multiplex testing in enhancing PID detection, as emphasized by Wiesenfeld et al. (2012) and Edsei Jr et al. (2013) (16,20).

Limitations Of The Study: The limitations of the study include a relatively small sample size, use of convenient sampling, and inability to assess long-term outcomes, which may limit the generalizability of the findings to broader populations.

Strengths Of The Study: The strengths of the study include the use of highly sensitive PCR-based molecular diagnostics, inclusion of both symptomatic and asymptomatic women, and combined testing strategy, enhancing early and accurate detection of subclinical PID.

CONCLUSION

We concluded that rapid molecular tests for Chlamydia trachomatis and Neisseria gonorrhoeae significantly aid in diagnosing subclinical PID, with high specificity and improved detection through combined

testing, supporting their utility in routine screening.

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