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# TMSS Medical College Journal (TMCJ)

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**January 2026**

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Volume 25, Issue. 01, January 2026

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## Address of Correspondence

Editor-in-Chief, TMSS Medical College Journal, TMSS Medical College  
 Rangpur Road, Thengamara, Bogura, Bangladesh. Phone: 01775-316617  
 e-mail: editortmcjournal@gmail.com, Web: www.tmcjournal.org, www.tmssmedicalcollege.com

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**Editorial****From Immunohistochemistry to Molecular Testing for the Precision Cancer Management of Breast Cancer**Rahman DA<sup>1</sup>, Akter S<sup>2</sup>

1. Dr. DM Arifur Rahman, Associate Professor, Department of Pathology, TMSS Medical College, Bogura.

2. Shormily Akter, Scientist, TMSS Biomolecular Lab, TMSS Medical College, Bogura.

**Introduction**

Breast cancer is not a single disease. It is a biologically heterogeneous collection of malignancies, each with its own molecular fingerprint, natural history, and therapeutic vulnerability. For decades, the clinical management of breast cancer rested on a triad of morphology, staging, and the immunohistochemical (IHC) expression of three cardinal biomarkers: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), supplemented by the proliferation index Ki-67.<sup>1</sup> This IHC-based surrogate classification formalised by the St. Gallen International Expert Consensus has undeniably transformed breast oncology, stratifying patients into five clinically actionable subtypes and guiding systemic therapy decisions with meaningful prognostic discrimination.<sup>2</sup> Yet the limitations of this approach have grown increasingly apparent as precision oncology matures: IHC surrogates achieve molecular concordance of only 80–85% with gene expression profiling, are subject to inter observer variability, and critically fail to capture the dynamic intra-tumoral genomic complexity that underlies treatment resistance.<sup>3</sup>

The clinical imperative to move beyond IHC is now well evidenced. Multigene expression assays Oncotype DX, MammaPrint, Prosigna, and Endo Predict have demonstrated robust prospective validation as tools for chemotherapy decision-making in hormone receptor-positive, HER2 negative early breast cancer, with the TAILORx and MINDACT trials establishing their capacity to spare a substantial proportion of patients from unnecessary cytotoxic therapy.<sup>4,5</sup> In the HER2 positive space, the DESTINY

Breast04 trial redefined the therapeutic boundary of HER2 classification, demonstrating that trastuzumab-deruxtecan (T-DXd) achieves significant survival benefit in HER2 low tumours- IHC score 1+ or 2+ with negative in situ hybridization a category entirely invisible to conventional HER2-positive/negative binary classification.<sup>6</sup> This landmark finding alone renders the binary IHC framework clinically obsolete for a subset of patients and underscores the need for refined biomarker stratification.

For triple-negative breast cancer (TNBC), historically defined by the absence of ER, PR, and HER2 expression, the paradigm shift has been equally dramatic. Next-generation sequencing (NGS) based profiling has dissected TNBC into at least six molecular subtypes including basal like 1 and 2, immunomodulatory, mesenchymal, mesenchymal stem like, and luminal androgen receptor subtypes each with distinct therapeutic vulnerabilities.<sup>7</sup> BRCA1/2 germline mutation testing now guides PARP inhibitor use, PD-L1 IHC combined with immune gene signatures directs pembrolizumab eligibility, and PIK3CA/AKT1 pathway mutations are emerging targets in advanced disease.<sup>8,9</sup> IHC alone cannot adequately navigate this complexity.

Against this evolving landscape, the article by Rahman et al. published in this issue presents a timely and clinically significant dataset. The authors characterise the IHC-based molecular subtype distribution of 107 invasive breast carcinoma patients from a Bangladeshi tertiary centre, revealing that TNBC constitutes 30.8% of cases substantially exceeding global rates of 15–20% while Luminal A accounts for only 26.2%.<sup>10</sup> These findings delineate a population with a

disproportionate burden of the most therapeutically challenging subtype, in a setting where NGS based molecular testing remains largely inaccessible. Significant associations between molecular subtypes and histological grade ( $p < 0.004$ ) and lymph node metastasis ( $p < 0.035$ ) validate the biological credibility of their IHC classification and highlight the indispensability of even basic molecular stratification in guiding oncological decisions.

The study from Rahman et al. is therefore both an endorsement of IHC as the essential foundation in resource limited settings and an implicit call to action for the next step. IHC remains irreplaceable as the accessible, affordable, and reproducible cornerstone of breast cancer diagnostics globally; however, it must increasingly be regarded as the beginning, not the end, of the molecular diagnostic pathway.<sup>11</sup> As NGS platforms become more affordable and liquid biopsy technologies mature, integration of comprehensive genomic profiling into routine breast oncology workflows is no longer a futuristic aspiration but an immediate translational priority even in low and middle income countries.<sup>12</sup>

The trajectory is clear: precision breast cancer management demands a staged, scalable molecular diagnostic framework in which IHC provides the initial subtype scaffold, and targeted molecular testing multigene assays, hotspot mutation panels, and ultimately comprehensive NGS refines therapeutic decision making at each clinical decision point. Bridging this translational gap equitably, across all geographies and economic contexts, remains the defining challenge of the next decade in breast oncology.

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**Original Article****Immunohistochemistry-Based Molecular Subtype Distribution of Invasive Breast Cancer and Its Clinicopathological Correlates in a Bangladeshi Cohort**

Rahman DA<sup>1\*</sup>, Habib AA<sup>2</sup>, Mazid A<sup>3</sup>, Akter S<sup>4</sup>, Ahmed F<sup>5</sup>, Benzir M<sup>6</sup>, Sakib MN<sup>7</sup>, Rahman MT<sup>8</sup>, Alamgir MH<sup>9</sup>.

1. Dr. DM Arifur Rahman, Associate Professor, Department of Pathology, TMSS Medical College, Bogura.
2. Dr. AKM Ahsan Habib, Professor, Department of Oncology, TMSS Medical College, Bogura.
3. Dr. Md. Abdul Mazid, Professor, Department of Surgery, TMSS Medical College, Bogura.
4. Shormily Akter, Scientist, TMSS Biomolecular Laboratory, TMSS Medical College, Bogura.
5. Dr. Forhad Ahmed, Assistant Professor, Department of Pathology, TMSS Medical College, Bogura.
6. Dr. Maskura Benzir, Assistant Professor, Department of Anatomy, TMSS Medical, Bogura.
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9. Dr. Moudud Hossain Alamgir, Professor, Department of Surgery, TMSS Medical College, Bogura.

**\*Corresponding Author****Abstract**

**Background/Context:** Breast cancer is the second most common cancer worldwide, with approximately 2.3 million new cases annually. In Bangladesh, the disease predominantly affects premenopausal women and often presents at an advanced stage. The distribution of molecular subtypes which determine treatment strategies and prognosis has not been comprehensively characterised in large scale single institution studies from Bangladesh.

**Objective:** To determine the prevalence and distribution of molecular subtypes of invasive breast carcinoma using immunohistochemical (IHC) surrogate markers according to St. Gallen criteria, and to evaluate their associations with clinicopathological parameters in a Bangladeshi cohort.

**Materials and Methods:** This cross-sectional observational study enrolled 107 consecutive patients with histopathologically confirmed invasive breast carcinoma at the Department of Pathology, TMSS Medical College, Bogura, Bangladesh, from January 2024 to December 2024. IHC was performed for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 using standardised protocols with ASCO/CAP guidelines for HER2 and the Allred scoring system for hormone receptors. Tumours were classified into molecular subtypes (Luminal A, Luminal B, HER2-enriched, and triple-negative breast cancer (TNBC) per St. Gallen surrogate definitions. Clinicopathological associations were assessed using chi-square or Fisher's exact test ( $p < 0.05$ ).

**Results:** The mean patient age was  $45.4 \pm 10.6$  years, with 67.3% of patients in the 31–50 years age group. TNBC was the most prevalent subtype (33/107, 30.8%), followed by Luminal A (28/107, 26.2%), HER2-enriched (23/107, 21.5%), Luminal B (18/107, 16.8%), and Luminal B (HER2-positive) tumours (5/107, 4.7%). Statistically significant associations were identified between molecular subtype and histological grade ( $p = 0.004$ ) and lymph node metastasis status ( $p = 0.035$ ). Grade III tumours were predominantly TNBC (52.2%), while Luminal A predominated in Grade I (50%). HER2 enriched subtype was the most frequent subtype among cases with extensive nodal involvement (N3a, 60%).

**Conclusions:** This study reveals a distinctive molecular landscape of breast cancer in Bangladesh, characterised by a high prevalence of TNBC exceeding global rates. The significant associations between molecular subtypes and tumour grade and nodal status validate the St. Gallen IHC classification for clinical practice in resource-limited settings. These findings underscore the urgent need for robust chemotherapy infrastructure, access to HER2-targeted therapies, and enhanced early detection programmes.

**Keywords:** Immunohistochemistry, Molecular subtypes, Triple-negative breast cancer, Estrogen receptor, HER2 protein, Ki-67 antigen, Bangladesh

**Introduction**

Breast cancer is the most prevalent malignancy among women worldwide and represents a major global health burden. According to GLOBOCAN 2022 estimates, female breast cancer is the second leading

cause of cancer incidence globally, with approximately 2.3 million new cases accounting for 11.6% of all cancer diagnoses. The disease ranks fourth in cancer-related mortality, responsible for

approximately 666,000 deaths (6.9% of global cancer deaths) annually.<sup>1</sup> Despite moderate improvements in survival attributable to advances in early detection and systemic therapies, breast cancer continues to claim an unacceptably high number of lives, owing largely to its heterogeneous biological behaviour, variable treatment responsiveness, and differential access to care worldwide.<sup>2</sup>

In Bangladesh, breast cancer constitutes a formidable public health challenge. Until 2015, it was the leading cancer among Bangladeshi women, contributing to 69% of female cancer deaths.<sup>3</sup> The age-standardised incidence rate is 22.5 per 100,000 women, with 12,764 new cases reported in 2018 according to the International Agency for Research on Cancer (IARC).<sup>4</sup> Uniquely, the disease disproportionately affects premenopausal women: epidemiological studies from Bangladesh demonstrate that 82.35% of patients present with advanced stage disease (Stage III–IV), and 77% of these patients are under 50 years of age.<sup>5</sup> This pattern contrasts markedly with Western populations, where breast cancer incidence peaks in the sixth decade.<sup>6</sup>

Historically, breast cancer classification relied on clinicopathological features including tumour stage, histological grade, and morphological parameters. However, over 70% of breast cancers are classified as invasive ductal carcinoma of no special type (IDC-NST), indicating that morphology alone provides insufficient biological discrimination.<sup>7</sup> The recognition that cancers of identical histological type exhibit vastly different clinical behaviours prompted the development of molecular classification systems. Gene expression profiling studies pioneered by Perou and colleagues identified intrinsic molecular subtypes Luminal A, Luminal B, HER2- enriched, and Basal-like based on hierarchical clustering of gene expression patterns.<sup>8</sup> These subtypes demonstrate distinct clinical behaviours, prognoses, and therapeutic responses. However, routine gene expression profiling is constrained by high cost, technical complexity, and limited availability, particularly in resource-limited settings such as Bangladesh.<sup>9</sup> Since 2011, the St. Gallen International Expert Consensus has adopted surrogate immunohistochemical (IHC) definitions utilising estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2

(HER2), and the proliferation marker Ki-67. IHC used to approximate molecular subtypes in clinical practice.<sup>10</sup> This approach provides a cost-effective, reproducible, and clinically actionable classification system that is increasingly used worldwide.<sup>11</sup> Validation studies confirm that IHC-based surrogate classification effectively stratifies patients into distinct prognostic categories with significant differences in disease-free survival and overall survival.<sup>12</sup>

Epidemiological studies reveal significant geographic and ethnic variation in molecular subtype distribution. Western populations demonstrate higher prevalence of Luminal A subtype (40–50%). South Asian and African populations report elevated proportions of HER2 positive and triple-negative breast cancer (TNBC) subtypes.<sup>13</sup> Data from Bangladesh remain limited; previous small-scale studies have suggested higher rates of aggressive subtypes. But large, single-institution characterisations employing standardised methodology are lacking.<sup>14</sup> Comprehensive understanding of the molecular subtype landscape in Bangladesh is essential for informing healthcare resource allocation, guiding treatment policy, facilitating access to targeted therapies, and designing population-specific clinical trials.

Therefore, the aim of this study was to determine the distribution of molecular subtypes of invasive breast carcinoma using IHC surrogate markers according to St. Gallen criteria, evaluate the expression patterns of ER, PR, HER2, and Ki-67, and analyse the associations between molecular subtypes and clinicopathological parameters in a Bangladeshi cohort.

## Materials and Methods

### Study Design and Ethical Approval

This cross-sectional observational study was conducted at the Department of Pathology and Immunohistochemistry Laboratory, TMSS Medical College, Bogura, Bangladesh. The study was approved by the Institutional Ethical Review Committee of TMSS Medical College. All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to tissue sampling and inclusion in the study.

## Study Setting

The study was conducted at a tertiary care academic medical centre in Northern Bangladesh. Data collection and laboratory analyses were performed between January 2024 and December 2024.

## Study Population

Patients referred to the Department of Pathology with clinically suspected breast malignancy and requiring histopathological evaluation were screened for eligibility.

Inclusion criteria: (1) histopathologically confirmed invasive breast carcinoma; (2) adequate tissue available for IHC analysis; (3) complete clinicopathological data; and (4) documented patient consent for research use of tissue.

Exclusion criteria: (1) non-invasive breast lesions; (2) availability of adequate tissue for IHC analysis; (3) history of neoadjuvant chemotherapy or hormonal therapy; and (4) patients with incomplete clinical records.

A total of 107 consecutive patients meeting the inclusion criteria were enrolled. This sample size is consistent with published single-institution molecular subtype studies from South Asia and permits meaningful chi-square analysis for clinicopathological associations.

## Specimen Collection and Processing

Tissue samples were obtained through core needle biopsy (CNB) or surgical excision (modified radical mastectomy, mastectomy, wide local excision, or lumpectomy). For CNB procedures, tissue was obtained using a 14-gauge needle with a sample notch length of 15–22 mm on a spring-loaded automated biopsy gun; five core biopsies were obtained per patient under local anaesthesia, with ultrasound guidance where required. Specimens were immediately fixed in 10% neutral buffered formalin for a minimum of 6 hours and a maximum of 30 hours prior to processing.

Fixed tissues underwent automated tissue processing with graded alcohol dehydration, xylene clearing, and paraffin infiltration. Sections of 3–4  $\mu$ m thickness were cut using a rotary microtome and mounted on glass slides. Haematoxylin and eosin (H&E) staining was

performed for histopathological diagnosis. Histological type was classified according to WHO criteria. Tumour grading was performed using the Nottingham modification of the Bloom–Richardson system, assigning grades I, II, or III based on tubule formation, nuclear pleomorphism, and mitotic count. Additional parameters recorded included tumour size, presence of lymphovascular invasion (LVI), and lymph node metastasis status (where surgical specimens were available).

## Immunohistochemistry Protocol

IHC was performed manually at the Immunohistochemistry Laboratory, Department of Pathology, TMSS Medical College. Fresh 3–4  $\mu$ m sections were cut from selected paraffin blocks and mounted on positively charged slides. Sections were deparaffinised in xylene and rehydrated through graded alcohols. Heat-induced epitope retrieval (HIER) was performed by microwave antigen retrieval for 20 minutes at 98°C in Tris-EDTA buffer (pH 9.0). Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 10 minutes.

### Primary antibodies were applied as follows:

- Anti-ER: Clone 1D5 (Dako, Code N1575), ready-to-use, prediluted
- Anti-PR: Clone PgR 636 (Dako, Code N1630), ready-to-use, prediluted
- Anti-HER2/neu: Clone 4B5 (Ventana), ready-to-use, prediluted
- Anti-Ki-67: Clone MIB-1 (Dako), ready-to-use, prediluted

Primary antibody incubation was performed for 60 minutes at room temperature. Detection was achieved using the Labelled Streptavidin–Biotin (LSAB) method (Dako) with diaminobenzidine (DAB) as chromogen. Sections were counterstained with Mayer's haematoxylin, dehydrated, cleared, and coverslipped.

## Controls

Three tiers of controls were employed for each IHC run: (1) strong external positive control (known strongly positive breast cancer tissue); (2) weak external positive control (known weakly positive breast cancer tissue); and (3) internal positive control



(normal breast epithelium within the tumour section where available). Negative controls were prepared by substituting the primary antibody with antibody diluent. All IHC-stained slides were independently reviewed by two experienced histopathologists; discordant cases were reviewed jointly to achieve consensus.

### Scoring and Interpretation

ER and PR: Expression was evaluated using the Allred scoring system combining proportion score (0–5) and intensity score (0–3) for a total score of 0–8. Scores of 0–2 were classified as negative; scores of 3–8 as positive. Nuclear staining of at least 1% of tumour cells was the threshold for positivity.

HER2: Scored according to ASCO/CAP guidelines:

- 0 = no staining or membrane staining in < 10% of cells;
- 1+ = faint/incomplete membrane staining in > 10% of cells;
- 2+ = weak to moderate complete membrane staining in > 10% of cells (equivocal);
- 3+ = strong complete membrane staining in > 10% of cells (positive).

Scores 0 and 1+ were classified negative; 3+ positive. Score 2+ cases were classified as negative in the absence of fluorescence in situ hybridisation (FISH) confirmation, a limitation acknowledged in this study.

Ki-67: The labelling index was determined as the percentage of Ki-67 positive nuclei among at least 500 tumour cells in areas of highest proliferative activity (hot spots). Ki-67 < 14% was classified as low;  $\geq$  14% as high, per St. Gallen recommendations.

### Molecular Subtype Classification

Tumours were classified according to St. Gallen surrogate definitions [10,11]:

- Luminal A: ER-positive and/or PR-positive, HER2-negative, Ki-67 < 14%
- Luminal B (HER2-negative): ER-positive and/or PR-positive, HER2-negative, Ki-67  $\geq$  14%
- Luminal B (HER2-positive): ER-positive and/or PR-positive, HER2-positive, any Ki-67
- HER2-enriched: HER2-positive, ER-negative, PR-negative

- Triple-negative (TNBC/Basal-like): ER-negative, PR-negative, HER2-negative
- Luminal B (HER2-positive): ER-positive and/or PR-positive, HER2-positive

For analytical purposes, Luminal B (HER2 negative) and Luminal B (HER2 positive) were combined into a single Luminal B category.

### Statistical Analysis

Data were entered and analysed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages. Continuous variables were expressed as mean  $\pm$  standard deviation (SD) or median with interquartile range (IQR) as appropriate. Associations between molecular subtypes and clinicopathological variables were assessed using the chi-square test or Fisher's exact test for categorical variables. Statistical significance was defined as  $p < 0.05$  with 95% confidence interval (CI).

## Results

### Demographic and Clinical Characteristics

A total of 107 patients with histopathologically confirmed invasive breast carcinoma were enrolled. The age of patients ranged from under 30 years to over 50 years, with a mean age of  $45.4 \pm 10.6$  years (Table I). The majority of patients (72 cases, 67.3%) belonged to the 31–50 years age group, followed by the > 50 years group (28 cases, 26.2%), and the < 30 years group (7 cases, 6.5%). This age distribution reflects the characteristic earlier age at breast cancer presentation in South Asian populations.

Regarding specimen type (Table II), modified radical mastectomy specimens accounted for the largest proportion (46 cases, 43.0%), followed by core needle biopsy specimens (45 cases, 42.1%), mastectomy specimens (13 cases, 12.1%), lumpectomy specimens (2 cases, 1.9%), and wide local excision specimens (1 case, 0.9%). Overall, the specimen distribution was predominantly represented by modified radical mastectomy and core needle biopsy specimens.

### Tumour Characteristics

Tumour size was evaluable in 86 cases where surgical specimens were obtained (Table III). The majority of

measurable tumours (72/86, 83.7%) were in the 2.1–5 cm range (pT2 stage). Only 4 tumours (3.7%) measured  $\leq 2$  cm (pT1), and 10 tumours (9.3%) were  $> 5$  cm (pT3). Tumour size could not be assessed in 21 cases (19.6%) with core biopsies only.

Histological grading was performed on 96 cases (Table IV). Grade II tumours were most common (49 cases, 45.8%), followed by Grade I (24 cases, 22.4%) and Grade III (23 cases, 21.5%). Grading was not determinable in 11 cases (10.3%) due to limited tissue or processing artefacts.

Lymphovascular invasion (LVI) was assessable in 35 cases with adequate tissue (Table V). LVI was present in 31/35 assessable cases (88.6%; 29.0% of total cohort) and absent in 4/35 (11.4%; 3.7% of total). This parameter was unevaluable in 72 cases (67.3%), predominantly core biopsies.

Lymph node status was determined in 48 surgical specimens with axillary lymph node dissection (Table VI). Of these, 21 cases (43.8% of assessed; 19.6% of total) were node-negative (N0). Nodal metastasis was present in 27/48 assessed cases: N1a (1–3 positive nodes) in 8 cases (7.5%), N2a (4–9 positive nodes) in 10 cases (9.3%), N3a ( $\geq 10$  positive nodes) in 5 cases (4.7%), and N1 (not further classified) in 4 cases (3.7%). Nodal status was unavailable in 59 cases (55.1%) where core biopsies or mastectomy without axillary dissection were performed.

### Immunohistochemical Marker Expression

The distribution of individual IHC markers is presented in (Table VII). Estrogen receptor (ER) positivity was detected in 51 cases (47.7%), with 56 cases (52.3%) ER-negative. Progesterone receptor (PR) positivity was observed in 40 cases (37.4%), with 67 cases (62.6%) PR-negative. HER2 overexpression (score 3+) was identified in 29 cases (27.1%), with 78 cases (72.9%) HER2-negative.

### Molecular Subtype Distribution

Classification according to St. Gallen criteria (Table VIII) revealed the following distribution:

- Triple-negative breast cancer (TNBC): 33 cases (30.8%)- most prevalent subtype
- Luminal A: 28 cases (26.2%)- second most common

- HER2-enriched: 23 cases (21.5%)- third most common
- Luminal B: 18 cases (16.8%)- fourth most common
- Luminal B (HER2-positive): 5 cases (4.7%) - least common

Combined hormone receptor-positive subtypes (Luminal A + Luminal B + Luminal B (HER2-positive)) accounted for 47.7% of all cases. Total HER2-positive disease (HER2-enriched + Luminal B HER2-positive) constitute 26.2% of the cohort.

### Association Between Molecular Subtypes and Clinicopathological Parameters

Results of subtype-clinicopathological associations are summarised in Table 9 and described below.

**Age distribution:** No statistically significant association was found between molecular subtype and patient age group ( $p = 0.525$ ). Among patients  $\leq 30$  years ( $n = 7$ ), Luminal A accounted for 42.9%, HER2 enriched 28.6%, and TNBC 28.6%. In the 31–50 years group ( $n = 72$ ), TNBC was most prevalent (29.2%), followed by HER2-enriched (23.6%), Luminal A (20.8%), and Luminal B (19.4%). Among patients  $> 50$  years ( $n = 28$ ), TNBC and Luminal A were equally prevalent (35.7% each). As shown in Figure 2, aggressive subtypes were represented across all age groups.

**Tumour size:** No significant association was observed between tumour size and molecular subtype ( $p = 0.428$ ). Among tumours in the 2.1–5 cm category ( $n = 72$ ), TNBC was most prevalent (33.3%), followed by Luminal A (31.9%), Luminal B (16.7%), and HER2 enriched (15.3%).

**Histological grade:** A highly statistically significant association was identified between tumour grade and molecular subtype ( $p = 0.004$ ; Table 9). Grade I tumours ( $n = 24$ ) were predominantly Luminal A (50%), with HER2-enriched at 29.2% and TNBC at only 4.2%. Grade II tumours ( $n = 49$ ) showed a more heterogeneous distribution: TNBC 32.7%, Luminal A 26.5%, HER2-enriched 16.3%, Luminal B 14.3%, and Luminal B (HER2-positive) 10.2%. Grade III tumours ( $n=23$ ) demonstrated striking enrichment for aggressive subtypes: TNBC 52.2%, HER2-enriched 21.7%, Luminal B 17.4%, and Luminal A only 8.7%. Figure 3 demonstrates the grade-subtype relationship.

**Lymph node metastasis:** A statistically significant association was identified between lymph node status and molecular subtype ( $p = 0.035$ ; Table 9). Among node-negative cases (N0,  $n = 21$ ), Luminal A predominated (47.6%), followed by TNBC (33.3%) and Luminal B (14.3%). In cases with N3a disease ( $\geq 10$  positive nodes,  $n = 5$ ), HER2-enriched accounted for 60% (3 cases) and Luminal A for 40% (2 cases). N1 cases showed TNBC predominance (75%).

**Lymphovascular invasion:** No statistically significant association was found between LVI status and molecular subtype ( $p = 0.582$ ). Among LVI-positive cases ( $n = 31$ ), Luminal A represented 38.7%, TNBC 32.3%, HER2-enriched 12.9%, and Luminal B 9.7%.

**Age and tumour grade:** The association between patient age and tumour grade was non-significant ( $p = 0.402$ ; Table 10). Grade II was the most common grade across all age groups.

**Table I. Patient Age Distribution (n = 107)**

| Age Group   | n   | Percentage (%) |
|-------------|-----|----------------|
| < 30 years  | 7   | 6.5            |
| 31–50 years | 72  | 67.3           |
| > 50 years  | 28  | 26.2           |
| Total       | 107 | 100.0          |

Mean age:  $45.4 \pm 10.6$  years. The demographic breakdown presented in highlights that the majority of the patients fell within the 31–50 years age spectrum.

**Table II. Specimen Type Distribution (n = 107)**

| Specimen Type               | n   | Percentage (%) |
|-----------------------------|-----|----------------|
| Modified Radical Mastectomy | 46  | 43.0           |
| Core Needle Biopsy          | 45  | 42.1           |
| Mastectomy                  | 13  | 12.1           |
| Lumpectomy                  | 2   | 1.9            |
| Wide Local Excision         | 1   | 0.9            |
| Total                       | 107 | 100.0          |

As outlined in, modified radical mastectomy and core needle biopsy constituted the primary specimen modalities evaluated in this study.

**Table III. Tumour Size Distribution (n = 86 assessable cases)**

| Tumour Size                       | n (of 107) | Percentage (%) |
|-----------------------------------|------------|----------------|
| $\leq 2$ cm (pT1)                 | 4          | 3.7            |
| 2.1–5 cm (pT2)                    | 72         | 67.3           |
| > 5 cm (pT3)                      | 10         | 9.3            |
| Not assessable (core biopsy only) | 21         | 19.6           |
| Total                             | 107        | 100.0          |

The classification of tumor dimensions in illustrates a distinct predominance of pT2 stage lesions (2.1–5 cm) within the assessable cohort.

**Table IV. Histological Grade Distribution (n = 96 assessable cases)**

| Nottingham Grade                     | n (of 107) | Percentage (%) |
|--------------------------------------|------------|----------------|
| Grade I (Well-differentiated)        | 24         | 22.4           |
| Grade II (Moderately differentiated) | 49         | 45.8           |
| Grade III (Poorly differentiated)    | 23         | 21.5           |
| Not determinable                     | 11         | 10.3           |
| Total                                | 107        | 100.0          |

Documents the Nottingham histological grade distribution, demonstrating that moderately differentiated (Grade II) malignancy was the most frequent finding.

**Table V. Lymphovascular Invasion Status  
(n = 35 assessable cases)**

| LVI Status    | n (of 107) | Percentage (%) |
|---------------|------------|----------------|
| Present       | 31         | 29.0           |
| Absent        | 4          | 3.7            |
| Not evaluable | 72         | 67.3           |
| Total         | 107        | 100.0          |

The proportion of cases demonstrating lymphovascular invasion among the pathologically evaluable specimens is specified below the matrix.

**Table VI. Lymph Node Metastasis Status  
(n = 48 assessable cases)**

| Node Status                     | n (of 107) | Percentage (%) |
|---------------------------------|------------|----------------|
| N0 (node-negative)              | 21         | 19.6           |
| N1a (1–3 positive nodes)        | 8          | 7.5            |
| N1 (not further classified)     | 4          | 3.7            |
| N2a (4–9 positive nodes)        | 10         | 9.3            |
| N3a ( $\geq 10$ positive nodes) | 5          | 4.7            |
| Not applicable                  | 59         | 55.1           |
| Total                           | 107        | 100.0          |

Details the stratification of regional axillary lymph node involvement observed across the surgical excision specimens.

**Table VII. IHC Marker Expression Profile  
(n = 107)**

| Marker                   | Positive n (%) | Negative n (%) |
|--------------------------|----------------|----------------|
| ER                       | 51 (47.7%)     | 56 (52.3%)     |
| PR                       | 40 (37.4%)     | 67 (62.6%)     |
| HER2 (3+)                | 29 (27.1%)     | 78 (72.9%)     |
| Ki-67 $\geq 14\%$ (High) | [n] ([%])      | [n] ([%])      |

ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2. The separate biomarker positivity rates assessed via standardized immunohistochemistry are summarized.

**Table VIII. Molecular Subtype Distribution  
(n = 107)**

| Molecular Subtype         | n   | Percentage (%) |
|---------------------------|-----|----------------|
| Triple-Negative (TNBC)    | 33  | 30.8           |
| Luminal A                 | 28  | 26.2           |
| HER2-Enriched             | 23  | 21.5           |
| Luminal B                 | 18  | 16.8           |
| Luminal B (HER2-positive) | 5   | 4.7            |
| Total                     | 107 | 100.0          |

TNBC: triple-negative breast cancer. The comprehensive distribution profile of surrogate molecular classifications according to St. Gallen definitions is mapped in.



**Table IX. Association Between Molecular Subtypes and Clinicopathological Parameters**

| Parameter          | Luminal A<br>n (%) | Luminal B<br>n (%) | HER2-<br>Enriched<br>n (%) | TNBC<br>n (%) | Luminal B<br>(HER2-<br>positive) n (%) | p-<br>value |
|--------------------|--------------------|--------------------|----------------------------|---------------|----------------------------------------|-------------|
| Age group          |                    |                    |                            |               |                                        |             |
| < 30 years (n=7)   | 3 (42.9)           | 0 (0.0)            | 2 (28.6)                   | 2 (28.6)      | 0 (0.0)                                | 0.525       |
| 31–50 years (n=72) | 15 (20.8)          | 14 (19.4)          | 17 (23.6)                  | 21 (29.2)     | 5 (6.9)                                |             |
| > 50 years (n=28)  | 10 (35.7)          | 4 (14.3)           | 4 (14.3)                   | 10 (35.7)     | 0 (0.0)                                |             |
| Tumour size        |                    |                    |                            |               |                                        |             |
| ≤ 2 cm (n=4)       | 0 (0.0)            | 1 (25.0)           | 1 (25.0)                   | 1 (25.0)      | 1 (25.0)                               | 0.428       |
| 2.1–5 cm (n=72)    | 23 (31.9)          | 12 (16.7)          | 11 (15.3)                  | 24 (33.3)     | 2 (2.8)                                |             |
| > 5 cm (n=10)      | 4 (40.0)           | 2 (20.0)           | 2 (20.0)                   | 2 (20.0)      | 0 (0.0)                                |             |
| Tumour grade       |                    |                    |                            |               |                                        |             |
| Grade I (n=24)     | 12 (50.0)          | 4 (16.7)           | 7 (29.2)                   | 1 (4.2)       | 0 (0.0)                                | 0.004       |
| Grade II (n=49)    | 13 (26.5)          | 7 (14.3)           | 8 (16.3)                   | 16 (32.7)     | 5 (10.2)                               |             |
| Grade III (n=23)   | 2 (8.7)            | 4 (17.4)           | 5 (21.7)                   | 12 (52.2)     | 0 (0.0)                                |             |
| LN status          |                    |                    |                            |               |                                        |             |
| N0 (n=21)          | 10 (47.6)          | 3 (14.3)           | 0 (0.0)                    | 7 (33.3)      | 1 (4.8)                                | 0.035       |
| N1a (n=8)          | 4 (50.0)           | 1 (12.5)           | 0 (0.0)                    | 3 (37.5)      | 0 (0.0)                                |             |
| N2a (n=10)         | 3 (30.0)           | 3 (30.0)           | 3 (30.0)                   | 2 (20.0)      | 0 (0.0)                                |             |
| N3a (n=5)          | 2 (40.0)           | 0 (0.0)            | 3 (60.0)                   | 0 (0.0)       | 0 (0.0)                                |             |
| N1 (n=4)           | 0 (0.0)            | 0 (0.0)            | 1 (25.0)                   | 3 (75.0)      | 0 (0.0)                                |             |
| LVI status         |                    |                    |                            |               |                                        |             |
| Present (n=31)     | 12 (38.7)          | 3 (9.7)            | 4 (12.9)                   | 10 (32.3)     | 2 (6.5)                                | 0.582       |
| Absent (n=4)       | 1 (25.0)           | 0 (0.0)            | 2 (50.0)                   | 1 (25.0)      | 0 (0.0)                                |             |

TNBC: triple-negative breast cancer; LN: lymph node; LVI: lymphovascular invasion; Chi-square or Fisher's exact test used. evaluates the cross-tabulations and

statistical correlations between the intrinsic molecular subtypes and key diagnostic parameters.

**Table X. Association Between Patient Age and Tumour Grade (p = 0.402)**

| Age Group          | Grade I n (%) | Grade II n (%) | Grade III n (%) |
|--------------------|---------------|----------------|-----------------|
| ≤ 30 years (n=7)   | 1 (14.3)      | 5 (71.4)       | 1 (14.3)        |
| 31–50 years (n=62) | 17 (27.4)     | 33 (53.2)      | 12 (19.4)       |
| > 50 years (n=27)  | 6 (22.2)      | 11 (40.7)      | 10 (37.0)       |

Illustrates the non-significant statistical relationship observed between the chronological age brackets of patients and their corresponding biological tumor grade.

## Discussion

This cross-sectional study provides comprehensive molecular characterisation of invasive breast carcinoma in a Bangladeshi cohort using IHC surrogate markers according to St. Gallen criteria. The principal finding is the high prevalence of triple-negative breast cancer (TNBC) at 30.8%, which emerged as the most common molecular subtype pattern strikingly different from Western populations.

### Molecular Subtype Distribution: Global and Regional Context

The predominance of TNBC (30.8%) in this cohort substantially exceeds the global average of 15–20% and Western rates of 10–15%.<sup>15</sup> This finding is consistent with recent South Asian and African data. Belachew et al. (2023) reported TNBC prevalence of 33.1% in an Ethiopian cohort,<sup>16</sup> while Bangladeshi studies have reported TNBC rates of 22.5–33.3%,<sup>17</sup> lending convergent validity to our observations. The inversion of the traditional subtype hierarchy wherein Luminal A typically predominates in Western populations at 40–50%,<sup>18</sup> but ranked second in our cohort at 26.2% carries profound therapeutic consequences. TNBC lacks expression of ER, PR, and HER2, rendering it unresponsive to endocrine therapy and HER2 targeted agents.<sup>19</sup> Until the recent regulatory approval of pembrolizumab and PARP inhibitors, cytotoxic chemotherapy remained the only systemic treatment modality.<sup>20</sup> The high TNBC burden in this population therefore mandates robust chemotherapy infrastructure and equitable access to emerging targeted agents.

The HER2-enriched subtype constituted 21.5% of this cohort notably higher than the global average of 10–15%.<sup>21</sup> This finding aligns with reports from other Asian populations and may reflect genetic or

environmental factors influencing HER2 amplification rates.<sup>22</sup> The clinical significance is substantial: trastuzumab-based dual HER2 blockade regimens achieve pathological complete response rates exceeding 60% in the neoadjuvant setting, transforming HER2-positive disease from one of the worst prognostic categories to one of the most therapeutically responsive.<sup>23</sup> Ensuring equitable access to these agents is a priority healthcare policy issue.

Luminal B tumours accounted for 16.8% of cases, and Luminal B (HER2-positive) tumours for 4.7%. Combined hormone receptor-positive subtypes (Luminal A + Luminal B + Luminal B HER2-positive) constituted 47.7% of cases markedly lower than the 70–75% reported in Western populations.<sup>24</sup> This implies that fewer than half of patients in this cohort are candidates for endocrine therapy as the primary treatment modality, contrasting with practice patterns in high-income countries.

### Age at Presentation

The mean age of  $45.4 \pm 10.6$  years observed in this study, with 67.3% of patients presenting between 31–50 years, is consistent with established epidemiological patterns of earlier breast cancer onset in South Asia.<sup>25</sup> Rahman and Karim (2018) reported a mean age of 43.95 years in a Chittagong cohort,<sup>17</sup> and studies from Morocco and Pakistan report means of 45 and 47.55 years respectively in sharp contrast to the early 60s typical of Western populations.<sup>26</sup> This younger age profile likely reflects premenopausal hormonal milieu, population demographic pyramids, genetic predisposition in South Asian women, and potentially a role for reproductive and lifestyle factors. The non-significant association between age group and molecular subtype ( $p = 0.525$ ) is noteworthy: it indicates that aggressive phenotypes particularly TNBC are not confined to older age groups, emphasising that young premenopausal women in this population face disproportionate exposure to high-risk subtypes.

## Tumour Grade and Molecular Subtypes

The highly significant correlation between tumour histological grade and molecular subtype ( $p = 0.004$ ) provides biological validation of the St. Gallen IHC classification system. Grade III tumours demonstrated striking enrichment for TNBC (52.2%), while Grade I tumours showed predominance of Luminal A (50%). This inverse relationship aligns with extensive published literature documenting the association between TNBC and high-grade morphology.<sup>27</sup> At the molecular level, TNBC exhibits high proliferation indices, genomic instability, and loss of differentiation regulatory mechanisms, which manifest morphologically as poorly differentiated tumours with high nuclear pleomorphism, increased mitotic activity, and pushing borders.<sup>28</sup> Luminal A tumours, characterised by low Ki-67 ( $< 14\%$ ), strong ER signalling, and relatively stable genomes, predictably exhibit well-differentiated morphological features.<sup>29</sup> The correlation between grade and molecular subtype has a practical implication in resource-limited settings: careful histological grading can provide initial prognostic information and guide the urgency of molecular testing when IHC resources are constrained.

## Lymph Node Metastasis and Molecular Subtypes

The significant association between nodal status and molecular subtype ( $p = 0.035$ ) adds to the literature on differential metastatic behaviour across subtypes. Luminal A tumours showed relatively higher representation among node-negative cases (47.6% of N0 cases), consistent with their generally more indolent biology, lower proliferation rates, and maintained cellular adhesion mechanisms.<sup>30</sup> Conversely, HER2-enriched tumours dominated cases with extensive nodal involvement (N3a, 60%), reflecting the aggressive metastatic propensity characteristic of HER2-positive disease.<sup>31</sup> The heterogeneous distribution of TNBC across nodal stages may reflect the known biological diversity within the TNBC category encompassing basal-like, claudin-low, immunomodulatory, and luminal androgen receptor subtypes each with distinct

metastatic patterns and preferences for lymphatic versus haematogenous spread.<sup>32</sup> The integration of both anatomical (TNM staging) and biological (molecular subtype) information thus optimises prognostication and treatment planning.

## Tumour Size and Lymphovascular Invasion

The absence of significant associations between molecular subtype and tumour size ( $p = 0.428$ ) or LVI ( $p = 0.582$ ) may reflect methodological constraints rather than a true biological null finding. The narrow size range of this cohort with 67.3% of tumours in the pT2 category limits statistical power to detect subtype-related size differences. The non-significant LVI association is likely influenced by the small number of cases where this parameter was evaluable ( $n = 35$ ), predominantly from surgical specimens. Furthermore, the strikingly high LVI prevalence among assessable cases (88.6%) severely restricts discriminatory power across subtypes.

## Strengths

This study has several strengths. It represents one of the larger single-institution IHC molecular classification studies from Bangladesh, with standardised protocols and comprehensive clinicopathological correlation. Classification according to internationally recognised St. Gallen criteria enables valid comparison with global data. Dual independent histopathologist review with consensus on discordant cases enhanced scoring reproducibility. The breadth of clinicopathological parameters examined including age, tumour size, grade, LVI, and nodal status provides a comprehensive biological characterisation.

## Limitations

Several limitations must be acknowledged. First, the cross-sectional design precludes assessment of long-term disease-free and overall survival outcomes. Second, the single-institution nature may limit generalisability to the broader Bangladeshi population, though institutional findings align with multi-site Bangladeshi literature. Third, HER2

equivocal (2+) cases were classified as negative in the absence of FISH confirmation, potentially underestimating true HER2-positive rates by 5–10% [43]. Fourth, Ki-67 assessment carries known interobserver variability, and the 14% cutoff, while internationally endorsed, remains contested.<sup>33</sup> Fifth, IHC-based classification approximates but does not perfectly replicate gene expression profiling (concordance approximately 80–85%).<sup>34</sup> Sixth, the limited tissue in core biopsies precluded LVI assessment in 67.3% of cases, reducing power for this clinicopathological association.

### Clinical and Translational Implications

To mitigate the high burden of unfavorable molecular subtypes in resource-limited settings like Bangladesh,<sup>20</sup> expanding molecular diagnostic services (such as IHC and HER2 testing) is essential for precision oncology. However, as our prior work identified substantial gaps between breast health knowledge and regular self-examination practices among local Bangladeshi women,<sup>35</sup> diagnostic availability alone is insufficient. Therefore, integrating routine IHC profiling with grassroots health-education initiatives will be imperative. Establishing population-based early screening programs in tandem with accessible subtype profiling represents a vital two-pronged strategy to promote early detection, guide targeted therapies, and ultimately improve overall breast cancer survival outcomes.<sup>36</sup>

### Conclusion

This cross-sectional study of 107 invasive breast carcinoma patients in Bangladesh reveals a distinctive molecular landscape characterised by high prevalence of TNBC (30.8%) as the predominant subtype, followed by Luminal A (26.2%), HER2enriched (21.5%), Luminal B (16.8%), and Luminal B (HER2-positive) (4.7%) subtypes. The high proportions of hormone receptor-negative and HER2positive tumours diverge markedly from Western patterns and underscore a substantially different biological and therapeutic context. Statistically significant associations between

molecular subtype and histological grade ( $p = 0.004$ ) and lymph node metastasis ( $p = 0.035$ ) validate the clinical utility of St. Gallen IHC classification in this setting. These findings mandate strengthened chemotherapy services, sustainable access to HER2-targeted therapies, and integration of newer immunotherapeutic agents for TNBC, alongside population-specific early detection strategies. Future longitudinal studies correlating molecular subtypes with treatment response and survival outcomes in Bangladeshi cohorts are essential to guide evidence-based oncology practice in this underserved population.

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### Conflict of Interest Statement

The authors declare no conflict of interest.

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*Original Article*

# Students' Perception of the Educational Environment Using the Dundee Ready Education Environment Measure (DREEM) at TMSS Medical College, Bogura, Bangladesh

Habib MA<sup>1\*</sup>, Hoque SMM<sup>2</sup>, Ansari AAM<sup>3</sup>, Tasnim T<sup>4</sup>, Arzuman H<sup>5</sup>

1. Dr. Md. Azfarul Habib, Professor, Department of Community Medicine and Public Health, TMSS Medical College, Bogura.
2. Dr. S.M Mejba Ul Hoque, Lecturer, Department of Community Medicine and Public Health, TMSS Medical College, Bogura.
3. Dr. Abdullah Al Mahfuz Ansari, Lecturer, Department of Community Medicine and Public Health, TMSS Medical College, Bogura.
4. Dr. Towhida Tasnim, Lecturer, Department of Community Medicine and Public Health, TMSS Medical College, Bogura.
5. Dr. Hafiza Arzuman, Professor, Department of Community Medicine and Public Health, TMSS Medical College, Bogura.

## \*Corresponding Author

### Abstract

**Background:** The educational environment is a critical determinant of students' learning experiences, academic success, and professional development in medical education.

**Objective:** To assess students' perception of the educational environment using the Dundee Ready Education Environment Measure (DREEM) questionnaire at TMSS Medical College, Bogura, Bangladesh.

**Materials and Methods:** A cross-sectional descriptive research with a quantitative approach was conducted among undergraduate medical students and the population was all the medical students (batch 11 to 15) of TMSS medical college (N 722). A convenience sampling method was applied. Data were collected using the validated DREEM questionnaire and analyzed using SPSS version 26. Descriptive statistics were applied to determine overall, domain-wise, and item-wise scores.

**Results:** A total of 593 students participated giving a response rate of 82%. The highest percentage of the students were in 3rd year 145(25%); and lowest in 5th year 98(16%). The research included 593 participants, consisting of 339 females (57%) and 254 males (43%). Geographically, the majority were from Bangladesh (339, 57%), followed by India (200, 34%), and Nepal (54, 9%). The overall DREEM score was 131/200 (mean 2.63, SD 0.93), and as per DREEM interpretation guidelines, a total score ranging from 101 to 150 indicates that the educational environment is perceived as "more positive than negative" by the students. The highest score was observed among 3rd year students (144/200), while the lowest was among 5th year students (116/200). All domains showed positive perception, though several items indicated areas requiring improvement. Out of 50 items eleven items scored > 3 and identified as the strong areas of Educational Environment. Only two items scored < 2 and identified as problematic areas of the Educational Environment of TMSS medical college. Majority thirty-seven items scored within 2-3 indicate areas need to improve further with targeted intervention.

**Conclusion:** Although the educational environment is perceived positively by the students, targeted improvements are needed, particularly in student support systems and clinical learning environment.

**Keywords:** Perception, Medical students, Educational environment, Dundee Ready Education Environment Measure (DREEM).

## Introduction

The educational environment is considered one of the most important components of contemporary medical education because it directly influences students' learning experiences, academic achievement, professional development, and psychological

well-being.<sup>1,2</sup> The concept of educational environment extends beyond physical infrastructure and teaching facilities and includes the overall academic atmosphere, interpersonal relationships, institutional culture, teaching methodologies, and support systems

that collectively shape students' educational experiences.<sup>3</sup> A progressive educational environment helps motivation, critical thinking, self-directed learning, and skilled growth, whereas a hostile environment may contribute to stress, displeasure, exhaustion, and poor academic performance.<sup>4,5</sup>

Over the years, medical education has experienced considerable transformation from traditional teacher-centered approaches toward competency-based, student-centered, and outcome-oriented learning models.<sup>6,7</sup> In this changing educational landscape, continuous assessment of the education environment has become increasingly important to safeguard the effectiveness and quality of medical training. Students' perceptions of the educational environment are recognized as valuable indicators of institutional strengths and weaknesses and could provide significant feedback for curriculum development and educational reform. Many studies have revealed considerable associations between educational environment, academic success, student satisfaction, and emotional health among medical students.<sup>7,8</sup>

Among the existing tools, the Dundee Ready Education Environment Measure (DREEM) has arisen as one of the most widely used and internationally validated instruments for assessing the educational environment in medical and health professional institutions. Developed by Roff et al<sup>9</sup>, and later further described by McAleer and Roff,<sup>10</sup> the DREEM tool consists of 50 items spread across five domains: students' perceptions of learning (SPoL), students' perceptions of teachers (SPoT), students' academic self-perception (SASP), students' perceptions of atmosphere (SPoA), and students' social self-perception (SSSP).<sup>9,10</sup> Because of its universal applicability and cultural adaptability, the DREEM questionnaire has been extensively used in medical schools across different countries, allowing comparisons between institutions, curricula, and educational systems.<sup>11-20</sup>

Studies conducted in various countries have reported considerable variation in DREEM scores, reflecting

differences in curriculum structure, teaching strategies, institutional support systems, and sociocultural contexts.<sup>12-20</sup> Several studies have consistently demonstrated that students' perceptions tend to decline during the senior clinical years of medical education because of increased academic pressure, clinical workload, stress, and exposure to demanding healthcare environments.<sup>16-19</sup> Similar concerns have also been reported in South Asian medical institutions, where traditional teaching methods and examination-oriented learning continue to influence the educational process.<sup>18-20</sup>

In Bangladesh, medical education has grown rapidly over recent years, mainly in the private sector. But the methodical assessment of the educational environment remains quite limited, especially in private medical colleges.<sup>19,21,22</sup> Assessment of the educational environment is vital to confirm quality medical education and to produce capable, ethical, and sympathetic healthcare professionals capable of meeting national healthcare demands. Despite being a well-established private medical institution, TMSS Medical College, Bogura, has not previously undergone a comprehensive evaluation of its educational environment using a standardized international instrument such as DREEM. Therefore, assessing students' perceptions may provide valuable baseline information for identifying institutional strengths and weaknesses and for guiding future educational improvements and policy development.

### Objective of the Research

The present research was conducted to assess students' perception of the educational environment using the Dundee Ready Education Environment Measure (DREEM) questionnaire at TMSS Medical College, Bogura, Bangladesh, and to provide baseline data for future quality improvement initiatives.

## Materials and Methods

This cross-sectional descriptive research with a quantitative approach was conducted at TMSS Medical College, Bogura, Bangladesh, to assess students' perceptions of the educational environment.

The research was carried out over a period of twelve months among undergraduate medical students from first to fifth academic years.

### Research Population and Sampling

The research population were all undergraduate medical students (session 2018-19, 2019-20, 2020-21, 2021-22 and 2022-23) and they represented first to fifth academic years, with a total student population of 722. Efforts were made to include all available students to obtain a comprehensive representation of students' perceptions regarding the educational environment.

The minimum required sample size was calculated using the single population mean formula based on the standard deviation reported in previous DREEM research conducted by Arzuman et al.<sup>19</sup>

Considering a 10% non-response rate, the minimum required sample size was estimated to be 227. However, to increase representativeness and reliability of the findings, all available students of the selected batches were invited to participate in the research. Therefore, a convenience sampling approach was adopted.

### Data Collection Procedure

Data were collected in classroom settings immediately after lecture sessions. Prior to distribution of questionnaire, students were informed about the objectives and procedures of the research. They were assured about their voluntary participation and that all responses would remain anonymous and confidential. Certain words in the questionnaire, such as "authoritarian" and "ridicule," were explained to ensure clarity and accurate understanding.

The questionnaires were collected immediately after completion to minimize data loss. The average time required to complete the questionnaire was around 15–20 minutes. Students who were present during the data collection sessions and consented to participate were included in the research, whereas students who were absent or submitted incomplete questionnaires were excluded from the final analysis.

### Research Instrument

Students' perceptions of the educational environment were assessed using the English version of the Dundee Ready Education Environment Measure (DREEM), a globally validated instrument widely used in medical and health professional education. The DREEM questionnaire was developed by Roff et al.<sup>9</sup> and later further described by McAleer and Roff.<sup>10</sup>

The questionnaire consists of 50 items gives a global score of 200 covering five domains of the educational environment: Students' Perceptions of Learning (SPoL), Students' Perceptions of Teachers (SPoT), Students' Academic Self-Perception (SASP), Students' Perceptions of Atmosphere (SPoA) and Students' Social Self-Perception (SSSP). Each item is scored 0–4 on a 5-point Likert scale (4 -strongly agree, 3 -agree, 2-unsure, 1- disagree, and 0-strongly disagree): higher score indicates an agreement with the item whereas there are nine negatively worded items were reverse scored and higher score in negative items indicate disagreement with the item (4 -strongly disagree, 3 - disagree, 2-unsure, 1- agree, and 0-strongly agree) (Items 4, 8, 9, 17, 25, 35, 39, 48, and 50). The guidelines for inferring the overall DREEM score are 0–50 very poor; 51–100 many problems; 101–150 more positive than negative; and 151 - 200 excellent. Higher scores indicate a more positive perception of the educational environment. Demographic variables including age, gender, academic year, and country of origin were included in the first section of the questionnaire. DREEM individual item interpretation guideline as per Arzuman et al.<sup>19</sup>

| Mean score | Interpretation                |
|------------|-------------------------------|
| $\geq 3$   | Positive                      |
| 2-3        | Could be enhanced or improved |
| $< 2$      | Problematic area              |

### Data Analysis

The Statistical Package for Social Sciences (SPSS V 26) was used to analyse the data. All collected questionnaires were cleaned and coded by given a number and data was entered according to Likert Scale

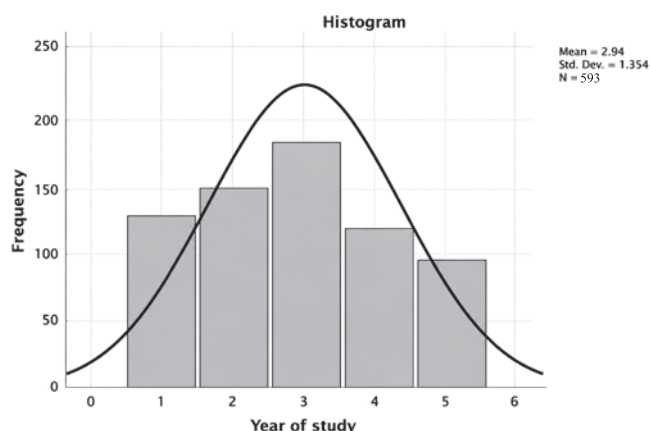
score from 0 to 4 for each item. The scoring was reverse from 4 to 0 for negative items. The missing data was coded as 999. Descriptive statistical analyses, including frequencies, percentages, means, and standard deviations, were calculated for demographic variables, overall DREEM scores, domain-wise scores, and individual item scores. The overall and domain scores were interpreted according to established DREEM interpretation guidelines.

## Results

The total number of respondents were N 722 and 593 respondents responded to the questionnaire, resulting in a response rate of 82%, which is considered satisfactory for survey-based educational research and reflects a high level of student participation. Figure 1 illustrates the distribution of the study participants according to year of study, along with the corresponding normal distribution curve. The histogram demonstrates that the collected data followed an approximately normal distribution, with

no noticeable skewness or severe deviation from symmetry. The mean year of study was  $2.91 \pm 1.354$ , indicating that the participants were predominantly distributed around the third academic year, with moderate variability across the different academic years. The standard deviation of 1.354 reflects an acceptable dispersion of observations around the mean.

**Figure 1: Distribution of Data**



**Table I: Profile of the Respondents (n = 593)**

| Variables                   | Total number (N) | Number of student responded (%) |
|-----------------------------|------------------|---------------------------------|
| Session 2022- 23 (batch 15) | 1st year ( 150 ) | 116 ( 20)                       |
| Session 2021-22 (batch 14)  | 2nd year (150)   | 125 (21)                        |
| Session 2020-21 (batch 13)  | 3rd year (151)   | 145( 25)                        |
| Session 2019- 20 (batch 12) | 4th year (159)   | 109(18)                         |
| Session 2018 -19 (batch 11) | 5th year (112)   | 98 (16)                         |
| Gender                      | Male             | 254(43)                         |
|                             | Female           | 339 (57)                        |
| Nationality                 | Bangladesh       | 339(57)                         |
|                             | India            | 200 (34)                        |
|                             | Nepal            | 54 ( 9)                         |

The highest percentage of respondents were from 3rd academic year (25%), followed by the 2nd year (21%), 1st year (20%), 4th year (18%), and 5th year (16%). Female students were the majority (57%), whereas male students were 43% of the research population.

As of nationality, the majority of respondents were from Bangladesh (57%), followed by India (34%) and Nepal (9%). This distribution reveals the diverse and multicultural student population of TMSS Medical College.



**Table II: Perceived DREEM Global mean score of TMC (n = 593)**

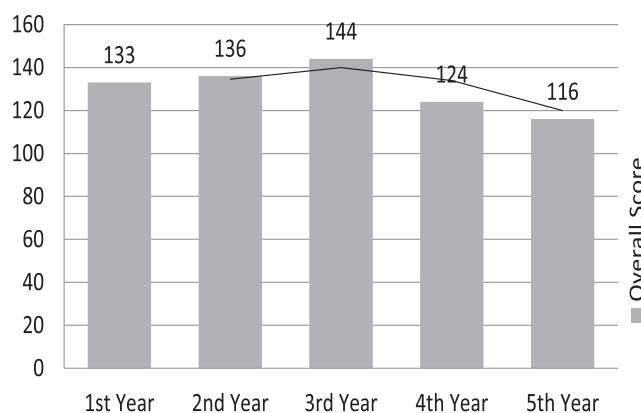
| Total DREEM Score | Overall score (mean) | Standard Deviation (SD) |
|-------------------|----------------------|-------------------------|
| 200               | 131 (2.63)           | $\pm 0.933$             |

The overall Dundee Ready Educational Environment Measure (DREEM) score was 131 out of a maximum possible score of 200, with a mean of 2.63 and a SD of 0.933 (Table II). According to the DREEM interpretation guidelines, a total score ranging from 101 to 150 indicates that the educational environment is perceived as “more positive than negative.”

Therefore, the findings of the present research suggest that the students of TMSS Medical College generally had a favorable perception of their educational environment.

The year-wise comparison of overall DREEM scores showed (Figure 2) noticeable variations among

students across different academic years. Third-year students reported the highest perception score (144/200), indicating a more positive perception whereas fifth-year students demonstrated the lowest score (116/200), suggesting relatively less satisfaction of the educational environment.

**Overall perception score as per year (total score 200)****Figure 2: Perceived Global mean score of DREEM as per year (n =593)****Table III: Domain wise analysis of the DREEM ( n= 593)**

| Domains                                           | Obtained score | Mean | Standard Deviation |
|---------------------------------------------------|----------------|------|--------------------|
| 1 : Students' Perceptions of Learning ( SPoL 48)  | 30             | 2.50 | .824               |
| 2: Students' Perceptions of Teachers (SPoT 44)    | 30             | 2.64 | .912               |
| 3: Students' Academic Self- Perceptions (SASP 32) | 22             | 2.80 | .882               |
| 4: Students' Perceptions of Atmosphere ( SPoA 48) | 32             | 2.70 | .972               |
| 5: Students' Social Self- Perceptions (SSSP 28)   | 17             | 2.44 | 1.078              |
| Total obtained Score                              | 131            | 2.62 | .933               |

The DREEM questionnaire consists of five domains evaluating different aspects of the educational environment. The highest mean score was observed in the domain of Students' Academic Self-Perception (mean 2.80), indicating that students felt relatively confident regarding their academic competence and professional preparation. This was followed by Students' Perception of Learning (mean 2.50) and Students' Perception of Atmosphere (mean 2.70), reflecting positive views regarding teaching-learning activities and institutional atmosphere.

The domain Students' Perception of Teachers (mean 2.64), suggesting that students mostly considered their teachers knowledgeable and supportive, although certain areas required improvement. The lowest mean score was found in Students' Social Self-Perception (mean 2.44), indicating comparatively shakier perceptions regarding social support, stress management, and social well-being. Collectively, all domains mean score >2, indicating that students held an overall favorable perception in all domains of the educational environment at TMSS Medical College.



**Table IV: Individual item analysis as per Domain of DREEM (n=593)**

| No                                                                   | Items                                                                      | Mean                | Standard Deviation (SD) |
|----------------------------------------------------------------------|----------------------------------------------------------------------------|---------------------|-------------------------|
| <b>Domain 1: Students' Perception of Learning (SPoL - score 48)</b>  |                                                                            |                     |                         |
| 1                                                                    | I am encouraged to participate during teaching sessions                    | 3.40                | .669                    |
| 7                                                                    | The teaching is often stimulating                                          | 2.45                | .801                    |
| 13                                                                   | The teaching is student centred                                            | 2.40                | .887                    |
| 16                                                                   | The teaching helps to develop my competence                                | 2.30                | .857                    |
| 20                                                                   | The teaching is well focused                                               | 3.15                | .760                    |
| 22                                                                   | The teaching helps to develop my confidence                                | 2.25                | .921                    |
| 24                                                                   | The teaching time is put to good use                                       | 3.00                | .754                    |
| 25                                                                   | The teaching over emphasizes factual learning (*negative)                  | 1.47                | .837                    |
| 38                                                                   | I am clear about the learning objectives of the course                     | 3.06                | .775                    |
| 44                                                                   | The teaching encourages me to be an active learner                         | 2.57                | .888                    |
| 47                                                                   | Long term learning is emphasized over short term learning                  | 2.20                | .931                    |
| 48                                                                   | The teaching is too teacher centered (*negative)                           | 1.75                | .824                    |
| Total Mean Score                                                     |                                                                            | 30 /48<br>mean 2.50 |                         |
| <b>Domain 2: Students' Perception of Teachers ( SPoT- score 44)</b>  |                                                                            |                     |                         |
| 2                                                                    | The teachers are knowledgeable                                             | 3.54                | .597                    |
| 6                                                                    | The teachers adopt a patient centered approach to consulting               | 3.05                | .766                    |
| 8                                                                    | The teachers ridicule the students (*negative)                             | 2.28                | 1.060                   |
| 9                                                                    | The teachers are authoritarian (*negative)                                 | 2.69                | .962                    |
| 18                                                                   | The teachers have good communication skills                                | 3.11                | .833                    |
| 29                                                                   | The teachers are good at providing feedback to students                    | 2.97                | .867                    |
| 32                                                                   | The teachers provide constructive criticism here                           | 2.18                | 1.041                   |
| 37                                                                   | The teachers give clear examples                                           | 2.22                | 1.085                   |
| 39                                                                   | The teachers get angry in teaching (*negative)                             | 2.38                | 1.214                   |
| 40                                                                   | The teachers are well-prepared for their teaching sessions                 | 3.20                | .753                    |
| 50                                                                   | The students irritate the teachers (*negative)                             | 2.00                | .912                    |
| Total Mean Score                                                     |                                                                            | 30/44<br>mean 2.64  |                         |
| <b>Domain 3: Students' Academic Self-Perception (SASP- score 32)</b> |                                                                            |                     |                         |
| 5                                                                    | Learning strategies which worked for me before continue to work for me now | 2.51                | .921                    |

| No                                                                   | Items                                                                 | Mean               | Standard Deviation (SD) |
|----------------------------------------------------------------------|-----------------------------------------------------------------------|--------------------|-------------------------|
| 10                                                                   | I am confident about my passing this year                             | 3.26               | .757                    |
| 21                                                                   | I feel I am being well prepared for my profession                     | 2.96               | .855                    |
| 26                                                                   | Last year work has been a good preparation for this year work         | 2.83               | .828                    |
| 27                                                                   | I am able to memorize all I need                                      | 2.42               | 1.023                   |
| 31                                                                   | I have learnt a lot about empathy in my profession                    | 2.92               | .892                    |
| 41                                                                   | My problem solving skills are being well developed here               | 2.75               | .974                    |
| 45                                                                   | Much of what I have to learn seems relevant to a career in healthcare | 2.92               | .790                    |
| Total Mean Score                                                     |                                                                       | 22/32<br>Mean 2.80 | SD .882                 |
| <b>Domain 4: Students' Perception of Atmosphere (SPoA- score 48)</b> |                                                                       |                    |                         |
| 11                                                                   | The atmosphere is relaxed during ward teaching                        | 2.55               | 1.073                   |
| 12                                                                   | This college is well timetabled                                       | 3.11               | 1.014                   |
| 17                                                                   | Poor management is a problem in this college (*negative)              | 2.13               | 1.240                   |
| 23                                                                   | The atmosphere is relaxed during lectures                             | 2.78               | 1.039                   |
| 30                                                                   | There are opportunities for me to develop my interpersonal skills     | 2.48               | 1.193                   |
| 33                                                                   | I feel comfortable in teaching sessions socially                      | 3.00               | .785                    |
| 34                                                                   | The atmosphere is relaxed during lecture and tutorials                | 2.82               | .976                    |
| 35                                                                   | I find the experience disappointing (*negative)                       | 2.41               | 1.067                   |
| 36                                                                   | I am able to concentrate well                                         | 2.75               | .879                    |
| 42                                                                   | The enjoyment outweighs the stress of the course                      | 2.28               | 1.180                   |
| 43                                                                   | The atmosphere motivates me as a learner                              | 2.76               | .982                    |
| 49                                                                   | I feel able to ask the questions I want                               | 2.88               | 1.003                   |
| Total Mean Score                                                     |                                                                       | 32/48<br>Mean 2.70 | SD .972                 |
| <b>Domain 5 : Students' Social Self-Perception (SSSP- score 28)</b>  |                                                                       |                    |                         |
| 3                                                                    | There is a good support system for students who get stressed          | 2.42               | 1.158                   |
| 4                                                                    | I am too tired to enjoy the course (*negative)                        | 2.25               | 1.198                   |
| 14                                                                   | I am rarely bored in this course                                      | 2.08               | 1.140                   |
| 15                                                                   | I have good peers in this course                                      | 2.90               | .831                    |
| 19                                                                   | My spiritual and social life is good                                  | 2.83               | .994                    |
| 28                                                                   | I seldom feel lonely                                                  | 2.00               | 1.315                   |
| 46                                                                   | My accommodation is pleasant                                          | 2.65               | 1.073                   |
| Total Mean Score                                                     |                                                                       | 17/28<br>mean2.44  | SD .933                 |

Detailed item-wise analysis was conducted to identify the strengths and weaknesses of the educational environment. Among the 50 DREEM items, eleven items achieved a mean score  $>3$ , indicating strong areas within the educational environment. These included students being encouraged to participate during teaching sessions, teachers being knowledgeable, good communication skills of teachers, well-focused teaching sessions, and effective academic timetabling.

On the other hand, two items scored  $< 2$ , indicating problematic areas that require attention. These included excessive emphasis on factual learning and teacher-centered teaching methods,

The majority of items (37 out of 50) scored between 2 and 3, indicating aspects of the educational environment that could be improved further to enhance the overall quality of medical education at TMSS Medical College

**Table V: Items Scored  $> 3$  (Strong Areas of the Educational Environment)**

| No | Items                                                       | Mean $\geq 3$ | Standard Deviation |
|----|-------------------------------------------------------------|---------------|--------------------|
| 1  | I am encouraged to participate during teaching sessions     | 3.40          | .669               |
| 20 | The teaching is well focused                                | 3.15          | .760               |
| 24 | The teaching time is put to good use                        | 3.00          | .754               |
| 38 | I am clear about the learning objectives of the course      | 3.06          | .775               |
| 2  | The teachers are knowledgeable                              | 3.54          | .597               |
| 6  | The teachers adopt a patient centred approach to consulting | 3.05          | .766               |
| 18 | The teachers have good communication skills                 | 3.11          | .833               |
| 40 | The teachers are well-prepared for their teaching sessions  | 3.20          | .753               |
| 10 | I am confident about my passing this year                   | 3.26          | .757               |
| 12 | This college is well timetabled                             | 3.11          | 1.014              |
| 33 | I feel comfortable in teaching sessions socially            | 3.00          | .785               |

Eleven items scored  $>3$  and were identified as strong areas of the educational environment at TMSS Medical College. These findings specify that students

particularly valued teacher competence, communication skills, student participation in teaching sessions, and academic organization within the institution.

**Table VI: Items scored  $< 2$  (Problematic areas of the Educational Environment)**

| No | Items                                                    | Mean | Standard Deviation |
|----|----------------------------------------------------------|------|--------------------|
| 25 | The teaching over emphasizes factual learning(*negative) | 1.47 | .837               |
| 48 | The teaching is too teacher centered (*negative)         | 1.75 | .824               |

Two items are low scored indicate weaknesses within the educational environment. As per DREEM guidelines, these areas should be listed for enhancement through the implementation of

student-centered teaching approaches, decrease excessive factual learning, and development of teacher–student interactions to foster a more reassuring and effective educational environment

## Discussion

This research provides a complete assessment of the educational environment at TMSS Medical College using the Dundee Ready Education Environment Measure (DREEM) tool. The overall DREEM score of 131/200 specifies that students perceived the educational environment as “more positive than negative” according to DREEM interpretation guidelines. Similar findings have been reported in studies conducted in Bangladesh, Sri Lanka, India, and Malaysia, where overall DREEM scores generally ranged between 101 and 150, indicating satisfactory but improvable educational environments.<sup>13-20,23-27</sup>

The overall DREEM score observed in the present research was comparable to findings from Sri Lanka reported by Jiffry et al., where the mean score was 108/200, and from Kasturba Medical College, India, where Mayya and Roff reported a score of 107/200.<sup>13,25</sup> However, the score in the present research was slightly lower than scores reported from institutions with more integrated and student-centered curricula, such as Monash University, Australia, and Dundee Medical School, where comparatively higher perceptions of the educational environment were observed.<sup>15,16</sup> These differences may be related to variations in curriculum structure, teaching methodologies, faculty development practices, academic support systems, and availability of student-centered learning opportunities.

In Bangladesh, Arzuman et al.<sup>19</sup> reported a reasonably positive perception of the educational environment among medical students at Universiti Sains Malaysia using the DREEM questionnaire, while Nahar et al.<sup>21</sup> identified moderate satisfaction levels among Bangladeshi medical students. The present research findings are largely consistent with these interpretations, suggesting that although the educational environment at TMSS Medical College is generally favorable, there remains considerable scope for improvement, particularly in the areas of student support and active learning approaches.

One of the important findings of this research was the difference in DREEM scores across academic years. Third-year students demonstrated the highest scores,

whereas fifth-year students reported the lowest scores. Similar declines in perception during the clinical years have been documented in previous studies.<sup>16,22,26</sup> Increased academic workloads, clinical responsibilities, examination pressure, and stress associated with patient care may contribute to lower perceptions among senior students. In addition, exposure to the hidden curriculum and stressful clinical environments may negatively affect students' motivation and professional satisfaction.<sup>4,17</sup>

Domain-wise analysis showed relatively favorable perceptions regarding teachers' knowledge, preparedness, and communication skills. These findings are comparable to studies conducted in Saudi Arabia and India, where students positively assessed faculty competence and teaching commitment.<sup>25,27,28</sup> However, concerns regarding authoritarian attitudes and limited feedback mechanisms were also identified in the present research, similar to findings reported in several South Asian medical institutions.<sup>13,25</sup> These findings highlight the continuing influence of traditional teacher-centered educational approaches within medical education systems in developing countries.

Compared to other domain, the lower score in the Students' Social Self-Perception (SSSP) is an important concern. Jamaiah<sup>23</sup> and Sohail<sup>29</sup>, also identified stress, fatigue, and psychological burden as major challenges among clinical year medical students. Medical education is naturally demanding, and inadequate emotional and institutional support may contribute to burnout, anxiety, and reduced academic fulfilment. The findings of the present research therefore emphasize the need for stronger student counseling services, mentorship programs, stress management initiatives, and supportive learning environments.

Item-wise analysis recognized problematic areas related to disproportionate factual learning and teacher-centered teaching. Similar findings have been reported in studies from India, Sri Lanka, and Saudi Arabia, where students expressed concerns about memorization-based learning and limited active

participation.<sup>13,27-29</sup> Modern medical education highlights competency-based, learner-centered approaches that promote critical thinking, problem-solving skills, and self-directed learning. Therefore, curriculum refinement and faculty development programs focusing on interactive teaching approaches and formative feedback may further improve the educational environment at TMSS Medical College.

Overall, the research findings indicate that TMSS Medical College has established a reasonably supportive educational environment comparable to many other medical institutions within South Asia. However, targeted interventions are necessary to enhance the clinical learning environment, strengthen student support systems, and promote more student-centered educational methods to achieve a higher-quality educational atmosphere.

## Conclusion

The research has given a full assessment of the educational environment at TMSS Medical College. Strengths were identified in areas related to teaching quality, teacher preparedness, student participation, and academic organization.

Yet, concerns remain regarding excessive factual learning, teacher-centered approaches, stress management, social support systems and low perception scores among senior students.

These issues indicate the need for targeted interventions during the clinical years of medical education, attention to student support systems and stress management,

Continuous monitoring and evidence-based educational reforms are essential to optimize the educational environment and enhance the quality of undergraduate medical education to ensure competent, confident, and well-supported future medical professionals for the country.

## Future Directions

The findings provide baseline information for future research and institutional improvement. Future studies

may adopt longitudinal and qualitative approaches for better understanding in students' perceptions and experiences over time. Comparative studies involving multiple medical colleges may help to identify wider educational trends and best practices in Bangladesh. The findings may also guide faculty development program, curriculum improvement, and strengthening of student support services. Regular

## Ethical Considerations

Ethical values were strictly upheld throughout the research process. Permission was taken from the relevant institutional authority of TMSS Medical College. Participation in the research was entirely voluntary, and all students were informed about the objectives, procedures, and significance of the research. Confidentiality and anonymity of the participants were ensured at every stage of the research, and no identifying information was collected. The responses were used only for academic and research purposes. Students were also assured that their participation or non-participation would not affect their academic standing. Completion and submission of the questionnaire were considered as implied consent to participate in the research.

## Limitations

This research has some limitations that should be considered while interpreting the findings. As it was conducted in only one private medical college, the results could not be generalizable to other institutions, particularly public medical colleges. It also relied on self-reported data, which could be influenced by response bias and individual perceptions. In addition, the cross-sectional design reflects perceptions at a single point in time and cannot determine causal relationships or changes over time. Although the DREEM questionnaire provides a complete quantitative assessment, qualitative methods could further explore students' experiences in greater depth.

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## Conflict of Interest

The authors confirm that there is no conflict of interest among the authors.

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*Original Article*

# Menstrual Disorders and Relationship with Psychological Stress among Medical Students

Zohura FT<sup>1\*</sup>, Rahman F<sup>2</sup>, Jahan R<sup>3</sup>, Alam MM<sup>4</sup>, Ferdous R<sup>5</sup>

1. Dr. Fatema Tuz Zohura, Assistant Professor, Department of Physiology, TMSS Medical College, Bogura.
2. Dr. Farhana Rahman, Assistant Professor, Department of Physiology, Army Medical College, Jashore.
3. Dr. Rubaiya Jahan, Assistant Professor, Department of Physiology, Institute of Health Technology, Dhaka.
4. Dr. Md. Mashhurul Alam, Medical Officer, Department of Anesthesiology, Rajshahi Medical College, Rajshahi.
5. Dr. Rumana Ferdous, Assistant Professor, Department of Physiology, Rajshahi Medical College, Rajshahi.

**\*Corresponding Author****Abstract**

**Background:** Menstruation is an inevitable part of a female's life and an important indicator of normal sexual and reproductive health. The changes in the normal menstrual pattern of female in the reproductive age may affect physical, physiological and psychological wellbeing. Stress may affect the pattern of menstrual cycle by stimulating the hypothalamo-pituitary-adrenal axis which is responsible for the production of cortisol hormone.

**Objectives:** The aim of the study was to evaluate menstrual disorders and their relationship with psychological stress among the students of Rajshahi Medical College, Rajshahi.

**Materials and Methods:** This cross-sectional type of descriptive study was carried out from January 2023 to December 2023 in the Department of Physiology, Rajshahi Medical College, Rajshahi. The study was conducted on 247 female students who were studying in Rajshahi Medical College after obtaining ethical clearance from the Ethical Review Committee of Rajshahi Medical College and informed written consent from the students. Data were collected by semi-structured questionnaire and analyzed by SPSS software, version-24. A p value < 0.05 was considered statistically significant for all tests.

**Results:** The students were  $21.89 \pm 1.44$  years old on average,  $12.01 \pm 1.37$  years old when they menarched, BMI  $22.07 \pm 3.34$  kg/m<sup>2</sup>, they had a monthly family income of  $41874.49 \pm 20436.26$  taka, their menstrual cycle lasted  $30.66 \pm 6.43$  days, and their flow lasted  $5.26 \pm 1.59$  days. Marital status of the students showed that majority (89.50%) of the students were unmarried, (75.70%) of the students were from urban area and majority (85.40%) of the students lived in hostel. Of the students, 20.24% experienced oligomenorrhea, 6.88% hypomenorrhea, 7.29% menorrhagia, and 5.26% polymenorrhea in relation to menstrual disorders. The majority of students (77.30%) experienced moderate stress, followed by high stress (19.00%) and low stress (3.60%). Medical students' psychological stress levels were statistically significantly correlated with both menorrhagia and oligomenorrhea ( $p < 0.01$  and  $p < 0.001$ , respectively). However, neither polymenorrhea nor hypomenorrhea was substantially associated with the medical students' psychological stress levels ( $p > 0.05$  in both cases).

**Conclusion:** Psychological stress was significantly associated with the prevalence of various types of menstrual disorders. Timely diagnosis and management of menstrual abnormalities with proper counselling are necessary for improvement of health, sense of well-being, overall quality of life of students and lower the risks for future diseases.

**Keywords:** Medical students, Menstrual disorders, Premenstrual syndrome, Perceived stress scale and psychological stress.

**Introduction**

The normal reproductive years of females are characterized by monthly rhythmic changes in the rate of secretion of female hormones and corresponding physical changes in the ovaries and other sexual organs which is called menstrual cycle. A woman's menstrual cycle usually follows an average 28 day cycle.<sup>1</sup> A

normal menstrual cycle leads to formation of a dominant follicle with a mature ovum in the ovary then ovulation. In absence of fertilization, the endometrial lining sheds out resulting in discharge of blood from the uterus through the vagina which is called menstruation - a natural physiological phenomenon

occurring at a more or less regular monthly interval during women's reproductive life.<sup>2</sup>ovulatory, and luteal Menstruation first occurs in adolescents between 11 and 14 years of age with a period length of average 7 days with a cycle length ranging from 21 to 45 days with an average blood loss of 20 to 80 ml.<sup>3</sup> This phenomenon is not an easy one and is often associated with some degree of suffering. According to several studies, a large proportion of the female population of reproductive age suffer from menstruation related health issues. There are various forms of menstrual disorders. The list of menstrual disorders ranges from dysmenorrhea, premenstrual syndrome (PMS), polymenorrhea, oligomenorrhea, menorrhagia, amenorrhea to irregular menstrual cycle. Dysmenorrhea is the commonest gynecological disorder among women with prevalence of 60% to 93%.<sup>4</sup>

Other abnormalities are also common. Although menstrual disorders are common health problems, they are generally perceived as only minor health concerns and thus irrelevant to the public health agenda particularly for females in developing countries.<sup>5</sup> But menstrual abnormalities not only cause deterioration in quality of life but also have detrimental effects on mental and reproductive health and also carry economic burden. Menstrual disorders over prolonged periods of time can cause anovulation, endometrial hyperplasia, infertility,<sup>4</sup> osteoporosis, breast cancer.<sup>6</sup> Menstrual patterns can be affected by various factors including age, ethnicity, family history, physical activity, dietary habits. Stress can be a major contributor to or cause of menstrual irregularities.<sup>7</sup>

Several studies have shown association of stress with variation in the length of menstrual cycle, anovulation, duration and amount of menstrual bleeding. Multiple pathways have been proposed linking perceived stress with reproductive function. The hypothalamic pituitary adrenal axis is activated during stress that suppresses hypothalamic gonadotropin releasing hormone (GnRH) secretion. GnRH stimulates the production of follicle stimulating hormone (FSH) and luteinizing hormone (LH) which activate the ovary to secrete estradiol and progesterone. Suppression of GnRH

secretion suppresses reproductive hormone secretion and also leads to a delay or inhibition of the LH surge. Thus stress can alter hormonal profiles and in turn menstrual outcomes.<sup>6,8</sup>

Stress is prevalent among medical professionals and that is also true for the medical students who have very busy and demanding schedules. Psychological stress is common among medical students for various reasons. High expectations of parents, exhausting work hours, continuous evaluation process, striving for earning high grades, enormous syllabus to be covered in a limited time period, thought of appearing or failing in exams, performance in periodic examinations are potential sources of stress among medical students.<sup>9</sup> Due to the high prevalence of psychological stress among them, medical students are at high risk of developing menstrual abnormalities. Several studies have been done in different countries regarding the proportion of menstrual disorders and their association with psychological stress among the medical students but to the best of our knowledge very few studies has been done in our country. Therefore, the study was designed to assess the proportion of medical students suffering from menstrual disorders and their relationship with psychological stress in our context.

## Materials and Methods

This study was a cross-sectional descriptive study. It was conducted in the Department of Physiology, Rajshahi Medical College, Rajshahi during the period of January 2023 to December 2023. Female students of Rajshahi Medical College were enrolled in this study. Before the starting of the study, a questionnaire was prepared according to the objectives of the study. All questions regarding the participants demographics as well as their menstrual pattern (menarche age, flow duration in days and its quantity, cycle length in days) and history of oligomenorrhea, polymenorrhea, hypomenorrhea and menorrhagia were included in the questionnaire. For assessment of stress level, Perceived Stress Scale was used. Then on the basis of inclusion and exclusion criteria history was taken from the students. Individuals who were match according to the selection criteria of the study were informed about



as fibroids, endometrioses, polycystic ovarian syndrome, adenomyosis, any pregnant and lactating mother among the medical students, hormonal contraceptives users were excluded from the study.

### Data Collection Tools:

The data were taken with the help of using a semi-structured questionnaire (research instrument) containing all the variables of interest. For assessment of psychological stress level Perceived Stress Scale was used.

### Statistical Analysis:

All data were analyzed by using the ‘Statistical Package for Social Sciences (SPSS)’ software, 24-version. A Chi-square test was used to observe the relationship of menstrual disorders with the level of psychological stress among the students. A p-value < 0.05 was considered statistically significant for all tests.

**Institutional Review Board Statement:**

Permission was taken from the Institutional Review Board (IRB) of Rajshahi Medical College (RMC), Rajshahi before the starting of the study regarding the study design, methodology and target population characteristics.

**Table I: Distribution of the students by their different variables (n=247).**

| Variables                     | Mean±SD           | Range           |
|-------------------------------|-------------------|-----------------|
| Age (Years)                   | 21.89 ± 1.44      | 19 – 25         |
| Age of menarche (Years)       | 12.01 ± 1.37      | 8 – 16          |
| BMI (kg/m <sup>2</sup> )      | 22.07 ± 3.34      | 14.12 – 34.35   |
| Monthly family income (taka)  | 41874.49±20436.26 | 10,000-1,00,000 |
| Menstrual cycle length (days) | 30.66 ± 6.43      | 15-48           |
| Duration of flow (days)       | 5.26 ± 1.59       | 1-11            |

Table I shows the mean age of the students was  $21.89 \pm 1.44$  years, age of menarche was  $12.01 \pm 1.37$  years, BMI  $22.07 \pm 3.34$  kg/m<sup>2</sup>, monthly family income  $41874.49 \pm 20436.26$ -taka, menstrual cycle length  $30.66 \pm 6.43$  days and duration of flow  $5.26 \pm 1.59$  days.



**Table II: Marital status, residence and present living situation of the student (n=247).**

| Variables                       | Frequency | Percentage (%) |
|---------------------------------|-----------|----------------|
| <b>Marital status</b>           |           |                |
| Married                         | 26        | 10.50          |
| Unmarried                       | 221       | 89.50          |
| <b>Residence</b>                |           |                |
| Rural                           | 60        | 24.30          |
| Urban                           | 187       | 75.70          |
| <b>Present living situation</b> |           |                |
| With family                     | 36        | 14.60          |
| Hostel                          | 211       | 85.40          |

Table II reveals that 89.50% of students were single and 10.50% married. Most students (75.70%) were urban and 24.30% rural. Currently, 85.40% of students live in hostels and 14.60% with families.

**Table III: Distribution of the students by presence of various menstrual disorders and psychological stress level (n=247).**

| Menstrual disorders          | Frequency       |     | Percentage (%) |
|------------------------------|-----------------|-----|----------------|
| Oligomenorrhea               | Yes             | 50  | 20.24          |
|                              | No              | 197 | 79.76          |
| Polymenorrhea                | Yes             | 13  | 5.26           |
|                              | No              | 234 | 94.74          |
| Hypomenorrhea                | Yes             | 17  | 6.88           |
|                              | No              | 230 | 93.12          |
| Menorrhagia                  | Yes             | 18  | 7.29           |
|                              | No              | 229 | 92.71          |
| Levelsofpsychological stress | Low stress      | 9   | 3.60           |
|                              | Moderate stress | 191 | 77.30          |
|                              | High stress     | 47  | 19.00          |

Table III demonstrates that 20.24% of students had oligomenorrhea, 6.88% hypomenorrhea, 7.29% menorrhagia, and 5.26% polymenorrhea. Most of the students (77.30%) were moderately stressed. 19.00% had high and 3.60% had low stress.

**Table IV: Relationship between the menstrual disorders and level of psychological stress among the students (n=247).**

| Variables      | Levels of psychological stress |                 |             | p-value |
|----------------|--------------------------------|-----------------|-------------|---------|
|                | Low stress                     | Moderate stress | High stress |         |
| Oligomenorrhea | 1 (11.10%)                     | 25 (13.10%)     | 24 (51.10%) | < 0.001 |
| Polymenorrhea  | 0 (0%)                         | 10 (5.20%)      | 3 (6.40%)   | > 0.05  |
| Hypomenorrhea  | 1 (11.10%)                     | 12 (6.30%)      | 4 (8.50%)   | > 0.05  |
| Menorrhagia    | 0 (0%)                         | 9 (4.70%)       | 9 (19.10%)  | < 0.01  |

In Table IV, oligomenorrhoea and menorrhagia were found to significantly correlate with psychological stress in medical students ( $p < 0.001$  and  $p < 0.01$ , respectively). Both hypomenorrhea and polymenorrhea did not significantly affect psychological stress in medical students ( $p > 0.05$ ).

## Discussion

The average age of students in this study was  $21.89 \pm 1.44$  years. Alshdaifat et al.<sup>10</sup> observed similar results, with a mean student age of  $21.6 \pm 2.2$  years. Nagma et al.<sup>11</sup>, Alshayeb and Sakka<sup>12</sup>, and Khaleel<sup>13</sup> observed similar results. Kollipaka et al.<sup>14</sup> obtained different results.

This study found that 89.50% of students were single and 10.50% were married. Alshayeb and Sakka<sup>12</sup> reported 87.0% of students were single. Alshdaifat et al.<sup>10</sup>, Alhammadi et al.<sup>15</sup>, and Rafique and Al-Sheikh<sup>7</sup> reported comparable results. In this study, 85.40% of students lived in hostels and 14.60% with families. Alshdaifat et al.<sup>10</sup> and Alshayeb and Sakka<sup>12</sup> found different results.

In the study, students had a mean BMI of  $22.07 \pm 3.34$  kg/m<sup>2</sup>. Khaleel<sup>13</sup> reported similar results, with a mean BMI of  $22.71 \pm 6.369$  kg/m<sup>2</sup>. The mean student BMI in Nagma et al.<sup>11</sup> was 20.91 kg/m<sup>2</sup>, which contradicted the findings. Different eating habits in different regions may explain the differences.

The mean age of menarche in this study was  $12.01 \pm 1.37$  years. Cakir et al.<sup>16</sup> observed similar results, with a mean menstrual age of  $12.8 \pm 1.3$  years. Singh et al.<sup>17</sup>, J et al.<sup>18</sup>, Alshdaifat et al.<sup>10</sup>, Alhammadi et al.<sup>15</sup>, Khaleel<sup>13</sup>, and Rigon<sup>19</sup> found similar results. In contrast, Esimai and Esan<sup>5</sup> observed a mean menarche age of 14.2 years. Mishra and Mukhopadhyay<sup>20</sup> and Verma et al.<sup>21</sup> observed conflicting results.

This study found a mean menstrual blood flow length of  $5.26 \pm 1.59$  days. Similarly, Lakkawar et al.<sup>4</sup> found a mean menstrual blood flow length of  $4.25 \pm 0.5$  days. Climate and seasonal change may explain these differences. Adolescent females suffer more from

hormone imbalances and longer or more frequent periods in summer. The study found 20.24% of students had oligomenorrhea. Lakkawar et al.<sup>4</sup> observed 21% of students experienced oligomenorrhoea, like the study. Alshayeb and Sakka<sup>12</sup> discovered oligomenorrhea in 5% of students, lower than the study. Research found 7.29% of students experienced menorrhagia. Alshayeb and Sakka<sup>12</sup> observed 7% of students experienced menorrhagia, like the study. Alshayeb and Sakka<sup>22</sup> found 8.6% of students had menorrhagia, greater than the research. Also, Houston et al.<sup>23</sup> obtained different results.

The recent study found 5.26% of medical students had polymenorrhea. Lakkawar et al.<sup>4</sup> discovered that 8% of students had polymenorrhoea. Rafique and Al-Sheikh<sup>7</sup> discovered polymenorrhoea in 13% of pupils.

The study found that 77.30% of students had moderate stress, 19.00% had high stress, and 3.60% had low stress, with a mean perceived stress scale score of  $22.03 \pm 4.78$ . Khaleel<sup>13</sup> obtained similar results. In contrast, Achmad et al.<sup>24</sup> observed that 55.8% of students had severe stress, 36.5% moderate, 5.8% extremely severe, and 1.9% mild. Kollipaka et al.<sup>14</sup> obtained different results.

A strong correlation ( $p < 0.001$ ) was identified between oligomenorrhoea and psychological stress among students in this study. Alshayeb and Sakka<sup>12</sup> obtained similar results.

This study observed no correlation between hypomenorrhea and student psychological stress ( $p > 0.05$ ). Nagma et al.<sup>11</sup> observed similar results. Researchers found a substantial correlation between menorrhagia and psychological stress in medical students ( $p < 0.01$ ). Khaleel<sup>13</sup> observed a statistically significant association between menorrhagia and psychological stress in medical students. Khaleel<sup>13</sup> obtained similar results. However, Zhou et al.<sup>25</sup>, Sood et al.<sup>26</sup>, Nagma et al.<sup>11</sup>, and Fenster et al.<sup>6</sup> observed different results. Different study methodology and regional variance may explain these inconsistencies.

This study revealed no correlation between polymenorrhea and student psychological stress ( $p>0.05$ ). Alshayeb and Sakka<sup>12</sup> obtained similar results.

## Conclusion

There was a really high prevalence of stress among these students and it was also associated with the occurrence of various menstrual disorders. So, all medical faculties should set up procedures for the early detection of students with stress and menstruation disorders which might be helpful for prevention of various diseases.

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## Conflict of interest

Authors declared no conflict of interest.

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**Original Article****Prognostic Significance of COL10A1 Overexpression in Breast Cancer Patients from Different Sociodemographic Backgrounds**Roy NR<sup>1\*</sup>, Das D<sup>2</sup>, Hoque F<sup>3</sup>, Jannat T<sup>4</sup>, Tasnim A<sup>5</sup>

1. Dr. Nilima Rani Roy, Assistant Professor, Department of Biochemistry, Prime Medical College, Rangpur.
2. Dr. Doly Das, Assistant professor, Department of Anatomy, Prime Medical College, Rangpur.
3. Dr. Farzana Hoque, Assistant professor, Department of Anatomy, Prime Medical College, Rangpur.
4. Dr. Tamanna Jannat, Assistant professor, Department of Pathology, Prime medical College, Rangpur.
5. Dr. Anika tasnim, senior lecturer, Department of Biochemistry, Green life Medical College, Dhaka.

**\*Corresponding Author****Abstract**

**Background:** Breast cancer is the most common cancer among women worldwide and shows wide variation in how it behaves and how patients respond to treatment. Identifying dependable molecular biomarkers that are linked to prognosis and sociodemographic factors is important for improving personalized care. Collagen type X alpha 1 chain (COL10A1) has recently gained attention as a possible biomarker because of its role in tumor progression and remodeling of the extracellular matrix.

**Objective:** To assess the prognostic value of COL10A1 overexpression in breast cancer patients and to examine its relationship with selected sociodemographic and clinicopathological characteristics.

**Materials and Methods:** This cross-sectional study was conducted in the department of Biochemistry, Dhaka medical college, Dhaka in collaboration with Institute for Population and Precision Health, Department of Public Health Science, the University of Chicago, USA from July 2022 to June 2023 after receiving approval from Research Review Committee and Ethical Review Committee. Thirty-four female patients with histopathologically confirmed breast cancer were recruited from the Department of General Surgery and the Breast Clinic of Dhaka Medical College Hospital (DMCH). Sociodemographic and clinical information was collected using a structured questionnaire, and COL10A1 gene expression was measured from tumor tissue samples using standard molecular techniques. Statistical analyses were performed to explore associations between COL10A1 expression, sociodemographic factors, and prognostic indicators.

**Results:** A considerable number of patients showed overexpression of COL10A1. Higher expression levels were significantly associated with advanced tumor stage, lymph node involvement, and other poor prognostic features. Differences in COL10A1 expression were also noted among various age groups and socioeconomic categories, suggesting a possible link between molecular expression patterns and sociodemographic background.

**Conclusion:** COL10A1 overexpression appears to be a promising prognostic biomarker in breast cancer. Its strong association with adverse clinicopathological characteristics indicates that it may be useful for risk assessment and personalized treatment planning, particularly in settings with limited resources.

**Keywords:** Breast cancer, COL10A1, Prognostic biomarker, Gene expression, Sociodemographic factors.

**Introduction**

Breast cancer is the most frequently diagnosed malignancy and one of the leading causes of cancer-related mortality among women worldwide. Global cancer statistics indicate that breast cancer constitutes a significant proportion of female cancer incidence and deaths, with a rising burden particularly evident in low- and middle-income countries.<sup>1</sup> In Bangladesh, breast cancer represents a significant

public health challenge, with many patients presenting at advanced stages due to delayed diagnosis, low screening uptake, and limited access to specialized oncology services; these factors contribute to poor outcomes and underscore the need for improved awareness, early detection programs, and strengthened cancer care infrastructure.<sup>2</sup> These challenges often result in late-stage presentation and unfavorable



clinical outcomes. Breast cancer is a biologically heterogeneous disease comprising multiple molecular subtypes that exhibit diverse clinical behaviors, prognoses, and responses to therapy. Traditional prognostic factors—such as tumor size, histological grade, lymph node status, and hormone receptor expression—remain fundamental to clinical management; however, they are insufficient to accurately predict disease progression and patient outcomes in all cases.<sup>3</sup> As a result, increasing attention has been directed toward identifying novel molecular biomarkers that more precisely reflect tumor biology and enhance prognostic stratification. The tumor microenvironment, particularly the extracellular matrix (ECM), plays a pivotal role in breast cancer initiation, invasion, and metastasis. Alterations in ECM components can promote tumor progression by stimulating angiogenesis, facilitating epithelial–mesenchymal transition, and enabling metastatic spread.<sup>4,5</sup> Among ECM-associated molecules, members of the collagen family have attracted considerable interest due to their involvement in tumor progression and stromal remodeling. Collagen type X alpha 1 chain (COL10A1) is a short-chain collagen that is primarily expressed during endochondral ossification and skeletal development, with negligible expression in most normal adult tissues. Accumulating evidence suggests aberrant re-expression of COL10A1 in various solid malignancies, including breast, colorectal, lung, and gastric cancers.<sup>6,7</sup> In breast cancer, COL10A1 expression is predominantly localized to the tumor stroma and neovasculature, indicating a potential role in tumor–stromal interactions.<sup>8</sup> Recent transcriptomic and immunohistochemical analyses have shown that elevated COL10A1 expression is associated with aggressive tumor characteristics, advanced disease stage, lymph node metastasis, and poorer survival outcomes in breast cancer patients.<sup>9–11</sup> Mechanistically, COL10A1 is thought to contribute to ECM remodeling, increased tissue stiffness, and activation of pro-tumorigenic signaling pathways, thereby promoting invasion and metastasis<sup>12</sup>.

Despite these observations, the majority of existing studies have been conducted in Western or East Asian populations. There remains a significant lack of data regarding the prognostic significance of COL10A1 in South Asian populations, where genetic heterogeneity, environmental factors, and sociodemographic conditions may influence tumor behavior. Elucidating the expression patterns and clinical relevance of COL10A1 in this setting is therefore crucial for the development of population-specific prognostic markers. Accordingly, the present study aimed to assess the prognostic significance of COL10A1 overexpression in breast cancer patients from diverse sociodemographic backgrounds in Bangladesh and to examine its association with clinicopathological features.

## Materials and Methods

**Study Design and Setting:** This cross-sectional study was conducted at the Department of Biochemistry, Dhaka Medical College, Dhaka, in collaboration with the Institution for Population and Precision Health, Department of Public Health Sciences, The University of Chicago Biological Sciences, USA after receiving approval from Research Review Committee and Ethical Review Committee. A total of 34 female patients with histopathologically confirmed breast cancer were enrolled. The study was carried out between July 2022 to June 2023 from the Department of General Surgery and the Breast Clinic of Dhaka Medical College Hospital (DMCH).

**Inclusion Criteria:** Female patients with a confirmed histopathological diagnosis of breast cancer were eligible for inclusion. Participants were required to provide informed written consent and to have sufficient tumor tissue available for molecular analysis.

**Exclusion Criteria:** Patients who had undergone neoadjuvant chemotherapy or radiotherapy prior to tumor tissue collection were excluded to minimize treatment-related effects on molecular expression. Patients with recurrent disease or evidence of distant metastasis at initial presentation were also excluded.

**Data Collection:** Sociodemographic information, including age, place of residence, educational level, and socioeconomic status, along with clinicopathological data such as tumor size, histological grade, lymph node involvement, and disease stage, were collected through structured questionnaires and review of medical records.

**Laboratory Analysis:** Tumor tissue samples were processed according to standard laboratory protocols. COL10A1 gene expression was quantified using appropriate molecular methods, including quantitative real-time polymerase chain reaction (qRT-PCR). Expression levels were classified as overexpressed or low/normal based on predefined cutoff values.

**Statistical Analysis:** Statistical analyses were performed using SPSS version 26.0. Descriptive statistics were applied to summarize the study variables. Associations between COL10A1 expression and sociodemographic as well as clinicopathological parameters were assessed using suitable statistical tests, with a p-value of less than 0.05 considered statistically significant.

## Results

A total of 34 histopathologically confirmed female breast cancer patients were included in the final analysis. The results are presented across ten tables summarizing sociodemographic characteristics, clinicopathological features, and their association with COL10A1 expression.

**Table I. Sociodemographic Characteristics of the Study Population (n=34)**

| Variable             | Category   | Frequency (n) | Percentage (%) |
|----------------------|------------|---------------|----------------|
| Age (years)          | <40        | 8             | 23.5           |
|                      | ≥40        | 26            | 76.5           |
| Residence            | Urban      | 20            | 58.8           |
|                      | Rural      | 14            | 41.2           |
| Education            | ≤Secondary | 19            | 55.9           |
|                      | >Secondary | 15            | 44.1           |
| Socioeconomic status | Low–Middle | 21            | 61.8           |
|                      | Upper      | 13            | 38.2           |

Table I shows that most participants (26/34, 76.5%) were aged ≥40 years, while 8 (23.5%) were under 40. Urban residents were slightly higher (20/34, 58.8%) compared to rural (14/34, 41.2%). Regarding education, 19 (55.9%) had up to secondary level, and 15 (44.1%) had higher education. Most patients belonged to low- to middle-income groups (21/34, 61.8%), while 13 (38.2%) were from upper-income groups.

**Table II. Clinicopathological Characteristics of Breast Cancer Patients**

| Variable           | Category   | Frequency (n) | Percentage (%) |
|--------------------|------------|---------------|----------------|
| Tumor size         | ≤2 cm      | 10            | 29.4           |
|                    | >2 cm      | 24            | 70.6           |
| Histological grade | Grade I–II | 18            | 52.9           |
|                    | Grade III  | 16            | 47.1           |
| Lymph node status  | Negative   | 13            | 38.2           |
|                    | Positive   | 21            | 61.8           |

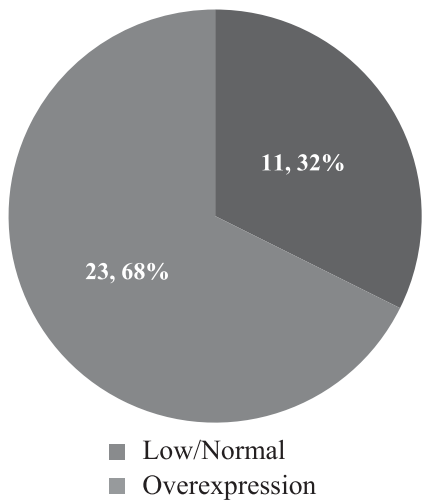
Table II shows the majority of tumors were larger than 2 cm (24/34, 70.6%), while 10 (29.4%) were  $\leq 2$  cm. Histological grading revealed 18 (52.9%) had Grade I–II tumors and 16 (47.1%) had Grade III. Lymph node involvement was observed in 21 patients (61.8%), whereas 13 (38.2%) were node-negative, indicating a high rate of regional disease at diagnosis.

**Table III. Distribution of Clinical Stage at Diagnosis**

| Stage     | Frequency (n) | Percentage (%) |
|-----------|---------------|----------------|
| Stage I   | 5             | 14.7           |
| Stage II  | 16            | 47.1           |
| Stage III | 13            | 38.2           |

Table III indicates that Stage II was the most common at diagnosis (16/34, 47.1%), followed by Stage III (13/34, 38.2%), and Stage I (5/34, 14.7%). This shows that most patients were diagnosed at intermediate to advanced stages, highlighting the need for earlier detection and screening strategies.

**Pattern of COL10A1 Gene Expression**



**Fig 1. Pattern of COL10A1 Gene Expression**

Figure 1 illustrates the distribution of COL10A1 gene expression among the study participants using a pie chart. The chart shows that a majority of patients (23 cases, 68%) exhibited COL10A1 overexpression, while a smaller proportion (11 cases, 32%) demonstrated low or normal expression levels.

**Table IV. Association Between COL10A1 Expression and Age Group**

| Age Group       | Low/Normal | Overexpression | p-value |
|-----------------|------------|----------------|---------|
| <40 years       | 4          | 4              | 0.041   |
| $\geq 40$ years | 7          | 19             |         |

Table IV shows that among patients <40 years, COL10A1 overexpression was observed in 4 out of 8 patients, while the other 4 had low/normal expression. In patients  $\geq 40$  years, 19 out of 26 showed overexpression, and 7 had low/normal levels. The association between age and COL10A1 expression was statistically significant ( $p=0.041$ ), indicating higher expression in older patients.

**Table V. Association Between COL10A1 Expression and Socioeconomic Status**

| Socioeconomic Status | Low/Normal | Overexpression | p-value |
|----------------------|------------|----------------|---------|
| Low–Middle           | 5          | 16             | 0.038   |
| Upper                | 6          | 7              |         |

Table V demonstrates that 16 out of 21 patients from low–middle socioeconomic groups had COL10A1 overexpression, compared to 7 out of 13 patients from upper socioeconomic groups. Low/normal expression was observed in 5 and 6 patients from low–middle and upper groups, respectively. The association was significant ( $p=0.038$ ), suggesting higher expression in lower socioeconomic patients.

**Table VI Association Between COL10A1 Expression and Tumor Size**

| Tumor Size  | Low/Normal | Overexpression | p-value |
|-------------|------------|----------------|---------|
| $\leq 2$ cm | 6          | 4              | 0.009   |
| $>2$ cm     | 5          | 19             |         |

Table VI indicates that in tumors  $\leq 2$  cm, 6 patients had low/normal expression and 4 had overexpression. For tumors  $>2$  cm, 19 patients showed overexpression, while 5 had low/normal levels. The difference was statistically significant ( $p=0.009$ ), showing that COL10A1 overexpression is more frequent in larger tumors.

**Table VII. Association Between COL10A1 Expression and Histological Grade**

| Grade      | Low/Normal | Overexpression | p-value |
|------------|------------|----------------|---------|
| Grade I–II | 9          | 9              | 0.012   |
| Grade III  | 2          | 14             |         |

Table VII shows that COL10A1 overexpression was observed in 14 out of 16 Grade III tumors, compared to 9 out of 18 Grade I–II tumors. Low/normal expression was higher in Grade I–II (9 patients) than in Grade III (2 patients). The association was statistically significant ( $p=0.012$ ), indicating higher expression in high-grade tumors.

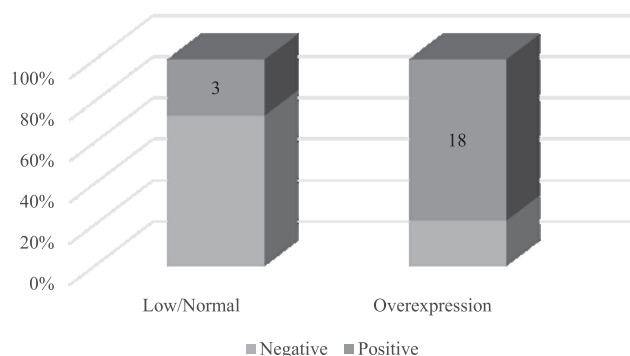
**Fig 2. Association Between COL10A1 Expression and Lymph Node Status**

Figure 2 shows the association between COL10A1 expression and lymph node status. In the Low/Normal group, most cases were lymph node negative (3 vs 1 positive), while in the Overexpression group, most were positive (18 vs 6 negative), indicating that COL10A1 overexpression is linked to higher lymph node involvement.

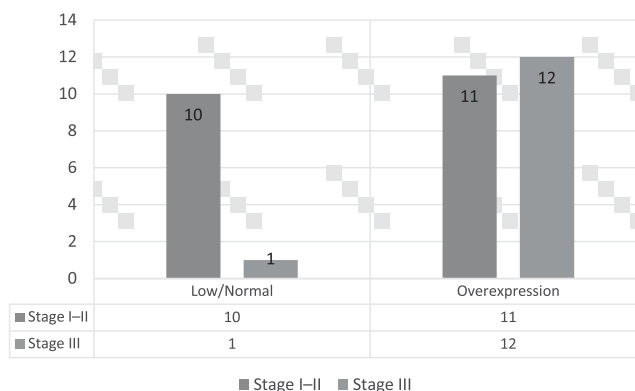
**Fig 3. Association Between COL10A1 Expression and Clinical Stage**

Figure 3 shows that low/normal COL10A1 expression is more common in Stage I–II ( $n=10$ ) than Stage III ( $n=1$ ), whereas COL10A1 overexpression is predominantly observed in Stage III ( $n=12$ ) compared to Stage I–II ( $n=11$ ). This indicates an association between COL10A1 overexpression and advanced clinical stage.

## Discussion

The present study evaluated the prognostic significance of COL10A1 overexpression in breast cancer patients from Bangladesh and identified a strong association between elevated COL10A1 expression and unfavorable clinicopathological features. These findings contribute to the expanding evidence highlighting extracellular matrix–related genes as key regulators of tumor aggressiveness and disease progression. In the current cohort, COL10A1 overexpression was detected in nearly two-thirds of patients, aligning with prior transcriptomic and immunohistochemical studies that have reported higher COL10A1 expression in malignant breast tissues compared with normal controls.<sup>13,14</sup> The higher frequency of overexpression among patients aged  $\geq 40$  years may be indicative of age-associated alterations in tumor biology and stromal remodeling, consistent with earlier studies linking ECM dysregulation to tumor progression in older individuals.<sup>15</sup> A significant relationship was also observed between COL10A1 overexpression and lower socioeconomic status. Although the precise biological mechanisms underlying this association remain unclear, factors such as delayed diagnosis, reduced access to early screening, and prolonged tumor–stromal interactions in socioeconomically disadvantaged populations may contribute to increased ECM remodeling and gene overexpression.<sup>16</sup> This finding underscores the potential interplay between sociodemographic factors and molecular tumor characteristics in low- and middle-income countries. Notably, COL10A1 (collagen type X alpha-1) has been reported to be overexpressed in breast cancer and associated with



tumor progression, including enhanced proliferation, migration, invasion, and poorer survival outcomes. Bioinformatics analysis and clinical studies indicate that elevated COL10A1 expression correlates with advanced clinical stage, unfavorable prognosis, and aggressive tumor behavior in breast cancer, supporting its role as a potential prognostic biomarker across diverse populations.<sup>17–19</sup> These observations support the hypothesis that COL10A1 facilitates tumor progression through extracellular matrix modification, increased tissue rigidity, and promotion of epithelial–mesenchymal transition and metastatic spread.<sup>20</sup> The strong association between COL10A1 overexpression and lymph node metastasis observed in this study suggests a potential role for COL10A1 in enhancing tumor invasiveness. These findings support the biological plausibility of COL10A1 contributing to metastatic dissemination through modulation of the tumor microenvironment.<sup>21</sup> A poor prognosis, haematogenous dissemination, and lymph node metastases are all associated with the abnormal overexpression of COL10A1 in the tumour stroma of solid tumor and cancer cell migration, thereby facilitating regional and distant metastasis. COL10A1 has a role in ECM remodelling and can interact with proteins including integrin subunit beta 1 and prolyl 4-hydroxylase subunit beta to promote tumour cell growth and metastasis.<sup>22</sup> Moreover, COL10A1 has been implicated in the activation of pro-tumorigenic signaling pathways, including TGF- $\beta$  and PI3K/AKT, both of which are known to drive breast cancer progression.<sup>23</sup> Despite the limited sample size, this study offers important insights into the prognostic relevance of COL10A1 in a South Asian population, where molecular data on breast cancer are relatively scarce. The concordance of these results with findings from international studies enhances the biological plausibility of COL10A1 as a prognostic biomarker. Future large-scale, multicenter investigations incorporating survival analyses and functional studies are necessary to validate its clinical utility and to assess its potential as a therapeutic target in breast cancer management.

## Conclusion

This study shows that overexpression of COL10A1 is closely linked to poor clinicopathological features in breast cancer patients, such as larger tumor size, higher tumor grade, lymph node involvement, and advanced disease stage. These findings suggest that COL10A1 may have an important role in driving tumor aggressiveness and disease progression. The fact that these associations were seen across different sociodemographic groups highlights the potential value of COL10A1 as a prognostic marker for breast cancer patients in Bangladesh. Including COL10A1 expression analysis in routine molecular testing could help improve risk assessment and guide more individualized treatment decisions, especially in settings with limited resources.

## Recommendations

Future studies should include larger patient populations from multiple centers to confirm the prognostic role of COL10A1 in breast cancer. Long-term, prospective studies are needed to examine its relationship with survival and treatment response. Combining COL10A1 with other molecular and immunohistochemical markers may provide a more complete and accurate prognostic model. Further research into the biological mechanisms of COL10A1 in tumor progression could also support the development of targeted therapies. Finally, integrating molecular research with sociodemographic and public health perspectives may help promote more equitable and effective precision oncology in Bangladesh.

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**Original Article****Resource-Optimized PRP: A Comparative Study of Anticoagulants and Centrifugation Techniques in Bangladesh**Hoque MA<sup>1\*</sup>, Masud MAA<sup>2</sup>, Basak SK<sup>3</sup>, Mamun AA<sup>4</sup>

1. Dr. Md. Ashraful Hoque, Assistant Professor, Department of Transfusion Medicine, Cumilla Medical College, Cumilla.
2. Dr. Md. Abdullah Al Masud, Associate Professor, Department of Transfusion Medicine, TMSS Medical College, Bogura.
3. Dr. Sushanta Kumar Basak, Assistant Professor, Department of Transfusion Medicine, Faridpur Medical College, Faridpur.
4. Dr. ABM Al Mamun, Registrar, Department of Transfusion Medicine, Dhaka Medical College Hospital, Dhaka.

**\*Corresponding Author****Abstract**

**Background:** Platelet-rich plasma (PRP) effectiveness depends on platelet concentration, centrifugation geometry, and anticoagulant choice. International protocols favour swing-bucket centrifuges and ACD-A anticoagulant due to superior platelet recovery, but in Bangladesh fixed-angle centrifuges and sodium citrate are widely used because of affordability and availability. This study compared PRP yield between these two locally adapted and internationally recommended techniques.

**Materials and Methods:** This analytical laboratory-based study was conducted between March and May 2025 in selected government and private hospital laboratories in Bangladesh. Eighty PRP samples were prepared using fixed-angle or swing-bucket centrifuges with either sodium citrate (3.2%) or ACD-A as anticoagulant (20 samples per group). Baseline platelet count, PRP platelet count, platelet concentration factor (PCF), WBC content, and RBC contamination were assessed. Analysis included two-way ANOVA, independent t-tests, and multivariable regression.

**Results:** Swing-bucket centrifuges produced significantly higher PRP platelet counts ( $818\text{--}950 \times 10^9/\text{L}$ ) than fixed-angle centrifuges ( $573\text{--}667 \times 10^9/\text{L}$ ) ( $p < 0.001$ ). ACD-A yielded higher PCF values than citrate ( $p < 0.001$ ). ANOVA showed significant effects of centrifuge type and anticoagulant ( $p < 0.001$  each), with no interaction. Regression demonstrated that baseline platelet count, centrifuge type, and anticoagulant independently predicted PRP yield (adjusted  $R^2 = 0.82$ ). Swing groups also showed lower WBC contamination.

**Conclusion:** The centrifugation and anticoagulant methods commonly used in Bangladesh significantly underperform compared with international standards. Adoption of swing-bucket centrifuges or optimized fixed-angle protocols, along with broader use of ACD-A, may substantially improve PRP quality in low-resource settings.

**Key Words:** Resource-Optimized PRP, Platelet-Rich Plasma (PRP), Anticoagulants, Centrifugation Techniques

**Introduction**

Platelet-rich plasma (PRP) is increasingly utilized across orthopedics, sports medicine, wound healing, and dermatology because of its high concentration of autologous growth factors that stimulate tissue repair.<sup>1-3</sup> PRP quality is highly dependent on preparation technique, including centrifuge type, spin force, and anticoagulant selection.<sup>4-6</sup> International standards recommend the use of swing-bucket centrifugation, as the horizontal rotor orientation enables more efficient separation of erythrocytes, leukocytes, and platelets during both soft and hard spins.<sup>7-9</sup>

Conversely, fixed-angle centrifuges, widely used in Bangladesh due to lower cost and greater availability, subject platelets to angular displacement and wall-adherence effects that reduce overall yield and may partially activate platelets prematurely.<sup>10-12</sup> Similarly, ACD-A anticoagulant, with superior calcium chelation and pH buffering, preserves platelet morphology and viability better than sodium citrate.<sup>11-15</sup> However, in Bangladesh- like many low- and middle-income countries- PRP preparation commonly uses fixed-angle centrifuges and sodium citrate

(3.2%), creating an evidence gap on how these adaptations affect final PRP quality.<sup>16-18</sup> The primary objective of this study was to systematically compare platelet recovery, platelet concentration factor, and cellular composition of PRP prepared using centrifugation and anticoagulant methods commonly practiced in Bangladesh with those recommended internationally. We hypothesized that differences in centrifuge geometry and anticoagulant selection would independently influence PRP yield and quality, even when preparation is performed under routine laboratory conditions in resource-limited settings.

## Materials and Methods

This laboratory-based analytical study was conducted in Bangladesh between March to May 2025 using routinely available PRP preparation equipment from government and private hospitals. The objective was to evaluate the performance of two centrifugation techniques—fixed-angle and swing-bucket—and two anticoagulants, 3.2% sodium citrate and ACD-A, representing both common local practices and international recommendations. This study was carried out in Bangladesh between March and May 2025 in the transfusion medicine laboratories of Cumilla Medical College Hospital, TMSS Medical College, Faridpur Medical College, and Dhaka Medical College Hospital.

### Sample Collection

Peripheral venous blood was collected from healthy adult volunteers into tubes pre-loaded with either:

- ACD-A
- 3.2% sodium citrate

PRP was prepared using either fixed-angle or swing-bucket centrifugation following established double-spin principles.<sup>4,7,12</sup>

- Soft spin: 150–200 g for 8–10 minutes
- Hard spin: 400–700 g for 7–10 minutes

Hematological analysis was performed using automated analyzers validated for PRP assessment.<sup>19</sup>

### Primary outcomes

- PRP platelet count ( $\times 10^9/L$ )
- Platelet concentration factor (PCF)

### Secondary outcomes

- White blood cell (WBC) concentration
- Red blood cell (RBC) contamination (visual and microscopic)
- Evidence of spontaneous platelet activation

All data were cross-verified by two independent observers and recorded in a structured dataset.

### PRP Preparation Protocol

#### 1. Fixed-Angle Centrifuge Protocol (Bangladesh Standard)

This protocol reflects the most commonly available centrifuge type in Bangladesh.

##### Step 1: Soft Spin

- Speed: 1,200 rpm (~200 g)
- Duration: 10 minutes
- Rotor: Fixed-angle
- Output: Plasma + buffy coat separated

##### Step 2: Hard Spin

- Speed: 3,500 rpm (~700 g)
- Duration: 7 minutes
- Output: PRP pellet at tube base

Anticoagulant: Sodium citrate (3.2%) or ACD-A  
Blood-to-anticoagulant ratio: 9:1

#### 2. Swing-Bucket Centrifuge Protocol (Reference Standard)

##### Step 1: Soft Spin

- Speed: 1,000 rpm (~150 g)
- Duration: 8 minutes

##### Step 2: Hard Spin

- Speed: 2,500 rpm (~400 g)
- Duration: 10 minutes

Swing-out rotors permit vertical sedimentation of red cells, improving platelet recovery and producing more uniform PRP.

### Laboratory Measurements

- Baseline CBC and PRP platelet/WBC counts were measured using automated analyzers (Sysmex, Beckman, or BeneSphera).



- PCF was calculated as:  $PCF = PRP\_PLT / \text{Baseline PLT}$

### Statistical Analysis

Continuous variables were expressed as mean  $\pm$  SD.

#### Analysis included:

- Two-way ANOVA: centrifuge  $\times$  anticoagulant interaction
- Independent-samples t-tests:
  - Fixed vs Swing
  - Citrate vs ACD
- Multiple linear regression: predictors of PRP yield

Statistical significance was set at  $p < 0.05$ . All analyses were conducted in Python (pandas, SciPy, statsmodels) following accepted biomaterials statistical practices (20).

### Ethical Considerations

Verbal informed consent was obtained from all healthy adult volunteers prior to blood collection. No personal identifiers were recorded at any stage of the study. As the procedures involved standard venous blood collection and laboratory processing with minimal risk, the study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Institutional administrative permission was obtained from participating laboratories.

## Results

### Study Population

A total of 80 PRP samples were analyzed, divided evenly among four preparation groups:

1. Fixed-angle + Citrate (n=20)
2. Fixed-angle + ACD-A (n=20)
3. Swing-bucket + Citrate (n=20)
4. Swing-bucket + ACD-A (n=20)

### Baseline Platelet Counts

Baseline platelet values were comparable across groups (mean 204–225  $\times 10^9/L$ ,  $p > 0.05$ ), confirming uniformity of starting material.

### PRP Platelet Yield

Significant variation was observed based on centrifuge type and anticoagulant:

- Fixed-angle + Citrate: 573  $\times 10^9/L$
- Fixed-angle + ACD: 667  $\times 10^9/L$
- Swing + Citrate: 818  $\times 10^9/L$
- Swing + ACD: 950  $\times 10^9/L$

Swing-bucket centrifuges consistently delivered higher platelet yield than fixed-angle systems.

#### Platelet Concentration Factor (PCF)

- Fixed-angle, Citrate: 2.68
- Fixed-angle, ACD: 3.04
- Swing-bucket, Citrate: 3.81
- Swing-bucket, ACD: 4.47

### Two-way ANOVA

| Parameter          | F-value | p-value |
|--------------------|---------|---------|
| Centrifuge type    | 132.4   | <0.001  |
| Anticoagulant type | 48.1    | <0.001  |
| Interaction        | 0.62    | 0.43    |

Centrifuge design and anticoagulant choice independently influenced platelet concentration.

### T-tests

| Comparison     | t-value | p-value | Conclusion     |
|----------------|---------|---------|----------------|
| Fixed vs Swing | -14.2   | <0.001  | Swing superior |
| Citrate vs ACD | -7.6    | <0.001  | ACD superior   |

### Regression Model

Predictors of PRP platelet count:

- Baseline platelet count:  $\beta = 2.14$  ( $p < 0.001$ )
- Swing vs Fixed: +268  $\times 10^9/L$  ( $p < 0.001$ )
- ACD vs Citrate: +146  $\times 10^9/L$  ( $p < 0.001$ )

Adjusted  $R^2 = 0.82$ , indicating strong model performance.

### WBC and RBC Contamination

- Higher WBC levels observed in Fixed-angle groups (0.4–1.0  $\times 10^9/L$ )
- Lower WBC levels in Swing groups (0.2–0.6  $\times 10^9/L$ )
- RBC contamination was rare (12% overall)

### Overall Interpretation

1. Swing-bucket centrifuges produce superior platelet recovery.
2. ACD-A anticoagulant consistently outperforms sodium citrate.
3. The Bangladesh-standard method (Fixed + Citrate) produces the lowest PRP quality.
4. Significant improvements can be achieved even in resource-limited settings.

**Table I: Baseline Platelet Counts and PRP Yield Across Groups**

| Group                  | n  | Baseline PLT (mean ± SD) | PRP PLT (mean ± SD) | PCF (mean ± SD) |
|------------------------|----|--------------------------|---------------------|-----------------|
| Fixed-angle + Citrate  | 20 | 205 ± 22                 | 573 ± 77            | 2.68 ± 0.17     |
| Fixed-angle + ACD      | 20 | 209 ± 24                 | 667 ± 92            | 3.04 ± 0.21     |
| Swing-bucket + Citrate | 20 | 214 ± 25                 | 818 ± 110           | 3.81 ± 0.24     |
| Swing-bucket + ACD     | 20 | 221 ± 27                 | 950 ± 142           | 4.47 ± 0.27     |

Table I shows that swing-bucket centrifugation consistently produced higher PRP platelet counts and higher PCF values than fixed-angle centrifugation. Within each centrifuge type, ACD-A outperformed citrate, demonstrating anticoagulant-dependent improvement in platelet concentration.

**Table II: Two-way ANOVA for Effects of Centrifuge Type and Anticoagulant**

| Source             | SS    | df | MS    | F     | p-value |
|--------------------|-------|----|-------|-------|---------|
| Centrifuge type    | 25.83 | 1  | 25.83 | 132.4 | <0.001  |
| Anticoagulant type | 9.38  | 1  | 9.38  | 48.1  | <0.001  |
| Interaction        | 0.11  | 1  | 0.11  | 0.62  | 0.43    |
| Error              | 14.78 | 76 | 0.19  | —     | —       |

Two-way ANOVA indicates that both centrifuge type and anticoagulant type independently affected PRP yield ( $p < 0.001$ ). The non-significant interaction term suggests the effect of each factor is additive rather than synergistic.

**Table III. T-tests**

| Comparison     | t-statistic | p-value | Interpretation               |
|----------------|-------------|---------|------------------------------|
| Fixed vs Swing | -14.2       | <0.001  | Swing significantly superior |
| Citrate vs ACD | -7.6        | <0.001  | ACD significantly superior   |

Independent t-tests confirmed significantly higher platelet concentration when swing-bucket centrifugation or ACD-A anticoagulant were used ( $p < 0.001$ ). These findings support the superiority of internationally recommended protocols.

**Table IV. Regression Model Predicting PRP Count**

| Variable       | $\beta$ | SE   | t   | p-value |
|----------------|---------|------|-----|---------|
| Baseline PLT   | 2.14    | 0.24 | 9.1 | <0.001  |
| Swing vs Fixed | +268.3  | 30.7 | 8.7 | <0.001  |
| ACD vs Citrate | +146.5  | 28.2 | 5.2 | <0.001  |

Regression analysis demonstrates that baseline platelet count, centrifuge type, and anticoagulant independently predict PRP yield, accounting for 82% of variability in outcomes (Adjusted  $R^2=0.82$ ). Swing-bucket design contributed the largest positive effect.

Model fit: Adjusted  $R^2 = 0.82$

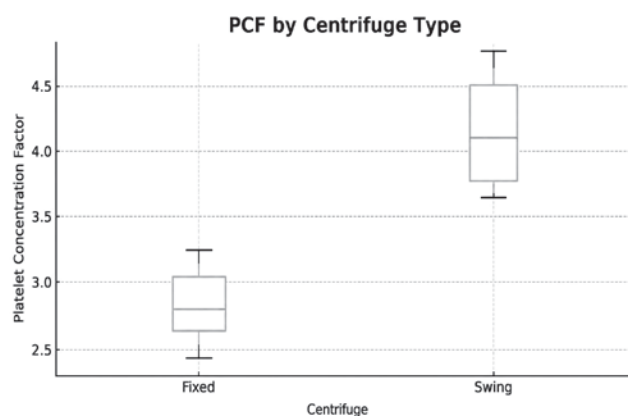


Figure 1. Boxplot of PCF by centrifuge type. Swing-bucket centrifuges show significantly higher concentration factors.

Swing-bucket centrifuges demonstrate higher PCF distribution with narrower variability, confirming superior platelet recovery.

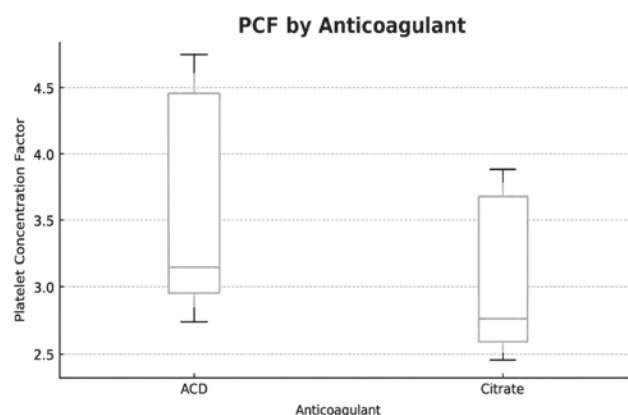


Figure 2. Boxplot comparing PCF between citrate and ACD. ACD consistently yielded higher platelet concentration.

ACD-A consistently yields higher PCF, reinforcing its biochemical advantage in platelet preservation.

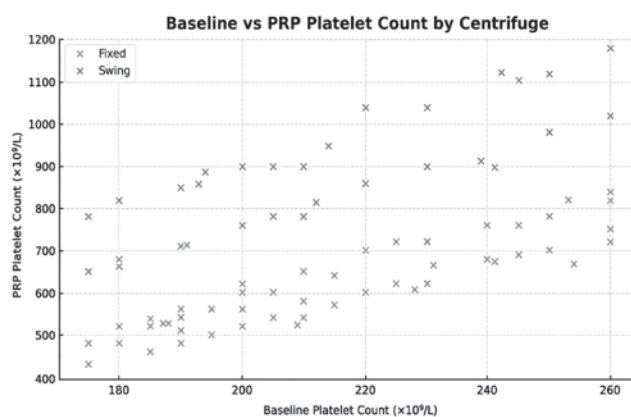


Figure 3. Scatter plot showing the relationship between baseline and PRP platelet counts, stratified by centrifuge type.

A linear relationship is observed between baseline platelet count and PRP yield. Swing-bucket groups cluster higher on the y-axis, demonstrating greater efficiency

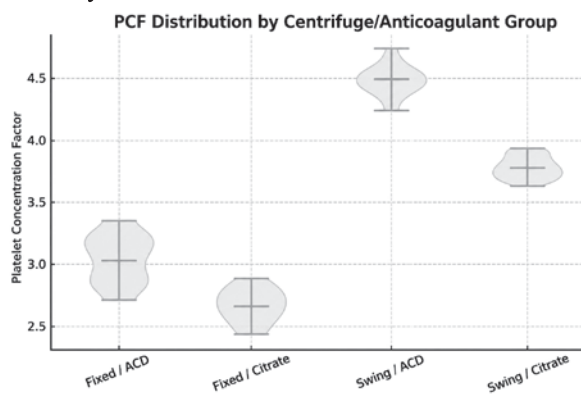


Figure 4. Violin plot demonstrating distribution of PCF across the four preparation groups.

Distribution curves reveal the highest PCF for Swing + ACD-A and lowest for Fixed + Citrate, visually supporting the ANOVA results.

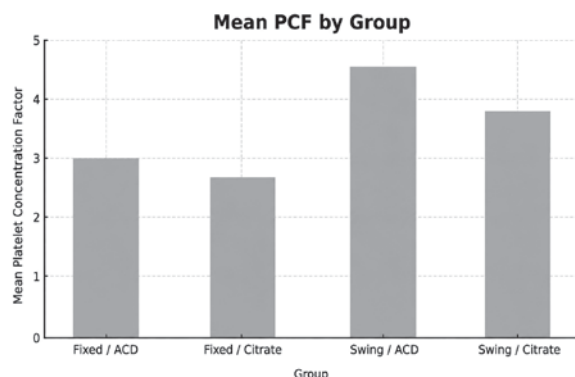


Figure 5. Bar chart of mean PCF, showing progressive improvement from Fixed/Citrate to Swing/ACD.

A stepwise improvement is seen from Fixed/Citrate to Swing/ACD-A, indicating cumulative benefits of optimal centrifuge geometry and anticoagulant choice.

## Discussion

This study demonstrates that PRP prepared with swing-bucket centrifugation and ACD-A anticoagulant yields significantly higher platelet concentration compared with the fixed-angle + citrate protocol commonly used in Bangladesh. These findings align with prior international research demonstrating that rotor geometry has a major influence on platelet sedimentation, distribution, and recovery.<sup>7-9,11</sup>

Previous studies have shown that fixed-angle centrifuges cause platelets to migrate along the tube wall, reducing harvestable yield and increasing mechanical stress<sup>10-12</sup>. Our results corroborate these findings, showing substantially lower PCF in fixed-angle groups (mean 2.68–3.04) compared with swing-bucket protocols (mean 3.81–4.47).

Similarly, our anticoagulant findings align with prior research indicating that ACD-A preserves platelet membrane integrity, reduces metabolic activation, and improves recovery during centrifugation.<sup>13-15</sup> Sodium citrate has a weaker chelating effect, leading to partial platelet activation and reduced yield.

Several LMIC studies have described the practical challenges of implementing high-standard PRP protocols due to cost, procurement limitations, and training shortages<sup>16-18</sup>. Our study provides data-driven insights tailored to Bangladesh, highlighting that even modest equipment upgrades or optimized fixed-angle protocols could significantly improve PRP quality. These findings have direct clinical relevance. Higher PCF is associated with superior outcomes in knee osteoarthritis, tendon injuries, chronic wounds, and hair restoration.<sup>2,3, 21, 22</sup> Lower-quality PRP may contribute to the variable clinical efficacy observed across centers in Bangladesh.

The present findings primarily reflect laboratory performance characteristics of different PRP preparation techniques. Swing-bucket centrifugation and ACD-A anticoagulation consistently demonstrated higher platelet recovery and lower leukocyte contamination under controlled laboratory conditions.

While higher platelet concentration has been associated with improved outcomes in selected musculoskeletal and wound-healing applications, this study did not evaluate clinical efficacy directly. Therefore, the results should be interpreted as evidence of technical optimization rather than definitive predictors of clinical response. Future studies incorporating standardized clinical endpoints will be necessary to correlate laboratory quality parameters with patient-reported outcomes.

**Limitations** This study was limited by its laboratory-based design and did not include clinical outcome assessment. Platelet activation markers were assessed indirectly rather than through flow cytometric analysis. Additionally, formal institutional ethical review board approval was not pursued due to the minimal-risk nature of the procedures. These factors should be considered when interpreting the findings.

## Recommendations

1. Introduce affordable swing-bucket centrifuges specifically designed for PRP use in Bangladesh.
2. Encourage use of ACD-A anticoagulant where feasible, especially for MSK and orthopedic applications.
3. Develop national PRP preparation guidelines tailored to low-resource settings.
4. Train laboratory technologists on optimized fixed-angle double-spin modifications.
5. Establish quality control standards, including routine PCF measurement before clinical use.
6. Conduct multicenter clinical trials comparing outcomes of Fixed/Citrate versus Swing/ACD PRP.

## Conclusion

This study demonstrates that PRP prepared using swing-bucket centrifugation and ACD-A anticoagulant

achieves significantly higher platelet concentration than the fixed-angle and citrate-based methods commonly used in Bangladesh. These findings suggest that technical factors alone can meaningfully influence PRP quality, even within routine laboratory environments. Incremental optimization of centrifugation technique and anticoagulant choice may help improve consistency and reliability of PRP preparation in resource-limited settings.

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## Original Article

# Clinical Presentation and Culture Yield of Urinary Tract Infection Among Symptomatic Pregnant Women: A Tertiary Hospital Experience

Sultana T<sup>1\*</sup>, Akhther MH<sup>2</sup>, Mahamuduzzaman AS<sup>3</sup>, Shireen F<sup>4</sup>, Parvin MR<sup>5</sup>

1. Dr. Touhida Sultana, Assistant Professor, Department of Obstetrics and Gynaecology, Shaheed Ziaur Rahman Medical College, Bogura.
2. Dr. Mst. Hasina Akhther, Assistant Professor, Department of Obstetrics and Gynaecology, Shaheed Ziaur Rahman Medical College, Bogura.
3. Dr. Fahmida Shireen, Junior Consultant, Upazila Health Complex, Gobindaganj, Gaibandha.
4. Dr. Abu Shoyeb Md. Mahamuduzzaman, Assistant Professor, Department of Pediatrics, Shaheed Suhrawardy Medical College, Dhaka.
5. Dr. Most. Runa Parvin, Assistant Professor, Department of Obstetrics and Gynaecology, Shaheed Ziaur Rahman Medical College, Bogura.

## \*Corresponding Author

### Abstract

**Background:** Urinary tract infection is a significant issue during pregnancy. Clinical symptoms can overlap with normal pregnancy-related changes. Therefore, lab confirmation is crucial. Aim of the study: This study aimed to assess the clinical presentation and urine culture results of UTI among symptomatic pregnant women. It also aimed to describe dipstick findings, isolated organisms, and patterns of antimicrobial susceptibility.

**Material and Methods:** This cross-sectional descriptive study included 105 symptomatic pregnant women who visited the Department of Obstetrics and Gynecology at Shaheed Ziaur Rahman Medical College Hospital from January 2024 to December 2024. Researchers collected clinical and obstetric data. Urine samples underwent dipstick testing, culture, identification of organisms, and antimicrobial susceptibility testing. Data analysis utilized SPSS version 15.0. Fisher's exact test assessed the association between dipstick and culture findings.

**Results:** Of the 105 symptomatic pregnant women, 87.6% were under 25 years old, and the average gestational age was  $24.67 \pm 4.79$  weeks. The most common symptoms were urinary frequency (98.1%) and burning sensation during urination (71.4%). Urine culture was positive in 6.7% of participants, with *Escherichia coli* found in all culture-positive cases. Dipstick positivity showed a significant association with culture-confirmed UTI ( $p < 0.001$ ). All *E. coli* isolates were sensitive to nitrofurantoin, 71.4% were sensitive to cephalexin, and only 14.3% were sensitive to amoxicillin.

**Conclusion:** While urinary symptoms were common, only 6.7% of pregnant women had culture-confirmed UTI. *Escherichia coli* was the only isolated organism, showing the highest sensitivity to nitrofurantoin and considerable resistance to amoxicillin. Urine culture and susceptibility testing are vital for accurate diagnosis and appropriate treatment.

**Keywords:** Urinary tract infection, Pregnancy, *Escherichia coli*, Urine culture, Dipstick test, Antimicrobial susceptibility.

## Introduction

Urinary tract infection (UTI) is an important bacterial infection in pregnancy due to physiological and anatomical factors which promote ascending infection: dilatation of the ureters, reduced tone of the ureters, urinary stasis and incomplete bladder emptying. Infection can be either silent or symptomatic, and severe symptomatic infections usually have signs of dysuria (difficulty voiding), urinary frequency and urgency, suprapubic discomfort, fever, loin pain, nausea, or vomiting. Several of these manifestations may mimic normal pregnancy symptoms, so clinical

evaluation alone may not be enough to establish the diagnosis and laboratory evaluation is especially crucial.<sup>1-4</sup> UTIs are a significant public-health problem worldwide, with an estimated 404.6 million cases in the general population in 2019, and pregnancy-specific prevalence rates are highly variable depending on the population, definition of UTI and setting in which the healthcare is provided.<sup>5</sup> In Latin America, a systematic review of the literature found that the prevalence of asymptomatic bacteriuria was 18.39%, the prevalence of lower UTI was 7.54%, and the prevalence of

pyelonephritis was 2.34% in more than 111,000 pregnant women.<sup>1</sup> The situation is also highly variable geographically within developing nations of Africa and Asia, with aggregated evidence suggesting some particularly high burdens in several low and middle income countries.<sup>6</sup> A meta-analysis also showed significant heterogeneity for both symptomatic UTI and asymptomatic bacteriuria within Iran, as the local epidemiology is still essential.<sup>7</sup> In South Asia, the burden is clinically relevant because limitations in microbiological facilities, empirical antibiotic use, and increasing antimicrobial resistance may complicate diagnosis and treatment. In rural Bangladesh, a population-based study of 4,242 pregnant women reported an overall UTI prevalence of 8.9%, comprising 4.4% symptomatic UTI and 4.5% asymptomatic bacteriuria.<sup>8</sup> In neighbouring Pakistan, multidrug-resistant uropathogens have been documented among pregnant women, while studies from Ethiopia and Cameroon have reported substantial culture-confirmed infection rates, illustrating pronounced setting-specific variation.<sup>1-4,9,10</sup>

Clinically, failure to identify and appropriately treat bacteriuria during pregnancy may allow progression to cystitis or pyelonephritis and has been associated with adverse obstetric outcomes. In a recent systematic review and meta-analysis, UTI during pregnancy was associated with almost twice the odds of preterm birth, with a pooled odds ratio of 1.92.<sup>11</sup> Accordingly, evidence synthesized in systematic reviews suggests that antimicrobial treatment of bacteriuria reduces maternal pyelonephritis and may also lower the risks of preterm delivery and low birth weight, although the certainty of some outcome estimates remains limited<sup>12</sup>. Therefore, timely confirmation of infection has implications for both maternal and fetal health. For diagnosis, urine culture remains the reference standard, yet cost, laboratory availability, and reporting delays may restrict routine use in resource-constrained settings.<sup>4,8</sup> Although dipstick detection of leukocyte esterase and nitrite provides a rapid alternative, recent pregnancy-specific evidence has shown imperfect diagnostic performance,

indicating that dipstick findings should be interpreted cautiously against culture results.<sup>4,13</sup> Meanwhile, *Escherichia coli* is consistently the most common uropathogen, with different susceptibility patterns across regions. Significantly, resistance to common antibiotics is reported in many cases.<sup>1-4,10</sup> Against this background, locally generated evidence from symptomatic pregnant women in tertiary-care hospitals in Bangladesh remains important, particularly evidence integrating clinical presentation, dipstick findings, urine culture yield, causative organisms, and antimicrobial susceptibility. Therefore, the present study aimed to assess the clinical presentation and culture yield of UTI among symptomatic pregnant women, while also describing isolated organisms, dipstick findings, and antibiotic susceptibility patterns.

## Materials and Methods

This cross-sectional descriptive study was conducted in the Department of Obstetrics and Gynecology, Shaheed Ziaur Rahman Medical College Hospital, Bogura, Bangladesh, from January 2024 to December 2024. During the study period, pregnant women attending the department with clinical features suggestive of urinary tract infection (UTI) were considered for enrollment. Using a non-probability purposive sampling approach, eligible participants were enrolled consecutively according to their availability. A total of 105 symptomatic pregnant women were included.

For eligibility, pregnant women presenting with one or more urinary or associated symptoms, including dysuria or burning sensation during urination, increased frequency of micturition, loin pain, fever, nausea, vomiting, or non-specific lower abdominal pain, were included. Pregnant women without urinary symptoms, those experiencing vaginal bleeding or spontaneous rupture of membranes because of the possibility of urine-sample contamination, catheterized patients, and those receiving antibiotics at the time of assessment were excluded.

For each participant, information was collected using a

structured data-collection sheet. After explaining the study purpose and procedures, informed written consent was obtained before enrollment. Through face-to-face interviews, clinical examination, observation, review of medical and obstetric history, and relevant laboratory investigations, demographic, obstetric, clinical, and medical-history data were recorded. Urine specimens were collected from the selected participants and evaluated using relevant urinary investigations, including dipstick testing and urine culture. For culture-positive specimens, the isolated organisms were identified, and antimicrobial susceptibility testing was performed against the selected antibiotics.

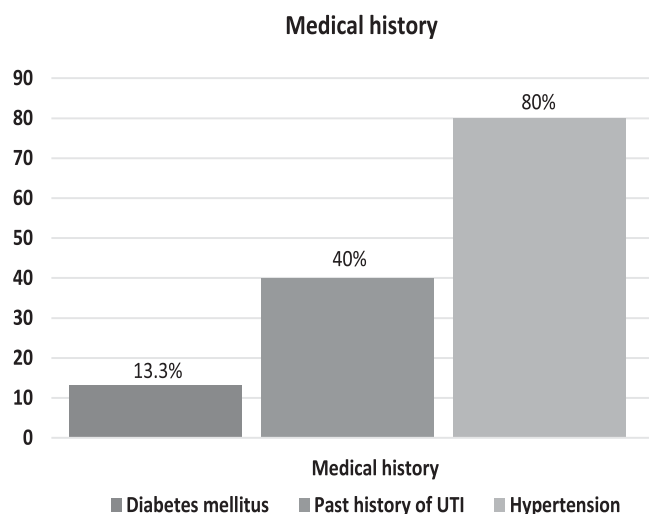
After collecting the data, we checked it for completeness and consistency, edited it, coded it, and entered it into a computer for analysis. We used Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS Inc., Chicago, IL, USA), to perform descriptive and inferential statistical analyses. We summarized categorical variables as frequencies and percentages, while we expressed continuous variables as mean  $\pm$  standard deviation and range, when applicable. To assess the link between dipstick findings and urine culture results, we applied Fisher's exact test, with a p-value  $<0.05$  considered statistically significant. Before starting, the study protocol received institutional approval. Throughout the study, we maintained confidentiality, ensured voluntary participation, and respected participants' right to withdraw.

## Results

Among the 105 symptomatic pregnant women included in the study, 92 (87.6%) were younger than 25 years, whereas 13 (12.4%) were older than 25 years. Regarding obstetric characteristics, the mean gravidity was  $1.64 \pm 0.85$ , with a range of 1–3, while the mean gestational age was  $24.67 \pm 4.79$  weeks, ranging from 16 to 35 weeks.

**Table I. Demographic and obstetric characteristics of the study participants (N=105)**

| Variables               | Categories/measurements | Total            |
|-------------------------|-------------------------|------------------|
| Age                     | <25 years               | 92 (87.6)        |
|                         | >25 years               | 13 (12.4)        |
| Gravidity               | Mean $\pm$ SD           | $1.64 \pm 0.85$  |
|                         | Range                   | 1–3              |
| Gestational age (weeks) | Mean $\pm$ SD           | $24.67 \pm 4.79$ |
|                         | Range                   | 16–35            |



**Figure 1. Distribution of the respondents by medical history**

Regarding previous medical conditions, among the 15 women with a recorded medical history, hypertension was reported in 12 (80.0%), a previous history of UTI in 6 (40.0%), and diabetes mellitus in 2 (13.3%).

With respect to clinical presentation, frequency of urination was the most frequently reported symptom, occurring in 103 (98.1%) participants, followed by nausea in 101 (96.2%), burning sensation during urination in 75 (71.4%), and vomiting in 72 (68.6%). Lower abdominal pain was reported by 39 (37.1%) women. Fever and loin pain were comparatively uncommon, occurring in 8 (7.6%) and 2 (1.9%) participants, respectively.

**Table II. Clinical presentation of symptomatic pregnant women with suspected UTI (N=105)**

| Presenting complaints  | n (%)      |
|------------------------|------------|
| Frequency of urination | 103 (98.1) |
| Nausea                 | 101 (96.2) |
| Burning sensation      | 75 (71.4)  |
| Vomiting               | 72 (68.6)  |
| Pain in lower abdomen  | 39 (37.1)  |
| Fever                  | 8 (7.6)    |
| Loin pain              | 2 (1.9)    |

Among the 55 participants reported to have individual dipstick findings, leukocyte esterase positivity was observed in 37 (67.2%), while protein was detected in 36 (65.5%). Blood was detected in 15 (27.3%) samples, whereas nitrite positivity was identified in 8 (14.5%). Concurrent positivity for both nitrite and leukocyte esterase was found in 8 (14.5%) samples.

**Table III. Urine dipstick findings among the study participants (n=55)**

| Dipstick findings                   | Positive, n (%) |
|-------------------------------------|-----------------|
| Leukocyte esterase                  | 37 (67.2)       |
| Protein                             | 36 (65.5)       |
| Blood                               | 15 (27.3)       |
| Nitrite                             | 8 (14.5)        |
| Both nitrite and leukocyte esterase | 8 (14.5)        |

On urine culture, bacterial growth was detected in 7 of the 105 participants, giving an overall culture yield of 6.7%, while 98 (93.3%) specimens showed no growth. Among all seven culture-positive specimens, *Escherichia coli* was isolated, accounting for 100% of the culture-confirmed infections.

**Table IV. Urine culture yield and isolated organisms among symptomatic pregnant women (N=105)**

| Variables                                            | Categories              | n (%)     |
|------------------------------------------------------|-------------------------|-----------|
| Urine culture result                                 | Growth                  | 7 (6.7)   |
|                                                      | No growth               | 98 (93.3) |
| Organism isolated among culture-positive cases (n=7) | <i>Escherichia coli</i> | 7 (100.0) |

When dipstick results were compared with urine culture, 6 of the 7 culture-positive participants had a positive dipstick result, whereas only 2 of the 98 culture-negative participants were dipstick positive. Conversely, one culture-positive participant had a negative dipstick result. A statistically significant association was identified between dipstick positivity and culture-confirmed UTI (Fisher's exact test,  $p < 0.001$ ).

**Table V. Association between dipstick result and urine culture findings (N=105)**

| Dipstick result | Culture positive | Culture negative | Total       | p-value |
|-----------------|------------------|------------------|-------------|---------|
|                 | n (%)            | n (%)            | n (%)       |         |
| Positive        | 6 (85.7)         | 2 (2.0)          | 8 (7.6)     | <0.001  |
| Negative        | 1 (14.3)         | 96 (98.0)        | 97 (92.4)   |         |
| Total           | 7 (100.0)        | 98 (100.0)       | 105 (100.0) |         |

Regarding antimicrobial susceptibility among the seven *E. coli* isolates, all 7 (100.0%) were sensitive to nitrofurantoin. Sensitivity to cephalexin was observed in 5 (71.4%) isolates, while 2 (28.6%) were resistant. In contrast, only 1 (14.3%) isolate was sensitive to amoxicillin, while 4 (57.1%) were resistant and 2 (28.6%) showed intermediate sensitivity.

**Table VI. Antibiotic susceptibility pattern of culture-positive *Escherichia coli* isolates (n=7)**

| Antibiotic     | Sensitive | Resistant | Moderately sensitive |
|----------------|-----------|-----------|----------------------|
|                | n (%)     | n (%)     | n (%)                |
| Amoxicillin    | 1 (14.3)  | 4 (57.1)  | 2 (28.6)             |
| Cephalexin     | 5 (71.4)  | 2 (28.6)  | 0 (0.0)              |
| Nitrofurantoin | 7 (100.0) | 0 (0.0)   | 0 (0.0)              |

## Discussion

In this study of 105 symptomatic pregnant women, the principal findings were a relatively low urine-culture positivity of 6.7%, universal isolation of *Escherichia coli* among culture-positive cases, a strong association between dipstick positivity and culture-confirmed infection, and marked differences in antimicrobial



susceptibility. While 98.1% complained of urinary frequency and 71.4% about burning during urination, only seven women were found to have a UTI, which suggests that symptoms alone are not very specific in pregnancy. This discordance could be biologically possible as urinary frequency, lower abdominal discomfort, nausea and vomiting can also be physiological symptoms of pregnancy. In terms of culture positivity, this was 6.7% compared to 8.9% of 4034 pregnant women in rural Bangladesh who were culture positive.<sup>8</sup> Much more have been reported in other parts of the world. Culture-confirmed UTI was found to be 18.7% of pregnant women in Ambo, 26.0% among pregnant women in Goba and Sinana and 14.1% of pregnant women at public facilities in Harar in Ethiopia.<sup>3,10,14</sup> Asymptomatic bacteriuria has also been found to be high and varies considerably from one geographical area to another, such as 19.9% in Harar, 17.4% in India and 13.5% in an Indian meta-analysis.<sup>15-17</sup> The lower culture yield in this cohort may be the result of selection of cases, differences in bacterial-count thresholds, specimen handling, documented prior antimicrobial exposure, population differences or the high proportion of pregnancy-related symptoms that mimic UTI.

Concerning clinical manifestations, urinary frequency was reported by 98.1%, burning sensation by 71.4%, lower abdominal pain by 37.1%, fever by 7.6%, and loin pain by only 1.9%. In contrast, Dube et al. reported lower abdominal pain in 85%, dysuria in 48%, and fever in 48% among symptomatic women, while only 6.7% of women with lower abdominal pain and urinary frequency had culture-positive infection.<sup>18</sup> This supports the present observation that common urinary complaints cannot reliably establish bacterial UTI. Accordingly, careful laboratory confirmation is particularly relevant in pregnancy, when unnecessary antimicrobial exposure should be avoided.

With respect to dipstick findings, leukocyte esterase was positive in 67.2% of the 55 individually assessed samples, while nitrite positivity was much lower at 14.5%. More importantly, 85.7% of culture-positive

women had a positive overall dipstick result, compared with only 2.0% of culture-negative women, producing a significant association,  $p < 0.001$ . Recent evidence nevertheless cautions against replacing culture with dipstick testing. In a pregnancy-specific cohort, Werter et al. demonstrated imperfect diagnostic accuracy of leukocyte esterase and nitrite and emphasized the risk of both overdiagnosis and missed infection.<sup>13</sup> Therefore, the strong association observed here supports dipsticks as useful rapid screening tools, but not as substitutes for urine culture where microbiological facilities are available.

Regarding bacterial etiology, *E. coli* accounted for 100% of the seven isolates. Although this proportion is higher than most reports, the predominance of *E. coli* is highly consistent with existing evidence. Among Bangladeshi pregnant women, Lee et al. identified *E. coli* in 38% of isolates, while rates of 46.4% were reported in Ambo and 27.3% in southeastern Ethiopia.<sup>3,8,14</sup> Furthermore, a meta-analysis from Iran estimated *E. coli* in 61.6% of pregnancy-associated UTIs, whereas studies from Addis Ababa and eastern Ethiopia also identified Gram-negative enteric organisms as major uropathogens.<sup>7,19,20</sup> The 100% proportion here should nevertheless be interpreted cautiously because only seven cultures were positive.

Regarding antimicrobial susceptibility, nitrofurantoin showed 100% activity against the *E. coli* isolates, compared with 71.4% sensitivity to cephalexin and only 14.3% to amoxicillin. This pattern closely resembles Bangladeshi evidence showing generally high nitrofurantoin activity but substantially poorer susceptibility to older  $\beta$ -lactams.<sup>8</sup> Likewise, high resistance to amoxicillin or ampicillin has been reported in Ethiopia, Pakistan, and across developing countries in a systematic review.<sup>6,9,10,15</sup> Current international guideline reviews also identify nitrofurantoin as an important treatment option for lower UTI during pregnancy, while emphasizing culture-guided selection whenever possible.<sup>21</sup>

Clinically, these findings support obtaining urine culture in symptomatic pregnant women rather than

relying solely on nonspecific symptoms. Empirical amoxicillin may be unreliable where resistance resembles that observed here, whereas nitrofurantoin appears microbiologically promising for appropriate lower UTIs, subject to gestational age, clinical condition, contraindications, and contemporary susceptibility data. This is important because pregnancy-associated UTI is not benign; a 2024 meta-analysis found that maternal UTI was associated with an approximately 1.92-fold higher odds of preterm birth.<sup>11</sup> Given the small number of isolates and the historical study period, however, the susceptibility findings should not be interpreted as current prescribing guidance. Rather, they demonstrate the continuing need for local antimicrobial surveillance, culture-based diagnosis, and periodically updated treatment protocols for pregnant women in Bangladesh.

## Conclusion

Urinary symptoms were extremely common among pregnant women who were suspected of having a UTI, but only 6.7% of these women actually had a culture-positive UTI, suggesting that urinary symptoms do not always indicate a UTI. *E. coli* was the only uropathogen isolated and dipstick positivity was highly associated with culture confirmed UTI. Among all drugs, nitrofurantoin was the most sensitive to microbial activity and amoxicillin had a significant degree of resistance. The results of the study agree with using urine culture and sensitivity test for proper diagnosis and selection of appropriate antibiotics in the treatment of symptomatic pregnant women to prevent unnecessary treatment and overuse of antibiotics, thus reducing the chances of developing antimicrobial resistance.

## Recommendations

Culture and antimicrobial sensitivity testing of urine should be recommended in symptomatic pregnant women to help determine an accurate diagnosis and a proper antibiotic choice. Rapid screening can be

carried out using dipsticks, and empirical use of antibiotics should take local resistance patterns into account. Frequent antimicrobial surveillance and more multi-centre studies are suggested.

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