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Value of p16 Expression in the Detection of High-Grade Squamous Intraepithelial Lesion of Cervix

Dr. Noor-E-Ferdous[1*], Dr. Farzana Sharmin[2], Dr. KM Mozibul Haque[3], Dr. Latifa Akhter[2], Dr. Rokshana Ahmed[4], Dr. Shakila Jahan Shimu[5], Shereen Yousuf[6], Dr. Ashrafun Nessa[7]

[1]Assistant Professor, Department of Gynaecological Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[2]Medical Officer, Department of Gynaecological Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[3]Associate Professor, Department of Anaesthesia, Holy Family Red Crescent Medical College and Hospital, Dhaka, Bangladesh
[4]Assistant Professor, National Institute of Neuroscience and Hospital, Dhaka, Bangladesh
[5]Research Assistant, National Centre for Cervical and Breast Cancer Screening and Training, Dhaka, Bangladesh
[6]Medical Officer, Department of Obstetrics & Gynaecology, Bangabandhu Sheikh Mujib Medical University Hospital, Dhaka, Bangladesh.
[7]Professor, Department of Gynaecological Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

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***Corresponding author:** Dr. Noor-E-Ferdous
Assistant Professor, Department of Gynaecological Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Abstract **Original Research Article**

Background: Cervical intraepithelial neoplasia (CIN) is usually a precancerous condition but, if left untreated, can develop into invasive cervical cancer. CIN2 and CIN3 are combinedly termed high-grade squamous intraepithelial lesions (HSIL). There are few screening tests based on cytology in detecting HSIL, but they are mostly less accurate. There is over-expression of p16 in pre-cancer and cancer of the cervix. The purpose of this study was to determine the value of p16 expression in the detection of HSIL. ***Methods:*** It was a cross-sectional study conducted in the colposcopy clinic of the Department of Obstetrics and Gynecology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from October 2021 to March 2022. The study included 72 HSIL patients detected by colposcopy and had subsequent cervical biopsy and p16 immunohistochemistry. ***Results:*** Majority (45.8%) of the patients were found within the 30-39 years age group, 44.4% of the participants were illiterate, and 76.4% came from the middle-income group. Colposcopy-directed cervical Punch or Loop electrosurgical excision procedure (LEEP) biopsy revealed that 70.5% of patients had HSIL, and the rest (64.3%) suffered from LSIL. Positive p16 results were significantly higher among the HSIL group (84.1%) compared to those other than the HSIL group (3.6%) (p<0.001). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the p16 in the detection of HSIL were 84.1%, 96.4%, 79.4%, and 88.9%, respectively. ***Conclusions:*** Immunohistochemistry for p16 has a high diagnostic value in detecting HSIL patients. Therefore, this could be recommended for appropriate management of patients with CIN to avoid misdiagnosis and the over or under-treatment.
Keywords: CIN, HSIL, p16, Immunohistochemistry, Cervix.

INTRODUCTION

Cervical cancer, a largely preventable disease, is the fourth most common cancer in women worldwide after breast, colorectal and lung cancer [1]. In 2020, cervical cancer caused approximately 340,000 deaths, with a further 600,000 new cases recorded, and this accounts for 3.4% of all deaths and 3.3% of all cancer incidents globally [2]. Around 85%of the global burden occurs in the less-developed regions, where it accounts for almost 12% of all female cancers [3] Among Bangladeshi women, cervical cancer is the second most prevalent cancer, with approximately 8,068 new cases detected yearly and causing 5214 deaths [4]. One effective means to decrease cervical cancer incidence and death is early detection of cancer and its precancerous lesions or cervical intraepithelial neoplasia (CIN) [5].

Approximately 80% of cervical cancers are squamous cells, and 15% are adenocarcinomas [7]. Cervical squamous intraepithelial neoplasia is classified into CIN1, CIN2 and CIN3 by the extent of epithelial involvement. The progression rates of CIN1 to CIN3 and to invasive carcinoma were 10% and 1%, respectively. The corresponding progression rates of CIN2 were 20% and 5%, and CIN3 to invasive cancer was greater than 12% [7]. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST Project) aimed to align terminology for HPV-associated squamous lesions of the LAT (lower anogenital tract) with current scientific knowledge by proposing a two-tiered system—low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) [8].

Human papillomavirus (HPV) infection is universally recognized as a causative agent in developing cervical intraepithelial neoplasia (CIN) and squamous intraepithelial lesions [9]. Epidemiologic risk factors for the development of carcinoma of the cervix include young age at first coitus, multiple sexual partners, high parity, and a history of other sexually transmitted diseases [6]. These lesions can be benign but are considered precancerous and often develop into invasive cervical carcinoma; over one-third of all HSILs and CIN grades II and III progress into cervical cancer over a period of between 10 and 15 years [2].

Several associated markers have been investigated for their potential utility in assisting the histopathologic classification of preinvasive lesions and in facilitating the distinction from non HPV induced alterations [10]. These pre-clinical cellular dysregulations could be evidenced clinically by the immunohistochemical study of some proteins, such as p16. p16 is a cell-cycle regulatory protein [11]. Its function is to regulate cell proliferation in the G1-S phase and negatively influence cell proliferation through a reciprocal relationship with another tumor suppressor protein, pRb. The overexpression of p16 could be found in cells with inactive pRb, commonly present in HPV infection [12]. The Rb tumor suppressor function is functionally inactivated by HPV E7 oncoproteins, which results in p16 overexpression in cervical cancers [13]. This p16 overexpression is a surrogate biomarker of HPV infection and helps evaluate HPV-associated squamous and glandular neoplasia of the lower gynecologic tract [3]. Hence, p16 immunoreactivity helps detect CIN because its expression could be affected by p16 mutations [14]. This is the reason why LAST Project suggested p16 staining as a preferred biomarker for cervical lesions [15]. Therefore, strong and diffuse block staining with p16 is interpreted as positive (i.e., p16-positive HSIL diagnosis), and patchy, incomplete p16 staining is interpreted as negative [8].

Although cervical cancer risk has substantially declined among women in developed countries due to effective cervical cytology screening programs, this cancer continues to be the most common cause of premature death among middle-aged women in their most productive years, and the largest single cause of year-life lost to cancer in developing countries [16]. In certain cases, the reactive changes, immature metaplasia or atrophic changes of the cervix may show similar morphologic features as intraepithelial lesions or discretion between the low-grade lesion and high-grade lesion is not possible by the routine hematoxylin and eosin stain of tissue, the study of this molecular biomarkers may be useful [17]. The correct diagnosis will certainly reduce an inappropriate surgical intervention, overtreatment, and psychological distress from unnecessary follow-up [18].

Hence, p16 immuno-staining might play an important role in distinguishing HSIL from LSIL and differentiating persistent infections from transient infections [19]. Therefore, to bring health benefits to the women, this study was conducted to estimate the diagnostic accuracy of p16 expression for the evaluation of high-grade squamous intraepithelial lesions for planning an appropriate management of patients with CIN; thus, unnecessarily aggressive and unsustainable treatment, as well as the morbidity associated with unnecessary intervention, can be avoided.

METHODS

Study Design and Participants

This was a cross-sectional observational study conducted among 72 women with HSIL diagnosed by colposcopy. The age range was 30 to 60 years. They had previously been detected as VIA (visual inspection of the cervix with acetic acid) positive and referred for colposcopy to the Colposcopy Clinic of BSMMU (Bangabandhu Sheikh Mujib Medical University) Hospital. The study period was from October 2021 to March 2022. Purposive and convenient sampling techniques were used to recruit the study participants.

Data Collection Procedure

The relevant clinical information was obtained by a preformed structured questionnaire. Colposcopy was performed after describing the full procedure and its complications, advantages and disadvantages to each study subject.

The colposcopy is a low resolution, stereoscopic, binocular, field microscope with a powerful light source. It is used to visually examine the cervix and the rest of the lower genital tract [20]. Colposcopy-directed biopsy was taken from all the suspected HSIL patients by punch biopsy forceps at the same sitting before thermocoagulation treatment. HSIL was diagnosed at colposcopy, using the Swede score system [21]. The Swede Score 0 – 4 means low grade / normal CIN 1, 5-6 means High grade/ non-invasive cancer CIN 2+ and 7-10 means High grade/ suspected invasive cancer CIN 2+.

In case of Loop Electrosurgical Excision Procedure (LEEP), the cervical tissue specimen was sent for the histopathological examination and p16 staining at the department of Pathology, BSMMU. LEEP is a treatment to remove precancerous cells from the cervix. The loop is inserted through the speculum and passed over the cervix, usually under local anesthesia.

Histological slides were made from blocks of paraffin, 4 mm thick, for immunocytochemical staining to identify p16 expression using the $p16^{INK4a}$ kit. p16 immunostaining was evaluated as a negative, focal/patchy, or diffuse staining pattern. $p16^{INK4A}$ (referred to as p16) immunohistochemistry (IHC) is widely used to facilitate the diagnosis of HPV-associated cervical precancerous lesions. LAST defines "block-positive" p16 as supporting a diagnosis of HSIL, provided the staining meets certain criteria: (1) demonstrates strong nuclear with or without cytoplasmic signal, (2) extends from the basal layers upward at least one-third of the epithelium, and (3) extends laterally over a significant distance. Following these criteria, most cases are straightforward to interpret strong/diffuse (positive) or no stain (negative). When the signal is weak/focal, pathologists generally interpret it as negative as well [22]. HSIL cells are easily missed (false-negative results) when they coexist with LSIL cells, metaplastic cells, repair cells, and atrophic cells [23].

Ethical Considerations

This study was performed after getting ethical clearance for the protocol from the Institutional Review Board and concerned authority of BSMMU. Informed written consent from each participant was taken before starting the interview.

Statistical Analysis

Socio-demographic characteristics, risk factors and obstetric history, contraceptive history, colposcopy impression by the Swede score, histopathological findings from colposcopy directed cervical biopsy, under- and over- treatment, the diagnostic accuracy of p16 related data of the study population were analyzed using descriptive statistics such as mean ±SD, frequency and proportion. Inferential analysis was done using SPSS version 26.0 and p-value <0.05 was considered statistically significant.

RESULTS

Socio-demographic Characteristics

Table 1 shows the socio-demographic characteristics of the patients with colposcopy. Here, most of the patients were within 30-39 years (45.8%). Most of them were married (91.7%). Above two-fifths (44.4%) were illiterates, while a large proportion (36.1%) were educated up to the primary level. Most (79.2%) of the women were homemakers, and most of the women (76.38%) came from the middle-income group.

Table 1: Socio-demographic characteristics of the study population

Characteristics	Number (n)	Percentage (%)
Age (in years)		
30-39	33	45.8
40-49	26	36.1
≥ 50	13	18.1
Marital status		
Married	66	91.7
Husband living abroad	4	5.6
Widowed	2	2.8
Educational status		
Illiterate	32	44.4
Up to Primary	26	36.1
Up to SSC	7	9.7
Up to HSC	6	8.3
Graduate and above	1	1.4
Occupation		
Housewife	57	79.2
Service holder	6	8.3
Garments worker	9	12.5
Monthly income status (in Taka)		
Low-income group	17	23.6
Middle income group	55	76.38

Low income < 6827 BDT and middle income =6828-26852 BDT (World Bank and UNDP, 2016)

Risk Factors, Obstetrics and Contraceptive History

Table 2 illustrates the distribution of risk factors, obstetrics and contraceptive history of the study population. Age of marriage at 12-14 years was found in above three-fifths (60.7%) of other than HSIL patients in comparison to 43.2% of the HSIL group.

Age at first delivery was noted in most women at <18 years (HSIL 79.5% vs. other than HSIL 75.0%). Most of the women were at their pre-/perimenopausal state (HSIL 86.4% vs. other than HSIL 82.1%). Multiparous women were (HSIL 88.6% vs. other than HSIL 89.3%), and above four-fifths of the patients of HSIL group (81.8%) were taking oral contraceptive pills for more than 5 years (78.6%) in comparison to 67.9% of other than HSIL group. There were no statistically significant differences in risk factors and obstetrics and contraceptive characteristics between the two groups (p>0.05).

Table 2: Distribution of risk factors, obstetrics and contraceptive history of the study population

Parameters	HSIL, *n* (%)	Other than HSIL, *n (%)*	*p*-value
Age at marriage			
12-14 years	19 (43.2)	17 (60.7)	0.096
15-17 years	16 (36.4)	4 (14.3)	
18-20 years	7 (15.9)	3 (10.7)	
≥ 21 years	2 (4.5)	4 (14.3)	
Age of first delivery			
< 18 years	35 (79.5)	21 (75.0)	0.651
≥ 18 years	9 (20.5)	7 (25.0)	
Menopausal status			
Pre-/peri menopause	38 (86.4)	23 (82.1)	0.627
Post-menopause	6 (13.6)	5 (17.9)	
Parity			
Nulliparous	5 (11.4)	3 (10.7)	0.932
Multiparous	39 (88.6)	25 (89.3)	
Contraceptive methods			
OCP	36 (81.8)	19 (67.9)	0.279
Condom	1 (2.3)	4 (14.3)	
IUCD	2 (4.5)	1 (3.6)	
Permanent	1 (2.3)	0 (0.0)	
None	4 (9.1)	4 (14.3)	
Duration of OCP use			
< 5 years	19 (43.2)	6 (21.4)	0.051
≥ 5 years	17 (56.8)	13 (78.6)	

*Other than HSIL indicates LSIL, chronic cervicitis and chronic cervicitis with squamous cell metaplasia.

Figure 1 demonstrates the distribution of the study population according to histopathological findings from colposcopy-directed cervical biopsy (punch and LEEP biopsy). Here high-grade lesion was found in nearly half (47.2%) of the patients, whereas the low-grade lesion was documented in 20.8%.

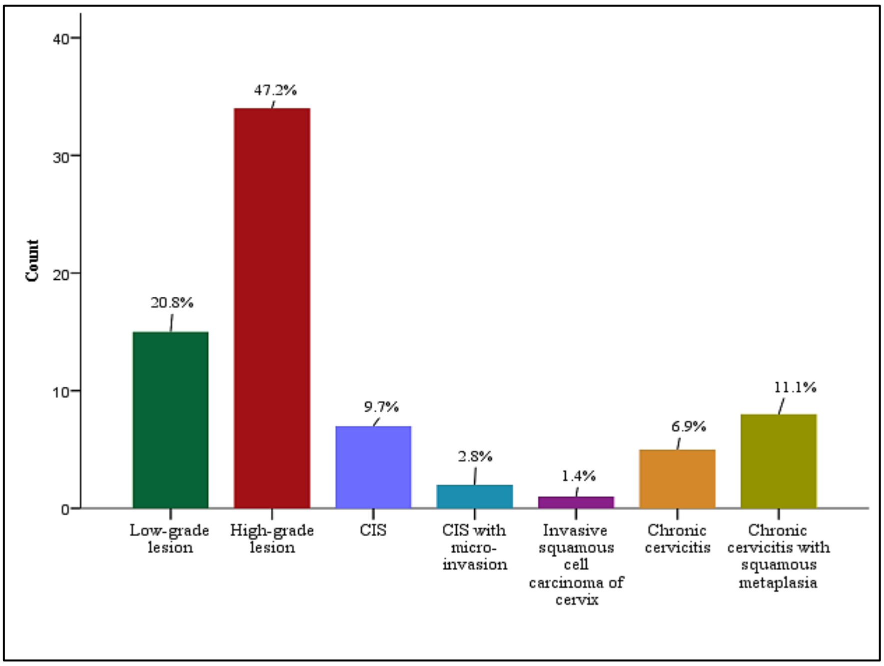

Figure 1: Bar diagram showing the distribution of study population according to histopathological findings from cervical biopsy

Treatment, under- and Over-Treatment and p16 Expression

Table 3 exhibits LEEP was performed more (70.5% vs 64.3%) in the HSIL group. The difference was not statistically significant. The HSIL patients had more under-treatment (6.4% vs 0%) and less overtreatment (2.1% vs 84.0%). Positive p16^{INK4a} results were more (84.1% vs 3.6%) in HSIL group respondents. Overtreatment and p16 expression difference in the distribution were statistically highly significant (p<0.001).

Table 3: Comparison of the study population according to treatment, under- and over-treatment as per see and treat protocol and p16 expression

Parameters	HSIL, n (%)	Other than HSIL, n (%)	*p*-value
Treatment			
Thermocoagulation	10 (22.7)	10 (35.7)	0.551
LEEP	31 (70.5)	18 (64.3)	
F/U colposcopy	1 (2.3)	0 (0.0)	
Radical hysterectomy	1 (2.3)	0 (0.0)	
Cone biopsy	1 (2.3)	0 (0.0)	
Undertreatment			
Yes	3 (6.4)	0 (0.0)	0.547
No	44 (93.6)	25 (100.0)	
Overtreatment			
Yes	1 (2.1)	21 (84.0)	<0.001
No	46 (97.9)	4 (16.0)	
p16			
Positive	37 (84.1)	1 (3.6)	<0.001
Negative	7 (15.9)	27 (96.4)	

Figure 2 shows that above half (54.2%) of the study population had a SWEDE score within 7-10, and the rest (45.8%) were between scores 5 to 6.

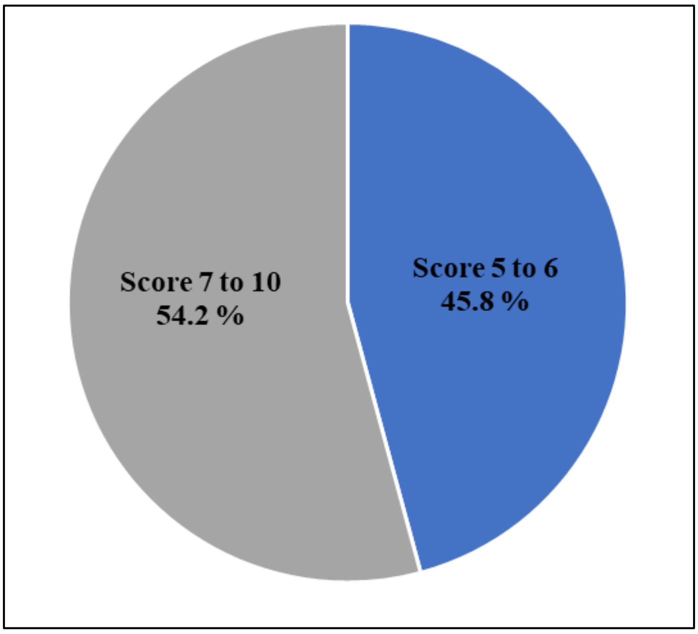

Figure 2: Percentage of colposcopy impression by Swede score

Diagnostic accuracy of the p16

Table 4 depicts the diagnostic accuracy of the p16^{INK4a} immunohistochemistry for detecting high-grade squamous intraepithelial lesions, sensitivity, specificity, and positive and negative predictive values for p16 expression were 84.1%, 96.4%, 97.4% and 79.4%, respectively.

Table 4: Distribution of study population according to and diagnostic accuracy of the p16^{INK4a} identification tests for high-grade squamous intraepithelial lesions

Diagnostic accuracy	p16	95% CI (lower-upper)
Sensitivity (%)	84.1%	69.9% to 93.4%
Specificity (%)	96.4%	81.7% to 99.9%
PPV (%)	97.4%	84.3% to 99.6%
NPV (%)	79.4%	66.1% to 88.4%
Accuracy	88.9%	79.3% to 95.1%

DISCUSSION

The increasing incidence of cervical cancer and pre-invasive lesions demands the need for accurate and reproducible diagnostic methods and terminology. The biopsy is the standard approach for accurate prediction of HSIL, and p16 is usually expressed at a low concentration in healthy cells but is overexpressed in the cervical cell of both HSIL and cancer. Therefore, this cross-sectional study focused on observing the diagnostic value of p16 expression in detecting HSIL of the cervix.

In this study, colposcopy-directed cervical Punch or LEEP biopsy revealed that 70.5% of patients had HSIL, and the rest (64.3%) suffered from LSIL. The Swede score is a good colposcopic scoring system used to screen and exclude high-grade lesions accurately. Above half (54.2%) of the patients had a Swede score of 7-10, and 45.8% had a score of 5- 6. The risk of overtreatment, or unnecessary treatment, is one of the main criticisms of the see-and-treat approach. The effectiveness of see-and-treat depends on colposcopic impression. Thus, patients may be unnecessarily exposed to bleeding and infection, the most common complications of the LEEP procedure. Women who are CIN 2, but exhibits p16 negative, are usually kept on conservative management and advised for follow-up. In this study, overtreatment was observed in 1(2.1%) HSIL and 21(84.0%) other than HSIL group patients (p<0.001). Nghiem *et al.*, in their study, had an average overtreatment rate of 7.1%. There was 78.8% chance of having lower (the 10% threshold) overtreatment rate [24]. Therefore, it is recommended that a see-and-treat strategy is only appropriate when an experienced colposcopist can differentiate low-grade from high-grade lesions, and the quality of colposcopic practice should be improved by setting appropriate standards [25].

In this study, positive p16^{INK4a} were among 84.1% of the HSIL group of patients compared to only 3.6% in other than HSIL group respondents. p16 expression differences in the distribution were statistically highly significant . Another study showed the expression of p16 in cervical cancer showed that p16 expression was more pronounced in HSIL (a proportion of 3þ or 4þ expression was seen in >90% of cases) as compared to LSIL (where only a proportion of 1þ or 2þ expression was seen) [3]. A statistically significant association of p16 with the histological diagnosis was noted. These findings were similar to Kim *et al.*, who suggested that p16 was mainly expressed in HSILs and carcinoma (>25% expression in 100% of cases) as compared to LSIL (<25% expression was seen) [26]. Volgareva *et al.*, and Wang *et al.*, also observed that the proportion of p16 positive samples increases in the following row: CIN I – CIN II – CIN III – carcinoma [27, 28]. Dijkstra *et al.*, demonstrated that using p16^{INK4a} immunohistochemistry significantly improves the accuracy of grading CIN lesions by a single pathologist, equaling an expert consensus diagnosis [29]. This can be said that p16^{INK4a} IHC provides greater accuracy of CIN grading with less variability and thus could help avoid unnecessary diagnostic and surgical procedures.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the p16INK4a in detecting HSIL were 84.09%, 96.43%, 79.41%, and 88.89%, respectively. This finding was supported by the study of Neih *et al.*, who reported that p16 immunoreactivity of atypical cells for detection of biopsy-proven significant lesions (HSIL or higher) was highly sensitive (sensitivity, 95%) and specific (specificity, 96%) and had favorable positive (91%) and negative (98%) predictive values [30]. In another study by Haidopoulos *et al.*, p^{16INK4A} immunostaining yielded 75% sensitivity, 62% specificity, 32% positive predictive value, and 91% negative predictive value in HSIL patients [31]. The accurate diagnostic rates of cancer and HSIL were significantly increased by p16 immunostaining plus cytology than by cytology alone. The false-negative or false-positive of p16 immunostaining occurred with a unicellular pattern. With a sensitivity of 96.0% and accuracy of 91.7%, the diagnostic performance of p16 immunostaining was much better than that of cytology alone, with a sensitivity of 36.0% and accuracy of 70.9% .They concluded that p16 immunostaining in cervical brushing cells might not only be used as an ancillary tool for cytological diagnosis of cervical neoplasia but also help to distinguish HSIL from LSIL [32].

This study had some limitations. The sample size was relatively small, and respondents were chosen only from the colposcopy clinic of BSMMU, Dhaka.

Therefore, the results of this study may not reflect the exact picture of the whole country. Also, the present study was conducted in a short time, and the sample was taken purposively. So, there may be a chance of bias that can influence the results.

CONCLUSIONS

p16 expression in detecting HSIL patients has high diagnostic performance with high sensitivity and specificity. p16 could be recommended for appropriate management of pre-cancer lesions of the cervix. It will help in proper diagnosis and avoid over- or under-treatment.

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Ethical Approval: The study was approved by the Institutional Ethics Committee.

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