



Research Article

# Prevalence of human papillomavirus infection in abnormal pap smears

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Received: 24 January 2021

Accepted: 19 July 2023

Published: 29 August 2023

**DOI**

[10.25259/Cytojournal\\_8\\_2021](https://doi.org/10.25259/Cytojournal_8_2021)

**Quick Response Code:**



## ABSTRACT

**Objectives:** This study was undertaken to study the prevalence of human papillomavirus (HPV) infection using the polymerase chain reaction (PCR) technique in abnormal cervical pap smears and to correlate the different cytological results with HPV infection.

**Material and Methods:** A total of 1788 cervical pap smears of women more than 30 years of age conducted over a period of 1 year 3 months (June 2015–August 2016) were screened by liquid-based cytology. High-risk (HR)-HPV testing was performed by PCR in abnormal lesions. Inflammatory smears and some atypical squamous cells of undetermined significance (ASCUS)-reactive cases were excluded from HPV testing. Histopathological correlation was done wherever possible.

**Results:** The overall prevalence of the intraepithelial lesions/malignancy was ASCUS. (ASCUS) - 79 (4.42%), atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) - 10 (0.56%), low-grade squamous intraepithelial lesion (LSIL) - 26 (1.45%), high-grade squamous intraepithelial lesion (HSIL) - 15 (0.84%), squamous cell carcinoma - 5 (0.28%), and adenocarcinoma - 1 case (0.06%). Overall, 136 (7.60%) samples were classified as abnormal. Seventy-seven samples were included for HR-HPV testing – 20 ASCUS, 10 ASC-H, 26 LSIL, 15 HSIL, and 6 malignant cases. A control group of ten samples with normal cervical cytology within the normal limit (Control) (WNLc) was tested for HR-HPV. HR-HPV was detected in 20% of samples of the WNLc group, 45% of the ASCUS group, 70% of the ASC-H group, 73.07% of the LSIL group, 86.67% of the HSIL, and 83.34% of the samples in the malignant group. Overall, HR-HPV was detected in 68.83% of abnormal cervical pap smears.

**Conclusion:** Our study shows that the percentage of HR-HPV-positive case increases with the severity of cytologic morphology. HPV had 4 times higher positivity in squamous intraepithelial lesion as compared to ASCUS.

**Keywords:** Cervical cancer, Liquid-based cytology, Human papillomavirus, Polymerase chain reaction

## INTRODUCTION

Cervical cancer is the third most common gynecologic cancer and causes of death among gynecologic cancers in Western countries.<sup>[1]</sup> However, in developing countries that lack access to cervical cancer screening, it remains one of the most common cancers among females with India alone accounting for one-quarter of the global burden of cervical cancer.<sup>[2]</sup>

Cytological examination remains a comparatively easy and less invasive method to screen these patients. However, there are numerous limitations to using cytology alone in screening protocols.

It has been illustrated that one in ten women positive for human papillomavirus (HPV) 16 and/or 18 have a high-grade cervical disease that was missed by cytology at baseline.<sup>[3]</sup> HPV is the most proven dominant factor in the development of cervical cancer, so it has naturally led to studies to recognize what role HPV testing could play in screening programs.<sup>[4]</sup> Several studies comparing HPV DNA testing and cytology showed that HPV testing was remarkably more sensitive for the detection of precancerous lesions.<sup>[2]</sup> A Pap test and a HPV test collected and co-tested at the same time is preferred over Pap cytology alone for screening in women ages 30–65 or as a triage test for atypical squamous cells of undetermined significance (ASCUS) cytology in women, who are 21 years of age and older.<sup>[5,6]</sup>

Most of the Indian women diagnosed with cervical cancer have never been screened for the disease and the majority of these cases are present in advanced stages due to lack of any ordered cervical cancer screening program. With this background, the present study was carried out to analyze the frequency of HPV infection in the abnormal Pap smears of women attending a tertiary care center in northeast India.

## MATERIAL AND METHODS

A prospective study was conducted over a period of 1 year and 3 months (June 2015–August 2016) after obtaining approval from the Institutional Ethical Committee.

### Inclusion criteria

The following criteria were included in the study:

1. All Pap smears diagnosed as cervical epithelial lesion on cytology as per the Bethesda system for reporting cervical cytology
2. All women, who were more than or equal to 30 years of age
3. All patients, who gave informed consent for both Pap and HPV testing.

### Exclusion criteria

The following criteria were excluded from the study:

1. Patients diagnosed with only inflammation without any epithelial abnormality
2. Patients with a history of hysterectomy
3. Women who were previously treated for cervical intraepithelial neoplasia (CIN) or cervical cancer.

### Collection and processing of liquid-based cytology (LBC) cervical specimens

Cervical samples were collected using the rovers Cervex-Brush® and the brush heads were transferred directly into

10 mL of 24% buffered ethanol solution (BD CytoRich preservative). 8 mL of which was used for LBC preparation for cytological diagnosis and the residual sample was preserved at –20°C for high-risk (HR)-HPV detection by polymerase chain reaction (PCR). The LBC technique was processed by BD SurePath™ (BD Diagnostics, TriPath, Burlington, NC, USA).

### HR-HPV detection by PCR

Genetix Nucleopore® Genomic DNA purification from tissue was used for DNA extraction and Amplisens® HR-HPV screen-Eph PCR kit was used to detect the presence of HR-HPV in residual LBC cervical samples. It was able to detect three main phylogenetic groups of HPV: A7, A6, and A9 that include 14 types: 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70, and responsible for more than 97% of severe cervical dysplasia and cervical cancer. This kit contains the internal endogenous control (Human genome DNA/ $\beta$ -globin gene) and primers against E1-E2 gene fragments of 14 types of HR-HPV. The cervical sample was considered positive for HR-HPV if the band of amplified DNA was present in agarose gel (1.7%) at the level from 267 to 325 base pairs (Bps) and  $\beta$ -globin showed amplified product at 723 Bps. The absence of beta-globin DNA results indicated inadequate sample collection or failure to adequately extract DNA.

### Biopsy sample for histopathological examination (HPE)

A punch biopsy was taken from cervical lesion wherever possible and fixed in formalin. The sample was stained with routine hematoxylin and eosin stain for HPE.

### Statistical analysis

Results on categorical measurements are presented in Number (%). Significance is assessed at a 5% level of significance. Chi-square/Fisher Exact test has been used to find out the significance of study parameters on a categorical scale between two or more groups. Diagnostic statistics such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy have been computed. The statistical software, namely, SPSS 15.0 and MedCalc 9.0.1 were used for the analysis of the data.

## RESULTS

A total of 1788 pap smears of women more than or equal to 30 years of age were screened to detect an epithelial abnormality. One hundred and thirty-six pap smears categorized as epithelial cell abnormalities fulfilling the inclusion and exclusion criteria were included in the study.

Furthermore, a randomly selected control group consisting of ten samples with normal cervical cytology within the normal limit (control) (WNLc) with the same age distribution as the test group was included in the study.

The age of the patients with epithelial cell abnormalities ranged from 30 to 83 years with a mean age of 47.5 years. Most of the cases were above para 3 (58%), followed by women with parity 1–3 (39%) and only 4% of cases were nulliparous. The majority of the cases were oral contraceptive pill (OCP) users of more than 5 years (53.7%), while non-OCP users were 26.4%. The most common presenting symptom was vaginal discharge in 42 cases (30.8%), followed by lower abdominal pain in 35 cases (25.7%), post-coital bleeding in 19 cases (13.7%), and irregular vaginal bleeding in 16 cases (11.8%). The other presenting symptoms were postmenopausal bleeding (8.9%), dyspareunia (3.8%), menorrhagia (2.9%), and primary infertility (2.2%). It was observed that 102 patients (75%) came from rural background with low socioeconomic status.

The overall prevalence of the intraepithelial lesions (IELs)/malignancy was as follows: ASCUS in 79 cases (4.42%), ASC cannot exclude HSIL (ASC-H) in 10 cases (0.56%), low-grade squamous intraepithelial lesion (LSIL) in 26 cases (1.45%), high-grade squamous intraepithelial lesion (HSIL) in 15 cases (0.84%), squamous cell carcinoma in 5 cases (0.28%), and adenocarcinoma in 1 case (0.06%). In total, 136 (7.60%) samples were classified as abnormal.

Interobserver variability and level of agreement between three pathologists were studied, as shown in Table 1 and Figure 1.

From the total group of 136 samples with IEL/malignancy, 77 samples were included for HR-HPV testing by PCR. The distribution of the samples was 20 cases of ASCUS, 10 cases of ASC-H, 26 cases of LSIL, 15 cases of HSIL, and 6 malignant cases. Furthermore, a randomly selected control

group (WNLc) consisting of ten samples with normal cervical cytology was subjected to HR-HPV testing by PCR [Figures 2 and 3].

HR-HPV was detected in 2 (20%) out of ten samples of WNLc, 9 (45%) out of 20 samples of ASCUS, 7 (70%) out of 10 samples of ASC-H, 19 (73.07%) out of 26 samples of LSIL, 13 (86.67%) out of 15 samples of HSIL, and 5 (83.34%) out of 6 malignant samples. Overall, HR-HPV was detected in 53 abnormal pap smears (68.83%).

The proportion of HR-HPV infection in each group is depicted in Table 2. Association between proportion of HR-HPV infection and the cytological groups revealed increased percentage of HR-HPV DNA-positive cases with increase in the severity of cytological interpretation, showing a high association with SIL, particularly with diagnoses of HSIL.

Histopathological correlation was done wherever possible [Figure 4]. The comparison of LBC with histopathological diagnosis is depicted in Table 3. p-value was strongly significant (0.006). Sensitivity, specificity, PPV, and NPV of LBC for prediction of dysplasia were 69%, 72%, 74.1%, and 66.7%, respectively. The accuracy of LBC for the prediction of dysplasia was 70.5%.

The comparison of HPV test results with histopathological diagnosis is depicted in Table 4. p-value was strongly significant (0.001). Sensitivity, specificity, PPV, and NPV of HPV test for the prediction of dysplasia were 92.3%, 58.8%, 77.4%, and 83.3%, respectively. The accuracy of the HPV test for the prediction of dysplasia was 75.5%.

## DISCUSSION

Early diagnosis of CIN and preclinical invasive cancer in adult is beneficial. Pap smear is one of the success stories in the field of preventive medicine. Infection with HPV is the most potent cause for the majority of pre-invasive lesions of

**Table 1:** Prevalence of cervical lesions by individual pathologist.

	P1			P2			P3		
	Cases	Prevalence	% n=133	cases	Prevalence	% n=97	Cases	Prevalence	% n=79
ASCUS	81	4.53	60.9	55	3.08	56.7	42	2.35	53.2
ASC-H	12	0.67	9.0	7	0.39	7.2	8	0.45	10.1
LSIL	22	1.23	16.5	18	1.00	18.6	13	0.73	16.5
HSIL	12	0.67	9.0	12	0.67	12.4	10	0.56	12.7
SCC	5	0.28	3.85	4	0.22	4.1	5	0.28	6.3
Adenocarcinoma	1	0.06	0.75	1	0.06	1.0	1	0.06	1.2
Total	133	7.44	100	97	5.43	100	79	4.42	100

P: Pathologist, n: Number of cases, ASCUS: Atypical squamous cells of undetermined significance, ASC-H: ASC cannot exclude high-grade squamous intraepithelial lesion, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade SIL, SCC: Squamous cell carcinoma

the cervix.<sup>[7]</sup> The global prevalence of HPV infection in the general population is estimated at 11.4%.<sup>[8]</sup>

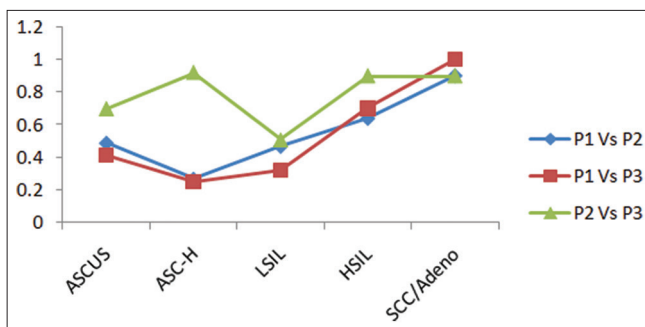
HPV is divided into HR-HPV and low-risk (LR-HPV). The former is composed of 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, and 73. The later was composed of 6, 11, 13, 32, 42, 54, and 70. HR-HPV was associated with malignant lesion and LR-HPV was associated with benign disease.<sup>[9]</sup> HPV DNA testing is a promising new technology for cervical cancer prevention. Large randomized clinical trials have manifested that HPV testing has a higher sensitivity but lower specificity than cytology for detecting high-grade cervical lesions in primary screening.<sup>[8]</sup> HPV testing, although expensive can be cost-effective in the long run as it can be executed with longer intervals between screening while at the same time minimizing cervical cancer incidence and deaths.<sup>[8]</sup>

In the present study, the mean age of HPV-positive cases was significantly younger (44.3 years) than the mean age of HPV-negative cases (50.7 years) similar to the study by Depuydt *et al.*<sup>[10]</sup> In the present study, most of the dysplastic

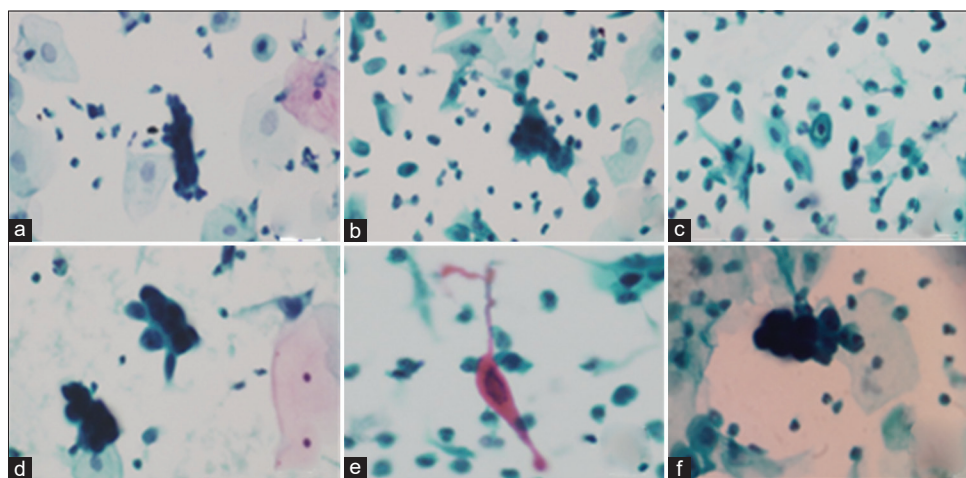
cases were above para 3 reflecting a higher incidence of dysplasia/cancer with increased parity corroborating the fact that married women are 2–4 times likely to develop cancer cervix than sexually active women.<sup>[11]</sup> This is related to the frequency of childbearing. The majority, in the present study, presented with vaginal discharge and belonged to low socioeconomic status similar to the study by Juneja *et al.*, who reported a higher incidence of cervical dysplasia and cancer in illiterate women of low socioeconomic status.<sup>[12]</sup>

The present study had a maximum prevalence of ASCUS of 4.42%. This finding was in concordance with Shrivastava *et al.*, who showed the prevalence of ASCUS as low as 4.96%.<sup>[13]</sup> The present study stated that HR-HPV positivity was seen as lowest with the ASCUS group (45%) and highest with the HSIL group (86.67%) among all abnormal pap smears. These findings were in concordance with most of the previous studies. Depuydt *et al.* showed that HR-HPV positivity was lowest in the ASCUS group (58.6%) and highest in the HSIL group (98.5%).<sup>[10]</sup> In another study by Al-Awadhi *et al.*, HR-HPV positivity was highest in the LSIL group (89%).<sup>[14]</sup>

The results of the present study showed high sensitivity of HR-HPV testing in detecting cervical dysplasia. The low sensitivity of cytology was mainly due to ASCUS and LSIL, which were normal in HPE. ASCUS and LSIL lesions, which were negative in HPE, most of them were HR-HPV negative and the lesions, which were HPE positive, were HR-HPV positive. Mayrand *et al.* stated that compared with cytology, HPV testing has greater sensitivity for the detection of CIN.<sup>[15]</sup> The sensitivity of HPV testing for CIN grade 2 or 3 was 94.6%, whereas the sensitivity of cytology alone was 55.4%.<sup>[15]</sup> The sensitivity of both the tests used together was 100%, and the

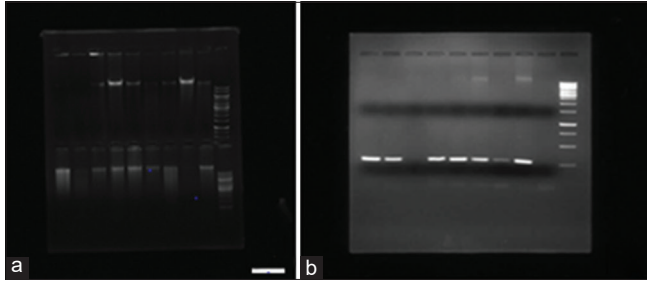


**Figure 1:** Agreement level using kappa statistics: Agreement level increases as grade increase.

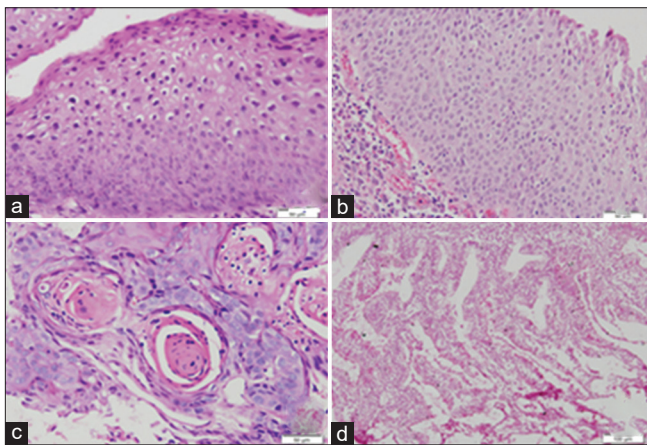


**Figure 2:** Liquid-based cytology cervical pap smears (a) atypical squamous cells (ASCs) of undetermined significance, (b) ASC cannot exclude high-grade squamous intraepithelial lesion, (c) low-grade squamous intraepithelial lesion, (d) high-grade squamous intraepithelial lesion, (e) malignant squamous cells carcinoma, and (f) adenocarcinoma.





**Figure 3:** Polymerase chain reaction (a) genomic DNA resolved in 0.8% agarose gel, (b) high-risk-human papillomavirus (HR-HPV) gene amplification – lane 1, 2, 4, 5, 6, 7, (+), lane 3 (-), lane 8 - positive control, and lane 9 - negative control (HR-HPV amplicon size – 283 bps).



**Figure 4:** Histopathology (a) low-grade squamous intraepithelial lesion, (b) high-grade squamous intraepithelial lesion, (c) keratinizing squamous cell carcinoma, and (d) adenocarcinoma.

**Table 2:** Association/contingency of cytology with HR-HPV status.

	Infected	Uninfected	Total	Proportion infected
WNLc	2	8	10	0.2
ASCUS	9	11	20	0.45
ASC-H	7	3	10	0.7
LSIL	19	7	26	0.73
HSIL	13	2	15	0.86
SCC/ Adenocarcinoma	5	1	6	0.83

WNLc: Within normal limit (Control), ASCUS: Atypical squamous cells of undetermined significance, ASC-H: ASC cannot exclude high-grade squamous intraepithelial lesion, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade SIL, SCC: Squamous cell carcinoma

specificity was 92.5%.<sup>[15]</sup> Cuzick *et al.* stated that HPV testing is markedly more sensitive in detecting CIN 2+ than cytology (96.1% vs. 53%) but is less specific (90.7% vs.96.3%).<sup>[16]</sup>

**Table 3:** Comparison of LBC with histopathological diagnosis.

	Dysplasia/malignancy in HPE	
	CIN	Normal
SIL/malignancy in cytology		
LSIL/HSIL/Malignancy	20	7
ASCUS/ASC-H	9	18

LBC: Liquid-based cytology, HPE: Histopathological examination, SIL: Squamous intraepithelial lesion, CIN: Cervical intraepithelial neoplasiam, LSIL: Low-grade SIL, HSIL: High-grade SIL, ASCUS: Atypical squamous cells of undetermined significance, ASC-H: ASC cannot exclude high-grade squamous intraepithelial lesion

**Table 4:** Comparison of HPV test with histopathology.

	Dysplasia/malignancy in HPE	
	CIN	Normal
HR-HPV status		
HR-HPV positive	24	7
HR-HPV negative	2	10

HPV: Human papillomavirus, HPE: Histopathological examination, HR-HPV: High-risk human papillomavirus

HPV DNA testing may not be a suitable gold standard in general because its use would make specificity and sensitivity prevalence-dependent.<sup>[17]</sup> This is true because HPV infection in negative pap smears varies from population to population. HPV infection is often transient in sexually active, young women with normal cervicovaginal cytology.<sup>[17]</sup> Hence, our study demonstrates the usefulness of HR-HPV testing as a triage method in ASCUS and LSIL.

## SUMMARY

In the present study, it was observed that the sensitivity of HPV testing is higher than cytology alone. Thus, in our hospital setting, HPV test appears to be more sensitive and NPV of HPV testing is higher than Pap test for detecting dysplastic lesions of the cervix. In a developing country like India with harsh constraints on resources, Pap test appears more feasible and economical. However, the choice of a test, in addition to cost, is also highly influenced by the test’s clinical performance. HPV testing may still not be available in all the centers but if added to the Pap cytology for detecting dysplastic lesions of the cervix.

## Acknowledgment

The present study was supported by a student research grant from the Department of Biotechnology, Ministry of Science and Technology, Government of India. We offer our thanks to clinical colleagues for providing cytological material and all the technical staff of Pap and Molecular laboratory for their help pertaining to laboratory work.

## COMPETING INTEREST STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interests.

## AUTHORSHIP STATEMENT BY ALL AUTHORS

All authors of this article declare that we qualify for authorship as defined by ICMJE <http://www.icmje.org/#author>. Each author has participated adequately in the work and takes public responsibility for appropriate portions of the content of this article. All authors read and approved the final manuscript. Each author acknowledges that this final version was read and approved.

## ETHICS STATEMENT BY ALL AUTHORS

This study was conducted with approval from the Institutional Ethical committee in NEIGRIHMS, Shillong, Dated: 13<sup>th</sup> April 2015, approval no. NEIGR/IEC2013/81. Authors take responsibility to maintain relevant documentation in this respect.

## LIST OF ABBREVIATIONS (In alphabetic order)

ASC-H – Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion  
 ASCUS – Atypical squamous cells of undetermined significance  
 Bps – Base pairs  
 CIN – Cervical intraepithelial neoplasia  
 H & E – Hematoxylin-eosin  
 HPE – Histopathological examination  
 HR-HPV – High-risk HPV  
 HSIL – High-grade squamous intraepithelial lesion  
 HPV – Human papillomavirus  
 IELs – Intraepithelial lesions  
 LBC – Liquid-based cytology  
 LR-HPV – Low-risk HPV  
 LSIL – Low-grade squamous cell intraepithelial lesion  
 NILM – Negative for intraepithelial lesion or malignancy  
 NPV – Negative predictive value  
 OCP – Oral contraceptive pills  
 PPV – Positive predictive value  
 PCR – Polymerase chain reaction  
 SCC – Squamous cell carcinoma  
 SIL – Squamous intraepithelial lesion  
 WNLc – Within normal limit (control).

## EDITORIAL/PEERREVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded

for reviewers and vice versa) through automatic online system.

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**How to cite this article:** Mishra J, Kalantri S, Raphael V, Dey B, Khonglah Y, Das A. Prevalence of human papillomavirus infection in abnormal pap smears. *CytoJournal* 2023;20:21.

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