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Cryotherapy in squamous intraepithelial lesions of cervix

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Abstract
Aims: In this study we studied the safety, effectiveness and acceptability of Cryotherapy in cases with abnormal squamous intraepithelial lesions.
Materials and Methods: In our study, 60 patients in reproductive age group attending gynecology OPD, with complaints of discharge per vaginum, lower abdominal pain, and post coital bleeding were screened for HPV DNA by paper smear method. Also these patients were subjected to detailed history, per abdomen, per speculum and per vaginum examination, VIA and papanicolou smear. Colposcopy and biopsy was done in patients where indicated. Then cryotherapy was given to these patients and patients were followed up by same.
Results: Cytological screening was done in 55 cases and it was found that 28(51%) were inflammatory, 15(27%) were LSIL, 2(8%) were AGUS, no ASCUS, 8(14%) were HSIL.VIA positive cases were 38 cases. On cytology they showe5% VIA negative were HPV positive and 95% cases were HPV negative. P-value is 0.038 which was statistically significant.d 17(45%) inflammatory, 13(34%) were LSIL, were 8(21%) were HSIL. pre-treatment 30(67%) cases had inflammatory smear, 11(24%) cases have LSIL cytology and 4(9%) case have HSIL. Post treatment none had LSIL& HSIL, 21(47%) converted to normal cytology and 24(53%) inflammatory smear. As the cases who had LSIL and AGUS initially after treatment turn normal or inflammatory. P value was 0.001, statistically significant. . 11 HPV positive cases were treated with cryotherapy and 8 cases turn negative showing P<0.001 which was clinically significant.
Conclusion: We concluded that in low resource settings, simple and safe diagnostic and therapeutic procedures like VIA, HPV DNA, cryotherapy are safe and effective methods to combat the killer disease of cervical cancer.

Keywords: Cryotherapy, squamous intraepithelial, cervix

1. Introduction
Cervical cancer; mainly caused by Human Papillomavirus infection, is a preventable disease but least prevented hence the leading cancer in Indian women. (ICMR2012) [1]. Using data from the WHO [2], United Nations, the World Bank and IARC GLOBOCAN, cervical cancer kills an estimated 275,000 women every year and 500,000 new cases are reported worldwide. Recent data released by India's Health Ministry based on the National Cancer Registry Programme (NCRP) report in 2009 the number of cervical cancer cases were 101938 which has increased to 107690 in 2012. Indian women face a 2.5% cumulative lifetime risk and 1.4% cumulative death risk from cervical cancer. At any given time, about 6.6% of women in the general population are estimated to harbour cervical HPV infection. HPV serotypes 16 and 18 account for nearly 76.7% of cervical cancer in India. (Sankaranarayanan et al 2009) [3].
There are different methods of screening for cervical cancer. Cervical cytology (Papanicolou smear) is a well-known and successful screening programme in reducing cervical cancer incidence. In low resource setting, VIA has been evaluated as an alternative to cytology since it is easy to perform, low in cost, does not require sophisticated equipment and be carried by field workers. (Bandit et al; J Med Assoc Thai 2006) [4].

Aims and Objectives
We screened the cases by cytological testing and VIA (visual inspection of cervix with acetic acid) for squamous intraepithelial lesions of cervix. Cervical biopsy was taken prior to cryotherapy to identify types of squamous intraepithelial lesions. Cryotherapy was given in cases with abnormal squamous intraepithelial lesions. Then Cases were followed up for safety, effectiveness and acceptability at 6 weeks, 3 months and 6 months wherever possible.

Inclusion Criterion
- Women in reproductive age group.
- Women using birth control method.

- Women having suspicious lesions.
- Women having persistant discharge per vaginum/low back pain or lower abdominal pain/post coital bleeding.
- Women having suspicious cervical lesions.
- Women with abnormal PAP report/ HPV DNA positive patients.

Exclusion criterion:

- Women who had menopause.
- Women who had undergone hysterectomy.
- Women using IUCD.
- Pregnant and lactating women.

45 of these patients were subjected to cryotherapy.

Materials and Methods

Patients and Study Design

The present study was conducted in the department of obstetrics and gynaecology and pathology, of Jawaharlal Nehru Medical College and Hospitals, Aligarh and Dr. B.R. Ambedkar Centre for Biomedical Research (ACBR), Delhi University from October 2011 to August 2013.

Written informed consent was obtained from all the subjects included in the study and was carried out in accordance with the principles of the Helsinki Declaration and Clinico-epidemiological and demographic details were taken from their clinical records. The study was approved by Institutional Ethics Committee

Total number of patients i.e. 6503 attended to our gynaecological OPD, for various complaints, out of which 55 patients were selected, who presented with symptoms of vaginal discharge, pelvic pain, post coital bleeding and on the basis of clinical examination. Different screening modalities such as VIA, HPV DNA, Cytology, Colposcopy and histology were applied and these 55 patients were enrolled in the study. Patients were excluded on the basis of cytology (high grade lesions), who did not give consent to be the part of study, or were not convinced for regular follow up.

Screening of patients by VIA, Cytology and Colposcopy were done in department of Obstetrics and gynaecology, JNMCH Aligarh. Pap smear and biopsy reporting were done in department of pathology JNMCH, Aligarh. Human Papilloma virus DNA testing of paper smear samples by PCR technique was carried out in ACBR, Delhi.

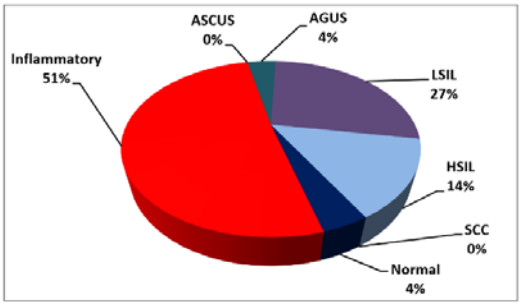
Data Analysis

Statistical analysis was performed by using the chi square test. P value was calculated.

Observation and Results

Cytological screening was done in 55 cases and it was found that 28(51%) were inflammatory, 15(27%) were LSIL, 2(8%) were AGUS, no ASCUS, 8(14%) were HSIL.

Graphical Representation 1

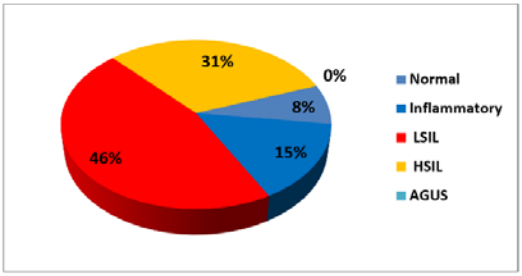


VIA positive cases were 38 cases. On cytology they showed 17(45%) inflammatory, 13(34%) were LSIL, were 8(21%) were HSIL.

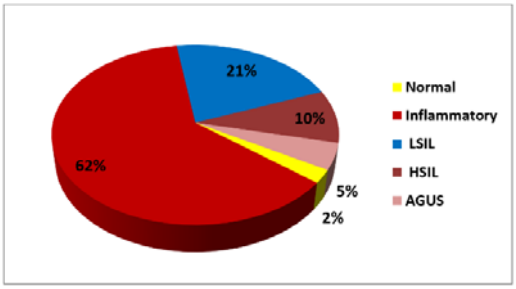
Among VIA negative cases 17, on cytology they showed 10(59%) inflammatory, 3 (17%) LSIL, 2(12%) AGUS. The p value came out to be 0.007, which was statistically significant. 31% VIA positive cases were HPV positive and 69% were HPV negative. 5% VIA negative were HPV positive and 95% cases were HPV negative. P-value is 0.038 which was statistically significant.

Co-Relation of HPV DNA Status & Cytology

Graphical Representation 2



HPV Positive



HPV Negative

2(15%) inflammatory smears were HPV positive, 6(46%) LSIL cytology were HPV positive, 4(11.1%) HSIL were HPV positive. P value is 0.028, which was statistically significant.

Table 1: Pre-treatment and Post treatment analysis of chief complaints in study (cryotherapy) group (n=45)

Chief Complaint	Pre Treatment (%)n=45	Post Treatment (%) n=45	P value
Pelvic pain	34(75)	13(28)	<0.001(significant)
Vaginal discharge	35(77)	16(35)	<0.001(significant)
Post coital bleeding	1(2)	1(2)	Not significant
Menstrual complaint	9(20)	7(15)	0.581(not significant)
No complaints	0(0)	25(56)	<0.001(significant)

Graphical Representation 3

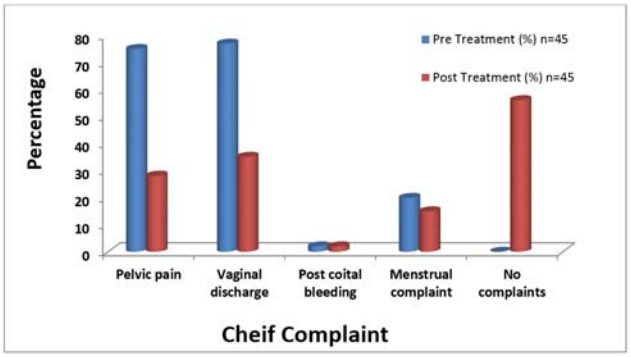


Table 1shows, 34(75%) cases had pelvic pain, 35(77 %) cases had vaginal discharge, 9(20%) had menstrual complaints and only 1(2%) had post coital bleeding. After treatment, pelvic pain persist in 13(28%) cases, vaginal discharge persist in 16(35%) ,menstrual complaints in 7(15%) cases, post coital bleeding in 1 and strikingly, 25(56%) had no complaints at all. 77% cases presents more than one complaints and 2% with more than two complaints. Upon application of chi-square p value <0.001 in pelvic pain and vaginal discharge which was statistically significant in comparison to other complaints like post coital bleeding and menstrual complaints.

Table 2: Pre and post treatment analysis of VIA in study (cryotherapy) group. (n=45)

Via status	Pre Treatment (%)	Post Treatment (%)	P value
Positive	32(71)	12(29)	<0.001(significant)
Negative	13(29)	33(71)	<0.001(significant)

Graphical Representation 4

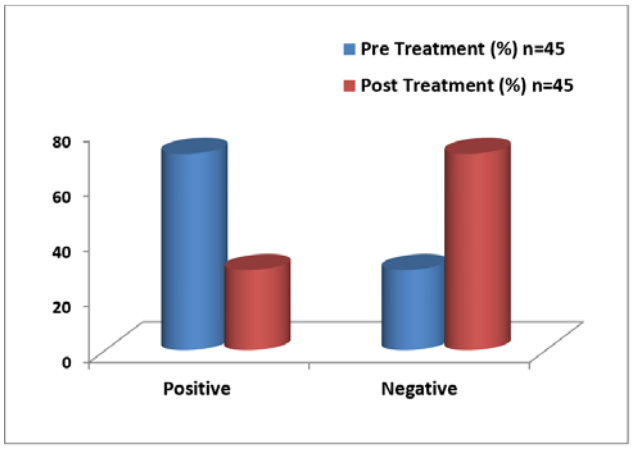


Table 2shows Pre-treatment VIA positive in study group were 32(71%) and VIA negative were 13(29%), after treatment only 12(29%) case remains VIA positive rest cases turned VIA negative. P value was significant statistically < 0.001.

Table 3: Cytological Analysis Pre & Post Treatment in Study (Cryotherapy) Group. (45)

Cytology	Pre Treatment (%)n=45	Post Treatment (%)n=45	P value
Normal	0	21(47)	<0.001(significant)
Abnormal	45	24(53)	

Table 3 Shows, pre-treatment 30(67%) cases had inflammatory smear, 11(24%) cases have LSIL cytology and 4(9%) case have HSIL. Post treatment none had LSIL& HSIL, 21(47%) converted to normal cytology and 24(53%) inflammatory smear. As the cases who had LSIL and AGUS initially after treatment turn normal or inflammatory. P value was 0.001, statistically significant.

Graphical Representation 5

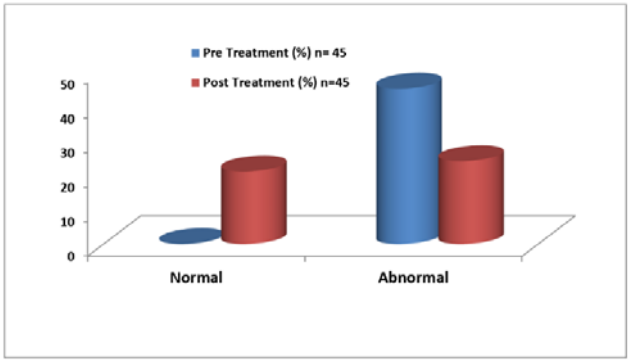


Table 4: HPV DNA status in case study (Cryotherapy) group (n=45)

HPV status	Pre treatment	Post treatment
Positive	11	3
Negative	34	34 +8

Graphical Representation 6

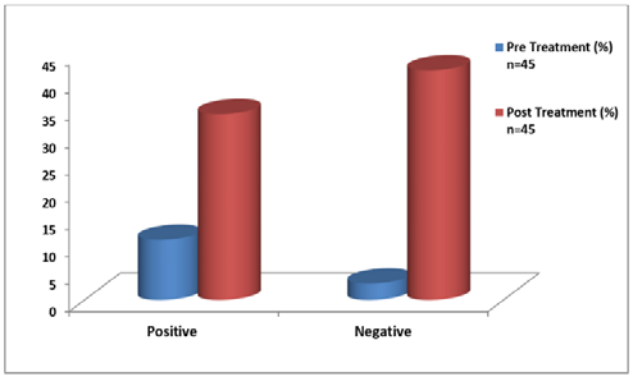


Table 4 shows pre and post treatment analysis of HPV status and cytology in cryotherapy group. 11 HPV positive cases were treated with cryotherapy and 8 cases turn negative showing P<0.001 which was clinically significant.

Table 5: Treatment Modalities Applied When Cryotherapy Treatment Failed (patient turn HPV negative but symptomatically not satisfied)

HPV	Repeat Cryo	Leep+Conisation
Positive	1	1
Negative	1	1

Table 5 shows, 2 cases underwent LEEP and Conisation one HPV negative and one positive due to AGUS on cytology. 2 Patients undergo repeat cryotherapy. Half of these were the cases which showed improvement in HPV infection clearance and cytology, but had vague symptoms of pelvic pain and cervical discharge due to hypertrophic cervix, or exposed cervical canal. Rest cases remained HPV positive and repeat cryotherapy was done due to big lesion size.

Discussion

India is the "global epicentre" of cervical cancer deaths. Cervical cancer being one of the most easily preventable cancer owing to the easy accessibility of the cervix to the clinician and easy screening methods available, is amenable to eradication one day. Owing to a substantial lag period of 10-20 years between the precancerous lesion and development of cervical carcinoma it is an easily preventable disease. A critical component of effective cervical cancer screening is the ability to offer women appropriate effective treatment for precancerous cervical lesions, thereby reducing overall cancer incidence and mortality.

In this present study, we have screened the high risk cases with VIA, cytology and HPV DNA testing by PCR method on paper smears, colposcopy and histopathology.

And precancerous lesions were treated with cryotherapy and followed up to know the Effectiveness, Safety and Acceptability of cryotherapy in clearing HPV infection and treating squamous intraepithelial lesions of cervix.

Cytology

In our study, on cytology 2(4%), were normal, 28(51%) were inflammatory, AGUS 2(4%), LSIL 15(27%) and HSIL 8(14%). In a study by (Denise et al 2009) [5], 403 adolescents between 1993 and 2006 showed aypical squamous cells of undetermined significance (ASCUS) in 5.5 % (22) of patients, LSIL 28% (113), HSIL in 3% (12).

Correlation of cytology findings with VIA

In our study, the prevalence of inflammatory smear was 45% in VIA positive and 59% in VIA negative group. Two patients had AGUS and both were VIA negative. Among the VIA positive cases, 34% were LSIL and among the VIA negative 17% were LSIL. All cases of HSIL were VIA positive. Thus sensitivity of VIA in our study is 68.72% and specificity is 70%. PPV is 49% and NPV is 61%. Thus according to our study LSIL can be missed by VIA but not HSIL.

Goel et al 2005 [6] found VIA to have a sensitivity of 96.7%, but the specificity is 36.4%. VIA is a sensitive method for ectocervical lesions, advantage of VIA is low cost and ease of use (can be used by paramedics). Its high sensitivity and immediate results (it is possible to see and treat approach at first visit). Main limitation is high false positive rates may lead to over treatment if a see and treat approach is applied.

Gravitt et al 2010 [7] found sensitivity of VIA to be 31.6%; specificity 87.5%. Despite use of available resources, infrastructure, and guidelines for cervical cancer screening implementation in resource limited areas, community participation and non-compliance remain the major obstacles to successful reduction in cervical cancer mortality in this Indian population. HPV DNA testing was more specific than Pap cytology and VIA. The use of a less invasive and more user-friendly primary screening strategy (such as self-collected swabs for HPV DNA testing) may be required to achieve the coverage necessary for effective reduction in cervical cancer mortality.

The above two mentioned prospective studies were done on Indian population, from Delhi and Andhra Pradesh respectively, and a drastic disparity is seen in the results in terms of sensitivity and specificity of VIA.

In our study, sensitivity of VIA was 68.72% and specificity of VIA was 70%. More over sensitivity and specificity of VIA was calculated in relation to cytology which is not a gold standard test for detection of cervical lesions because cytology had its own limitation, which is subjected to high inter-observer and intraobserver error. VIA can be applied as a screening test in low resource setting, because of its low cost and low level of expertise, but where facilities are available, it should always be coupled with cytology and HPV DNA testing, as they are more sensitive and specific than VIA alone. VIA seems to had low accuracy in differentiating cervicitis and low risk cervical intraepithelial lesion. VIA may had high accuracy in detecting high grade lesions.

Correlation of HPV and VIA status

In our study, 31% VIA positive cases were HPV positive and 69% were HPV negative. 5% VIA negative were HPV positive and 95% cases were HPV negative. P-value is 0.038 which is statistically significant. In our study, we found sensitivity of VIA for detection of HPV infection to be 92.31%, specificity 38.10%, PPV 31.58%, NPV 94.12%, Though no literature is available to compare our result with other studies, but it is inferred from our study that, women who were VIA negative and HPV DNA positive belongs to age group >30 years, that's why HPV DNA screening is recommended in this age group as VIA negative will not exclude HPV DNA negativity.

Correlation of HPV and cytology

In our study, 2(15%) inflammatory smears were HPV positive, 6(46%) LSIL cytology were HPV positive, 4(11.1%) HSIL were HPV positive. P value is 0.028, which is statistically significant. Sensitivity 43.48%, specificity 93.62%, PPV 76.92%, NPV 69.05%. If we compare the accuracy of VIA and HPV DNA testing, then HPV testing is more accurate. Nuovo et al 2000 [8] Compared with screening by cytology alone, double testing with cytology and for type-specific HPV persistence resulted in a 35% (95% confidence interval [CI] = 15% to 60%) increase in sensitivity to detect CIN3+, without a statistically significant reduction in the PPV (relative PPV = 0.76, 95% CI = 0.52 to 1.10). Several strategies that incorporated screening for high-risk HPV subtypes were explored, but they resulted in reduced PPV compared with cytology. Compared with cytology, primary screening with HPV DNA testing followed by cytological triage and repeat HPV DNA testing of HPV DNA–positive women with normal cytology increased the CIN3+ sensitivity by 30% (95% CI = 9% to 54%), maintained a high PPV (relative PPV = 0.87, 95% CI = 0.60 to 1.26), and resulted in a mere 12% increase in the number of screening tests (from 6257 to 7019 tests). It concludes that Primary HPV DNA–based screening with cytology triage and repeat HPV DNA testing of cytology-negative women appears to be the most feasible cervical screening strategy.

In a study by Gravitt et al 2010 HPV testing had a higher sensitivity (100%) and specificity (90.6%) compared to Pap cytology sensitivity (78.2%) specificity (86.0%)

Pretreatment and post treatment analysis of chief complaints in study (cryotherapy) group. In our study we found 60% were relieved of pelvic pain and discharge per vaginum. Menstrual complaint were only relieved in 22% of cases as the cause for abnormal vaginal bleeding were due to other reasons. Symptom of post coital bleeding in patients with low grade intraepithelial lesions was 2.2%, which was not relieved initially after treatment with cryotherapy. P value was significant in complaints of vaginal discharge and pelvic pain.

Pre and post treatment analysis of VIA in study (cryothearpy) group. In our study, in cryotherapy group 45 cases were treated with cryotherapy. 32 cases were VIA positive before treatment and after treatment 20 of these turned VIA negative.

When P value calculated for this, it was <0.001 and was significant.

Cytological Analysis Pre and post treatment analysis in study (cryothearpy) group. Pre-treatment 30(67%) cases had inflammatory smear, 11(24%) cases have LSIL cytology and 4(9%) case have HSIL. After giving cryotherapy treatment none had LSIL& HSIL, 21(47%) converted to normal cytology and 24 (53%) inflammatory smear. As the cases who had LSIL and AGUS initially after treatment turn normal or inflammatory. P value was 0.001, statistically significant. 2 Patients undergo repeat cryotherapy. Half of these were the cases which showed improvement in HPV infection clearance and cytology, but had vague symptoms of pelvic pain and cervical discharge due to hypertrophic cervix, or exposed cervical canal. Rest cases remained HPV positive and repeat cryotherapy is done or planned due to big lesion size. Possibility of persistent source of infection (infected partner) cannot be ruled out.

Ferency (1985) [9] demonstrated that if disease in the endocervical canal is treated with cryotherapy, the failure rate is higher.

The most common cause of treatment failure are an inadequate freeze because of low gas pressure, poor probe application, insufficient freeze time, large three or four quadrant lesions or extension of disease into end ocervical canal.(Morrow 1987) [10].

Cryotherapy is usually effective in the management of CIN because it usually destroys to a depth of 5 mm as well as 5 mm lateral to the edge of cryoprobe. (Boonstra H, 1990) [11].

HPV DNA status in study (Cryotherapy) group

In our study 11 HPV positive patients were given cryotherapy treatment. 8 out of 11 patients turned to be HPV DNA negative. 3 patients are in close follow up. Post treatment HPV DNA turned negative with significant with P<0.001. Prusty & Das 2004 [12] studied that, the transcription factor AP-1 plays a central role in the transcriptional regulation of specific types of high risk HPVs such as HPV 16 and HPV 18, which are etiologically associated with the development of cancer of the uterine cervix in women.

Conclusion

Cancer of the cervix has been the most important cancer in women in India over the past two decades. Most cases are found in women younger than 50. The most important risk factor for cervical cancer is infection by the human papillomavirus (HPV). In our study, it has been observed, that HPV DNA detection was an important tool in cervical cancer screening. Its specificity and accuracy was much higher than cytology and VIA. Colposcopy is sensitive but needs expertise. (ASCP Cervical Cancer Guideline Committee (2012), American Cancer Society, American Society for Colposcopy and Cervical Pathology) [13]

Our study shows cervical cancer is a disease of lower income group primarily which can be prevented by nutrition, hygiene and most important by counselling the people for use of contraception and the long pre invasive phase of disease in which patients can turn up for testing and medical or surgical treatment.

According to ACCP [14] Cryotherapy is a simple, less expensive and OPD based procedure. It along with VIA for diagnosis can be used in fields. Patients treated with cryotherapy cure rate can be high with proper technique, training and adequate pressure of gas. Thus in low resource setting, simple and safe diagnostic and therapeutic procedures like VIA, HPV DNA, cryotherapy may be the only way to combat the killer disease.

Unfortunately, due to non-compliance on the part of patient follow up has been a major problem, as many of them visited till they had problem. Some were unable to come either they were unaware of the importance of the follow up or have social or monitory problems. It can be reduced by appropriate counselling and information. However patients were tried to contact telephonically to come for follow up and asked about the symptoms.

This is a primary study and too small, to come to definite conclusion at the moment. More studies need to be conducted on a larger scale to come to a definite conclusion.

Take Home Message

Cancer of the cervix has been the most important cancer in women in India over the past two decades. Most cases are found in women younger than 50. (Government of India / WHO collaboration programme, June 2006) [15]. Cryotherapy is a simple, less expensive and OPD based procedure. It along with VIA for diagnosis can be used in fields. Patients treated with cryotherapy cure rate can be high with proper technique, training and adequate pressure of gas. Thus in low resource setting, simple and safe diagnostic and therapeutic procedures like VIA, HPV DNA, cryotherapy and curcumin may be the only way to combat the killer disease.(Cervical Cancer Prevention Guidelines for Low-Resource Settings, Baltomore, MD: JHPIEGO Corporation (July 2000) [16].

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