Diagnosing Abnormal Intraepithelial Lesion of Cervix to Screen for Cervical Cancer

Dr. Uma Jain, Dr. Aditya Jain, Dr. Dilip Jain

Abstract: Introduction: cervical cancer is the most common genital cancer among women in India. Cervical intraepithelial neoplasia (CIN) is a premalignant transformation and abnormal changes in squamous cells on the surface of the cervix. The precursor can be readily detected by a painless, reproducible, and reasonably accurate screening test, the Pap smear, which has been the basis for the significant decrease in cervical cancer mortality. Aim of the study: The role of pap smear in detecting premalignant and malignant lesions of the cervix and to determine the prevalence of various lesions. Materials and methods: this was a retrospective study done in a private clinic and pathology lab in district Shivpuri (MP). The study was done during the period from 1st October 2018 to 30th September 2020. Result: In this study out of 500 cases, 2.8 % of cases were reported as unsatisfactory. Non-neoplastic smears were reported in (87.2) %, out of which, Inflammatory smear was 77.2 % normal smear were 6.8 % and atrophic smear was 0.4 %. Epithelial cell abnormalities were found in 10 % of cases. The most common epithelial abnormalities was ASCUS 4.8 % followed by LSIIL 3 %, ASCH 1.2 %, AGUS 0.4 %, HSIL 0.4 % and SCC 0.2 %. IN our study the epithelial abnormalities were most commonly found in the patient of 41-50 years of age group. Conclusion: Premalignant lesions of the lower genital tract can be picked up in routine screening. Early diagnosis of cancer in the pre-invasive state has a better prognosis and advanced-stage morbidity is prevented. Pap smear is cheaper as compared to cancer treatment in the long run.

Keywords: Epithelial cell abnormalities, cervical cancer, Papanicolaou Pap smears

1. Introduction

Cervical cancer is the second most common cancer in women worldwide after breast cancer. Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease.

Cervical cancer is responsible for 25.9% of all cancer cases and 23.3% of cancer deaths among Indian women. Cervical cancer can be prevented as it is easy to identify and treat its precursor lesions in most cases.

Cervical intraepithelial neoplasia (CIN) is a premalignant transformation and abnormal changes in squamous cells on the surface of cervix. CIN is believed to originate in transformation CIN. The Transformation zone is susceptible to Oncogenic Factors especially during menarche or after pregnancy when the metaplasia is most active. Oncogenic factors are introduced at sexual intercourse. The main etiological agent is human papillomavirus (HPV) (16, 18, 31, 33) infection. Other agents may play & secondary role and are 1. Infections: HIV infection, Herpes simplex, Chlamydia, Trichomoniasis and others, 2. Early sexually intercourse (<16years), 3.Early age of first pregnancy, 4. Multiple sex partners, 5. Multiparty, 6. Dietary deficiency of Vit A, C, E, folic acid, 7. Oral contraceptives users, 8. Smoking, 9. Low socioeconomic conditions, 10. Immunosuppressed individuals.

Precancerous changes of the cervix usually do not cause the pain or any other symptoms and are not detected unless a woman undergoes Screening. Symptoms generally do not appear until abnormal cervical cells become cancerous and invade nearby tissue. The most common symptoms are Abnormal vaginal bleeding or Bleeding or spotting between periods, Vaginal bleeding after menopause, Vaginal bleeding after sex, Longer or heavier menstrual periods than usual, abnormal vaginal discharge, pain during sexual intercourse, urinary symptoms or backache. Various methods are available for screening of cervical cancer.

Cytology based screening programme is the mainstay of early detection and prevention of cervical cancer.

Papanicolaou Papanicolaou [Pap] test developed by George Papanicolaou in 1950. Pap test is used primarily as a tool for screening healthy women for pre-invasive cervical cancer (CIN) and early invasive cancer. Screening for cervical cancer using the paps test was successful in reducing the incidence of cervical cancer by 79% and mortality by 70%. According to different studies, cytology has a sensitivity that varies from 47 to 62% and specificity between 60 to 95% for the detection of high-grade cervical intraepithelial neoplasia (CIN2/CIN3), Errors of sampling, fixation, interpretation and follows up may be responsible for missed cases.

Liquid-based cytology is a thin layer slide preparation technology and thus gives better morphological assessment. This will alleviate errors in sampling and preparation and increase the sensitivity to the original goal of 80%.

Automated computerized image processor: It eliminates 25% of most likely negative smears and 75% are selected for cyto technician screening.

Cervicography: this technique was described in the 1990s where a photograph of the cervix is taken and sent for evaluation.

Visual inspection with acetic acid (3-5%) (VIA) and Visual inspection with Lugol's iodine (VILI): In Indian scenario VIA and VILI test for screening of cancer cervix and pickup of pre-invasive disease is proving to be highly sensitive but less specific and very cost-effective as compared to Pap smear. The sensitivity of VIA ranges 66 and 96% and specificity between 64 and 98% . This is as good as or better than conventional cytology the sensitivity

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and specificity of VILI was 87.2% and 84.7%, respectively as noted in a cross-sectional study involving 4444 women.\textsuperscript{9}

**Colposcopy** was introduced by Hinselmann in 1972. The purpose of colposcopy is to detect abnormal areas so that selective biopsy can be done. It is not done routinely in all patients. Only patients with positive cervical cytology for malignant cells or suspicious cells with a normal looking cervix need a colposcopy.\textsuperscript{10} Conservative surgery and its follow up can be done under colposcopic guidance. Abnormal areas appear under colposcopy as acetowhite areas, mosaics, punctuation and abnormal vessels. For final diagnosis, any one of the biopsy techniques e.g. cervical punch biopsy, wedge biopsy, loop biopsy or cone biopsy can be used.

**Cone Biopsy** is both diagnostic and therapeutic which can be conventional wedge biopsy or loop electrosurgical excision procedure (LEEP).\textsuperscript{11}

An **endocevical curetage (ECC)** is indicated when the lesion extends up the endocervical canal or if the upper extent of the lesion cannot be visualized.

**HPV Testing**
HPV Testing is an ideal primary screening modality for cervical cancer. This is attributed to its high sensitivity coupled with a high negative predictive value.\textsuperscript{12}

**Co-Testing With HPV and Cytology**
Most countries with established cytology screening programs first introduced HPV testing as a co-test alongside with cytology.

**Viral Oncoprotiens and Biomarkers**
Viral E6 and E7 oncoproteins are necessary for malignant conversion the abilities of high-risk HPV E6 and E7 associated with the tumour suppressors p53 and pRB, respectively have been suggested as a mechanism by which these viral proteins induce tumours.\textsuperscript{13} P16 and Ki-67 have emerged as important biomarkers for the detection of high-risk human papillomavirus and in confirming the histopathological diagnosis.\textsuperscript{14} AgNOR is a new molecular tumour marker which stands for silver-stained nuclear organizer regions.\textsuperscript{15}

**Screening Protocol:** \textsuperscript{11,15,16}
- When to start: >21 years, Repeat every – 3 yearly, if co-testing (pap smear + HPV DNA testing) – 5 yearly (HPV DNA testing should begin >30 years of age)
- When to stop screening – 65 years (if regular screening in the past 10 years)
- If the total hysterectomy was done for benign causes other than CIN / Cervical cancer – no Screening
- Women who have had a subtotal hysterectomy still have a cervix – Screened regularly.
- HIV positive women – Screened annually
- Vaccinated women – Screened regularly.

The **terminology** has been changed over the years. The terminology, carcinoma in situ (CIS) was introduced by Rubin (1910), ‘Dysplasia’ by Walters and Regan (1956) and cervical intraepithelial neoplasia (CIN) by Richart (1967). WHO (1975) redefined CIN into three categories.\textsuperscript{16} Persistent infection with high-risk HPV types may lead to precursor lesions of the cervix, referred to as CIN, which is epithelial cellular change, where the ratio nucleus to the size of the cell is increased. CIN is graded as CIN 1 (mild), CIN 2 (moderate), or CIN 3 (severe) depending on the proportion of thicness of the epithelium showing mature, differentiated and undifferentiated cells. Most of the CIN 1 and 2 lesions regress spontaneously; however, untreated in which 4% reach the invasive stage by 1 year, 11% by 3 years, 22 % by 5 years and 30% by 10 years. CIN is now replaced with the low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL)\textsuperscript{7}

The Bethesda System (TBS) in 1988 and modified in 2014, introduced a uniform terminology for reporting Pap test results (Table 1)\textsuperscript{17}

<table>
<thead>
<tr>
<th>Table 1: The 2014 Bethesda System</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECIMEN TYPE:</td>
</tr>
<tr>
<td>Indicate conventional smear(Pap smear) vs. liquid-based preparation vs. other</td>
</tr>
<tr>
<td>SPECIMEN ADEQUACY</td>
</tr>
<tr>
<td>• Satisfactory for evaluation (describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g. partially obscuring blood, inflammation, etc.)</td>
</tr>
<tr>
<td>• Unsatisfactory for evaluation… (specify the reason)</td>
</tr>
<tr>
<td>• A specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify the reason)</td>
</tr>
<tr>
<td>GENERAL CATEGORIZATION (optional)</td>
</tr>
<tr>
<td>• Negative for Intraepithelial Lesion or Malignancy</td>
</tr>
<tr>
<td>• Other: See Interpretation/Result (e.g. endometrial cells in a woman≥45 years of age)</td>
</tr>
<tr>
<td>• Epithelial Cell Abnormality: See Interpretation/Result (specify ‘squamous’ or ‘glandular’ as appropriate).</td>
</tr>
<tr>
<td>INTERPRETATION/RESULT</td>
</tr>
<tr>
<td>NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY</td>
</tr>
<tr>
<td>(when there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report whether or not there are organisms or other non-neoplastic findings)</td>
</tr>
<tr>
<td>Non-Neoplastic Findings (optional to report)</td>
</tr>
<tr>
<td>❑ Non-neoplastic cellular variations</td>
</tr>
</tbody>
</table>

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For Prevention of cancer of cervix regular screening to find out any pre-cancerous lesion and treat them, and to get the HPV vaccine if the patient is eligible is a must. According to CDC recommended age for HPV vaccination is 11-12 years (before sexual activity) and can be given between 9 to 26 years.

2. Materials and Methods

This was a retrospective study done in a private clinic and pathology lab in district Shivpuri (MP) the study was done during the period from 1st October 2018 to 30th September 2020.

Inclusion criteria:- Patients complaining of vaginal discharge, post-coital bleeding dyspareunia intermenstrual bleeding postmenopausal bleeding were included in this study the age of the patients was between from 21 to 65 years.

Exclusion criteria: Women with invasive cervical cancer and those who are not giving consent for pap smear were excluded from the study.

In this study, the results of pap smear collected from 500 women were analyzed. Data were collected from the records and reports from the clinic and pathology lab. Data were
searched for detailed history, personal information for each case, literacy, socio-economic status, parity, various complaints, any associated high-risk factor, clinical finding and other relevant information. Reporting was according to the guideline of 2014 Bethesda system.

**Technique**
No vaginal examination was performed before taking the smear. Women were advised to abstain from intercourse, douching and any medicinal creams for at least 24 hours before the test, the patient is placed in the dorsal position and a Cusco speculum is inserted. Sample was taken with Ayre’s spatula and cytobrush from transformation zone and end cervix, Slides are made, spray fixation was done and send to the pathology lab.

A total of 500 cases were analyzed during a period of two years. Different cases were studied and all the data were analyzed using IBM SPSS ver.20 software. Frequency distribution and cross-tabulation was used to prepare tables, data is expressed as a percentage.

### 3. Results

#### Table 2: Socio-demographic characteristics the age of women ranged from 21 to 75 years

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>No. of cases</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;21-30</td>
<td>42</td>
<td>8.4</td>
</tr>
<tr>
<td>31-40</td>
<td>209</td>
<td>41.8</td>
</tr>
<tr>
<td>41-50</td>
<td>153</td>
<td>30.6</td>
</tr>
<tr>
<td>51-60</td>
<td>72</td>
<td>14.4</td>
</tr>
<tr>
<td>&gt;61</td>
<td>24</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
<td>416</td>
<td>83.2</td>
</tr>
<tr>
<td>Urban</td>
<td>84</td>
<td>16.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital Status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>497</td>
<td>99.4</td>
</tr>
<tr>
<td>Unmarried</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>18</td>
<td>3.6</td>
</tr>
<tr>
<td>Primiparous</td>
<td>54</td>
<td>10.8</td>
</tr>
<tr>
<td>Multiparous</td>
<td>428</td>
<td>85.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Educational level</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Illiterate</td>
<td>23</td>
<td>4.6</td>
</tr>
<tr>
<td>Primary</td>
<td>59</td>
<td>11.8</td>
</tr>
<tr>
<td>Middle</td>
<td>57</td>
<td>11.4</td>
</tr>
<tr>
<td>HSC</td>
<td>258</td>
<td>51.6</td>
</tr>
<tr>
<td>Graduation</td>
<td>77</td>
<td>15.4</td>
</tr>
<tr>
<td>Post Graduation</td>
<td>26</td>
<td>5.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 2 shows in our study most of the women were of 31 to 40 year 41.8% age group, most of them were rural 83.2 and married 99.4% of women were multiparous 85.6%.

Out of 500 cases, 87.2% (436) patients were reported as negative for the intraepithelial lesion. 6.8% were reported as normal (NILM) The most common non-neoplastic cytological finding was inflammatory 77.2% out of which nonspecific inflammation 52.2%, chronic erosive cervicitis 25% and other inflammatory lesion were 2.8%.

![Figure 1: Presenting Symptoms](image1)

Fig. 1 shows that the most common presenting symptoms were abnormal vaginal discharge. 26.4%

![Figure 2: Per speculum examination findings of the cervix](image2)

The most common finding on per speculum examination was healthy-looking cervix 35.8% followed by unhealthy with erosion 26.4%.

#### Table 3: Non-neoplastic cytological diagnosis in pap smear (436 cases)

<table>
<thead>
<tr>
<th>Non-neoplastic cytological diagnosis</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NILM Normal</td>
<td>34</td>
<td>6.8%</td>
</tr>
<tr>
<td>Atrophy</td>
<td>2</td>
<td>0.4%</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>386</td>
<td>77.2%</td>
</tr>
<tr>
<td>Nonspecific inflammation</td>
<td>261</td>
<td>52.2%</td>
</tr>
<tr>
<td>Chronic erosive cervicitis</td>
<td>125</td>
<td>25%</td>
</tr>
<tr>
<td>Others</td>
<td>16</td>
<td>2%</td>
</tr>
<tr>
<td>Squamous metaplasia</td>
<td>4</td>
<td>0.8%</td>
</tr>
<tr>
<td>Gardnerella</td>
<td>6</td>
<td>1.2%</td>
</tr>
<tr>
<td>Trichomonas</td>
<td>2</td>
<td>0.4%</td>
</tr>
<tr>
<td>Candida</td>
<td>2</td>
<td>0.4%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>436</td>
<td><strong>87.2%</strong></td>
</tr>
</tbody>
</table>
ASC-US: Atypical squamous cells of undetermined significance; ASC –H: Atypical squamous cells; that cannot rule out high- grade lesion; AGUS Atypical glandular cell of undetermined significance LSIL: Low – grade squamous intraepithelial lesion HSIL: High grade squamous intraepithelial lesion; SCC: Squamous cell carcinoma;

In our study, 2.8% of cases were reported as unsatisfactory. 10% of reports showed epithelial abnormalities. The most common epithelial abnormalities was ASCUS 4.8% followed by LSIL 3%, ASCH 1.2%, AGUS 0.4%, HSIL 0.4% and SCC 0.2%.

Table 4: Categorization of epithelial abnormalities based on Cyto diagnosis

<table>
<thead>
<tr>
<th>Cytodiagnosis</th>
<th>No. of cases</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>14</td>
<td>2.8</td>
</tr>
<tr>
<td>Epithelial cell abnormalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC-US</td>
<td>24</td>
<td>4.8</td>
</tr>
<tr>
<td>ASC-H</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td>AGUS</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>LSIL</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>HSIL</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>SCC</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Total Epithelial cell abnormalities</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

ASC-US: Atypical squamous cells of undetermined significance; ASC –H: Atypical squamous cells; that cannot rule out high- grade lesion; AGUS Atypical glandular cell of undetermined significance LSIL: Low – grade squamous intraepithelial lesion HSIL: High grade squamous intraepithelial lesion; SCC: Squamous cell carcinoma;

In our study, the epithelial abnormalities were most commonly found in Perimenopausal (41-50) age group. The most common age of the patient in our study for ASCUS 21-30 years, LSIL was 31- 40 years, HSIL was 41-60 years, SSC more than 60 years.

Table 5: Age-wise distribution of epithelial cell abnormalities

<table>
<thead>
<tr>
<th>Epithelial abnormalities</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>60+</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>24.4</td>
</tr>
<tr>
<td>ASC –H</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>6.2</td>
</tr>
<tr>
<td>AGUS</td>
<td>--</td>
<td>1</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>2.4</td>
</tr>
<tr>
<td>LSIL</td>
<td>0</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>15.3</td>
</tr>
<tr>
<td>HSIL</td>
<td>--</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>SCC</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 6: Various parameters of pap smear in the present study compared with other studies.

<table>
<thead>
<tr>
<th>Cytodiagnosis</th>
<th>Present Study</th>
<th>Tejaswini et al</th>
<th>Kalyani et al</th>
<th>Chandni B Patel</th>
<th>Saravani P</th>
<th>Eka Rani</th>
<th>Shaki O</th>
<th>Bal MS et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>2.8%</td>
<td>0.95%</td>
<td>17.80%</td>
<td>8.9%</td>
<td>6.50%</td>
<td>1.73%</td>
<td>1%</td>
<td>-</td>
</tr>
<tr>
<td>AGUS</td>
<td>0.4</td>
<td>0.48</td>
<td>0.24</td>
<td>0.4</td>
<td>0.10</td>
<td>0.06</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>LSIL</td>
<td>3</td>
<td>0.95</td>
<td>0.24</td>
<td>0.6</td>
<td>2.43</td>
<td>0.06</td>
<td>6.8</td>
<td>2.7</td>
</tr>
<tr>
<td>HSIL</td>
<td>0.4</td>
<td>1.43</td>
<td>0.41</td>
<td>1.2</td>
<td>1.26</td>
<td>0.06</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>SCC</td>
<td>0.2</td>
<td>0.48</td>
<td>0.41</td>
<td>0.1</td>
<td>0.48</td>
<td>0.43</td>
<td>2.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

In our study, the epithelial abnormalities were most commonly found in the age group of (41-50) which was consistent with the study of J Mishra 23 in which Cytopathological changes were mostly associated with the onset of menopause But our study was not consistent with the study of Geethu G. Nair 22 in their study the most frequent epithelial abnormalities was LSIL 2.7%.

In our study, the epithelial abnormalities were most commonly found in the age group of (41-50) which was consistent with the study of J Mishra 23 in which Cytopathological changes were mostly associated with the onset of menopause But our study was not consistent with the study of Geethu G. Nair 22 in their study the most frequent epithelial abnormalities was LSIL 2.7%.

5. Conclusion

Several tests can be used for screening of cervical cancer. The Pap smear (cytology) is the only test that has been used extensively and has been pivotal in reducing cervical cancer mortality and incidence. Other tests like VIA, VILI and HPV also hold immense potential but there isn’t significant evidence on their effectiveness. Some researchers are trying to study other tests in depth.

Cervical cancer should be diagnosed and treated at the pre-invasive phase at it lasts several years before it becomes invasive and incurable. Early diagnosis of cancer in the pre-invasive state has a better prognosis and advanced-stage morbidity is prevented. Cytology based screening program is still the mainstay in the prevention of cervical cancer. Pap test is the most effective, very simple, easy to perform, painless, cost-effective, reproducible and reasonably accurate screening test to detect cervical epithelial lesions.

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and thus helpful in reducing the morbidity and mortality of cervical cancer.

Medical Doctors Nursing Staff ANM Asha Anganwadi Worker and other health Workers Should be made aware about importance of Pap smear. Health Checkup camps should be conducted in order to raise awareness about Pap smear test and cervical cancer prevention.

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