Sentinel Lymph Node Detection Using Methylene Blue in Iranian Patients with Early Stage Cervical Cancer

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ABSTRACT

Cervical cancer is one of the most common diseases in women and is associated with high mortality. The aim of this study is to evaluate the feasibility of Sentinel Lymph Node (SLN) detection in patients with cervical cancer using the methylene blue dye. Patients in the early stages of cervical cancer were enrolled in this study. Methylene blue, 4 mL, was injected into the cervical peritumoral area in 24 cases. Surgically removed lymph nodes were examined by pathology for the blue lymph nodes that were considered as SLNs. Sensitivity and specificity of this test are, respectively, 89% and 83%. Detection rate based on the different nodes with the highest rate of 37.5% was observed in obturator. Various studies have shown that concurrent use of methyl blue method has the highest rate of SLN identification.

Key words: Cervical cancer, SLN, sentinel lymph node, methyl blue

INTRODUCTION

Cervical cancer is one of the most common neoplastic that according to the World report, half-million new people are infected by this disease annually. Prevalence of Cervical cancer is significantly high after breast cancer (Franco et al., 2003; Anderson and Simonot, 2001). It affects about 16 per 100,000 women per year and kills about 9 per 100,000 per year (IARC., 2002). In this disease both genetic and environmental factors, such as smoking, parity, hormone therapy, nutrition and infection are effective. Although, Human papillomavirus (HPV) infection appears to be a necessary factor in the development of almost all cases of cervical cancer (Madeleine et al., 2001; Merill et al., 2005; Moreno et al., 2002). Based on studies, cervical cancer is the sixth most common cancer in women in united state. So, it has a high prevalence in developed countries (Krivak et al., 2007). In 1998, about 12,800 women were diagnosed in the US and about 4,800 died (Canavan and Doshi, 2000). In the Iranian population, the prevalence of cervical cancer is about 0.18-1.02 (Jafari et al., 2008; Talebi and Poor, 1999).

General and local extensions are two important ways for the cervical cancer to metastasize, although distant metastasis is most frequently in advanced stages. So, local treatment, such as surgery and radiotherapy, has been the main management of cervical cancer. Overall, the incidence of pelvic lymphatic metastasis in patients with stages I and II are in the range of 16-0 and 31-25%, respectively (Malur et al., 2001).
Generally lymphadenectomy for patients without metastatic lymph nodes are not necessary due to complications from surgery. Sentinel Lymph Node (SLN) is the first node to be involved in lymphatic spread. The pathologic status of this specific node presumably reflects the overall status of the whole regional drainage area. Surgical decisions and treatment based on SLN for reproductive cancers, breast and melanoma has been associated with great success (Morton et al., 1999; Giuliano et al., 1994; Veronesi et al., 1997; Ung, 2004; Sandelimm, 2004; Ikeda et al., 2004; Leong, 2004; Levenback et al., 1995, 2001; Moore et al., 2003a, b).

Since 2000, the use of this method for cervical cancer was studied. Methyl blue was used initially (Medl et al., 2000; Dargent et al., 2000; O'Boyle et al., 2000; Dargent and Ennia, 2003) then radiocolloid (Kamprath et al., 2000; Lanttsch et al., 2001; Martinez-Palones et al., 2004; Angiolia et al., 2005). The studies indicated that hybrid model has been verified and effective (Malur et al., 2001; Verheijen et al., 2000; Levenback et al., 2002; Plante et al., 2003; Buist et al., 2003; Lambaudie et al., 2003; Barranger et al., 2004; Niikura et al., 2004; Gil-Moreno et al., 2005). In these methods, patients should be in the early stages of cancer (Levenback et al., 2002; Plante et al., 2003; Buist et al., 2003; Lambaudie et al., 2003; Barranger et al., 2004; Niikura et al., 2004; Gil-Moreno et al., 2005).

In developing countries, due to the high incidence of cervical cancer has been attempted on such a low cost method to be used for diagnosis. So methyl blue instead of radiocolloid has been investigated in several studies. Use of radiocolloid is time consuming and costly (O'Boyle et al., 2000; Martinez-Palones et al., 2004; Wang et al., 2004).

The response rate of methyl blue is not high but many studies indicated that if there is proper injection rate can be associated with an appropriate response (Dargent et al., 2000). Various colored powder has been used in different countries. So according to studies we can say, respectively patent blue, isosulfan blue, methylene blue used in Europe, USA, China (Franco et al., 2003; Veronesi et al., 1997; Ung, 2004; Medl et al., 2000; Dargent et al., 2000; O'Boyle et al., 2000; Kamprath et al., 2000; Martinez-Palones et al., 2004). So the aim of this study is SLN examination by methyl blue.

MATERIALS AND METHODS

In this cross-sectional study, from 2012-2014, 25 patients with early stage (I-IIA) cervical cancer diagnosed in the oncology units of Firuzoogar and Baghiollah hospitals (Iran) were enrolled. All patients undergoing radiotherapy were excluded so one patient was excluded of study. And, we excluded patients treated with neoadjuvant and enlarged lymph nodes. The study was performed after approval by the Scientific and Ethical Committee of Iran University of Medical Sciences and explained to the patients before surgery. Informed consent was obtained from every patient enrolled in this study. Before surgery, under direct observation, 4 mL methylene blue was injected into the cervix peritumorally (mainly at 3, 6, 9 and 12 o’clock position) in cases. In each of these parts 0.5 cc superficial and 0.5 cc deep was injected. Interval between the injection and the incision was between 20-35 min. Pelvic lymphadenectomy was carried out. All blue nodes were considered sentinel nodes through intraoperative direct inspection. The number and location of SLNs were documented. All removed nodes were sent for pathology testing. So, the pathologic diagnosis was as the gold standard in this study. All removed nodes were examined through routine hematoxylin and eosin (H and E) staining. And also for more information, immunohistochemistry was another test that using in this examination.

SLNs step-sectioned at the interval of 1 mm and the non-sentinel lymph nodes (non-SLNs) removed from 41 patients, were examined through routine hematoxylin and eosin (H and E) staining. For SLNs diagnosed as tumor-free by routine H and E staining, one additional slide of each SLN was immunostained using an anticytokeratin antibody (ZM-0069, Zymed, USA). In the other 40 patients, only routine H and E staining (one or two sections per node) was done for all removed nodes.

**Statistical analysis:** Statistical analysis was performed using SPSS software (version 18.0). The p-value<0.05 was considered statistically significant. The following equations were used:

\[
\text{Detection rate} = \frac{\text{Patients with successful SLN identification}}{\text{Patients enrolled}}
\]

Positive Predictive Value (PPV) = \(\frac{\text{No. of patients (methyl blue and pathology)}}{\text{No. of patients with positive test (methyl blue)}}\)

Negative Predictive Value (NPV) = \(\frac{\text{No. of healthy subjects (methyl blue and pathology)}}{\text{No. of subjects with a negative test (methyl blue)}}\)

Limitations of this study are the lack of access to patients with inclusion and exclusion criteria, time-consuming and costly process.

RESULTS

Clinical characteristics of the studies population are presented in Table 1. The median and range of patients age was 10.24±44.50 and 29-65 years old, respectively. Average tumor size is 1.06±3.46. And tumor size was varied from 1-5 cm. Also the average time between injection and surgery was 27.8±4.67 min.

Reproductive characteristics of the study population are shown in Table 1.

The parity was divided in two groups (≤4 and >4). About 54.2% women had children more than 4.

As you can see in Fig. 1, 37.5, 33.3 and 29.2% of people are located in IB1, IB2 and IIA1, respectively.
Fig. 1: Rates in different staging of tumor categories (%)

<table>
<thead>
<tr>
<th>Characteristic groups</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28-35</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>36-45</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td>46-55</td>
<td>10</td>
<td>41.7</td>
</tr>
<tr>
<td>&gt;56</td>
<td>6</td>
<td>25.0</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4 cm</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>&gt;4 cm</td>
<td>16</td>
<td>66.7</td>
</tr>
<tr>
<td>Staging of tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB1</td>
<td>9</td>
<td>37.5</td>
</tr>
<tr>
<td>IB2</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>IIA1</td>
<td>7</td>
<td>29.2</td>
</tr>
</tbody>
</table>

Reproductive characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity</td>
<td>7-2</td>
<td>4.33±1.66</td>
</tr>
<tr>
<td>Parity</td>
<td>7-2</td>
<td>3.96±1.73</td>
</tr>
<tr>
<td>abortion</td>
<td>2-0</td>
<td>0.40±0.64</td>
</tr>
<tr>
<td>Live child</td>
<td>6-2</td>
<td>3.90±1.60</td>
</tr>
</tbody>
</table>

Table 2: Rate of positive staining with methyl blue and pathologic response in different nodes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of positive responses methyl blue</th>
<th>Number of positive responses Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Right external iliac</td>
<td>6</td>
<td>25.0</td>
</tr>
<tr>
<td>Left external iliac</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>Right obturator</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td>Left obturator</td>
<td>10</td>
<td>41.7</td>
</tr>
<tr>
<td>Right inguinal</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Left inguinal</td>
<td>1</td>
<td>4.2</td>
</tr>
</tbody>
</table>

In total, people are categorized in 3 levels based on the coloring of SLN, one level without color, second level with single color and third level to more than one color.

There is no significant difference in the overall SLN detection efficiency and tumor size. Though there is no significant relation between overall detection rates and time injection until surgery too.

According to Table 2 and Fig. 2 the differences between rates of positive staining with methyl blue and pathologic response in different nodes is evident.

The results indicated that PPV in right external iliac, left external iliac, right obturator and left obturator lymph nodes was 33, 50, 40 and 90%, respectively. Overall, PPV of methyl blue testing in the evaluation of the SLN was 58%. And also, NPV in right external iliac, right obturator and left obturator lymph nodes was 100% and about left external iliac reported 87%. Totally negative predictive value is 97%. Sensitivity and specificity of this test are respectively 89 and 83%. Detection rate based on the different nodes with the highest rate of 37.5% was observed in obturator (Table 3).

**DISCUSSION**

Based on the results of studies the detection rate of SLN by blue dye has reported 83.3% (Di Stefano et al., 2005). In the present study, 24 patients were evaluated by methyl blue and the results indicated that the detection rate is the highest rate in obturator lymph node.

In various studies, doses of methyl blue has been considered as a variable and the results have shown that low doses of this substance can reduce the level of detection (Di Stefano et al., 2005; Sheng et al., 2004). It was experimentally observed that the full 4 cc injection of methyl blue, increased detection rate.

In several studies blue dye was injected into the cervix peritumorly, mainly at 2, 4, 8 and 10 o’clock position. In present investigation injection is done at 3, 6, 9 and 12 o’clock position. The results show that the injection in this position is
practical and comfortable. And also in these hour position, probability of injection in lymph nodes increased. In this study, the mean time injection of methyl blue to surgery is 27.8±4.67. And there is no significant relation between detection rate and this time. It is noteworthy, that the study was conducted have reported similar results (Di Stefano et al., 2005).

The highest detection rate observed in obturator lymph node that, the studies by Sheng et al. (2004) and Yuan et al. (2007) have reported similar results. Although, individual studies showed different results (Angiolia et al., 2005; Nikura et al., 2004; Gil-Moreno et al., 2005; Rob et al., 2005; Rhim et al., 2002).

In the present study, the sensitivity of this test was 89%. A study was conducted in New York by Beatrice Cormier the sensitivity of methyl blue was 87.5%. Generally, in several studies, the sensitivity of methyl blue varies from 77-100% (Cormier et al., 2011; Roy et al., 2011). Also, in evaluating the specificity of methyl blue, 83% was reported.

Various studies have shown that concurrent use of methyl blue and radioisotope methods has the highest rate of SLN identification (Roy et al., 2011). In the present study, due to the limitations just methyl blue has been investigated. Therefore, a further study with a mixed procedure is recommended.

In the present study, Enlarged and macroscopic lymph nodes were excluded. Because these kinds of nodes may be blocked by tumor cells, thus preventing the migration of the injected dye or because of blockage of the lymph node capsule by tumor cell emboli again is preventing the dye from entering the node (Plante et al., 2003).

SLN identification on each side of the pelvis represents its involvement but there is exception in parametrial lymph node. These nodes are very close to the cervix so there is problem to identify nodes (Plante et al., 2003). This point increases the false positive nodes. So in the present study, parametrial lymph nodes have not been evaluated.

Based on the present study is the first national assessment, the positive predictive value is 58%. It seems that one of the limitations of this study is the sample size, so further studies with larger sample size are recommended.

Finally, we can by SLN Mapping as the gold standard, diagnosis and treat patients with early cervical cancer. The present data demonstrate that methylene blue is the high quality and cost less test.

On the other side, removing out the looking healthy tissue (baft) also is an invasive action which will be decreased.

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REFERENCES


