EDITORIAL
Current Status of Lymphatic Mapping with Sentinel Lymph Node Biopsy (SLNB) in Cutaneous Melanoma

ORIGINAL ARTICLES
Effectiveness of Nutritional Intervention in Reduction of Gastrointestinal Toxicity during External Beam Radiotherapy in Women with Gynecological Tumors
Disparities in Breast Cancer Characteristics in Mexico
Impact of a Protocol for the Prevention and Care of Oral Mucositis in Pediatric Patients Diagnosed with Cancer
Presence of Human Papillomavirus and Epstein-Barr Virus in Breast Cancer Biopsies as Potential Risk Factors
Interleukin-6 Induces Epithelial-Mesenchymal Transition in Breast Cancer Cells

REVIEW ARTICLES
Multidisciplinary, Multi-Institutional Consensus on Cetuximab Usefulness in the Treatment of Patients with Head and Neck Squamous Cell Carcinoma
Perioperative Pain Management in Gynecologic Oncology Surgery

CLINICAL CASES
Dedifferentiated Parosteal Osteosarcoma of the Ulnar Diaphysis
Sarcomatous Degeneration of an Arteriovenous Malformation
Magnesium Deficiency in a Patient on Chemotherapy-Radiotherapy Treatment for Cervical Cancer: Case Report and Review
Corneal Intraepithelial Neoplasia as a Cause of Visual Acuity Decrease: a Low-Cost Approach
EDITORIAL

Current Status of Lymphatic Mapping with Sentinel Lymph Node Biopsy (SLNB) in Cutaneous Melanoma

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Received for publication: 10 November 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Sentinel lymph node biopsy (SLNB), a procedure known as lymphatic mapping with sentinel lymph node biopsy1, was developed, described and published by Donald Morton and Alistair Cochran in 19922. It is based on Cabañas’ early works in patients with penile cancer, and is current standard procedure for the staging of patients with cutaneous melanoma with Breslow depth greater than 1 mm without palpable lymph nodes in the lymphatic drainage zone3. It is one of the most important advances in oncologic surgery in the last decades: not only did it put an end to the existing controversy to decide between elective and therapeutic dissection in intermediate thickness melanoma, but its implications have been such, that the technique is already essential for lymph node staging in many other neoplasms, especially breast cancer, non-melanoma skin cancer and head and neck mucosal cancer3.

It is a minimally-invasive technique that, although not without complications, these are obviously less common compared with those occurring in patients undergoing elective lymph node dissection thus allowing, with a lower rate of complications, to classify patients with stage I and II cutaneous melanoma by risk based on the presence or absence of subclinical (not previously identified) lymph node metastases.

However, for lymphatic mapping and SLNB to be useful and meet its purpose, and for staging to be real, it has to meet some requirements; at least 5:

1. Taking care of technical aspects that enable a high rate of sentinel lymph node (SLN) identification and minimal false negative rate.
2. Low rate of complications.
3. Adequate histological assessment, including immunohistochemistry.
4. Cautious interpretation of the study sensitivity.
5. Adequate selection of patient candidates for the procedure, including the diagnostic procedure.

The technique lymphatic mapping with SLNB is carried out with is essential to ensure adequate identification of the first node that drains the tumor. The combined technique (dye and radiocolloid) is with no doubt the standard procedure and has to be carried out during the primary tumor resection. If the pigmented lesion has been previously split, the biopsy should not be broad and no reconstruction of the area should be made (flaps or grafts).

The pursued objectives with an adequate biopsy and mapping technique are to obtain a high identification rate and a minimal false negative rate.

Currently, SLN accepted identification rate is 99.4%, and false-negative rate depends on primary tumor Breslow level, with 4.8% in patients with intermediate melanoma and 10.5% in those with thick melanoma, based on the results obtained in D. Morton’s MSLT-1 trial4.

The critical points to be followed for an adequate identification of the true SLN are: the first station lymph node zone must be free of palpable lymph nodes; it is advisable to have an ultrasound not showing lymph nodes with suspected metastasis and, if this is the case, ultrasound-guided fine aspiration biopsy should be performed; biopsies with broad margins, with grafts or with flap rotation of any kind should be avoided; use of perilesional colloidal rhenium sulfide on the eve of the intervention or even two hours prior to the

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doi:10.24875/j.gamo.17000015
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procedure; performance of preoperative scintigraphy (bidi-

mensional image), which allows for anatomic localization (tridimensional identification can be made with SPECT-CT; especially at sites with complex drainage such as the head and neck), which allows for the drainage site to be identified on the skin; use of perilesional dye at least 10 minutes prior to the procedure and once the incision on the lymph node area is made; and intraoperative palpation of it to rule out by touch the presence of suspicious lymph nodes that have not received the colloid or dye (Dr. Merrick Ross verbal communication). Following these steps enables the highest identification rate possible.

The false negative rate is minimized by following the above steps and adding an adequate histopathological study that allows for sub-microscopic metastases to be identified. Avoiding the study with frozen sections is recommended and waiting for definitive assessment. Negative SLNs with conventional testing (hematoxylin and eosin staining) should be analyzed with immunohistochemical staining (HMB-45 and/or Melan-A).

The probability of having a metastatic SLN ranges from 5% to up to 40% and depends mainly on Breslow’s level and, although other factors have been associated, these are not yet clearly defined and are the subject of controversy; these include Clark’s level, anatomic site (higher risk in head and neck melanomas), primary tumor regression, ulceration (probably the second most important factor after tumor thickness), lymphovascular invasion, mitotic index (in those with Breslow’s level lower than 1 mm) and age (it is metastatic more frequently in subjects younger than 80 years)2.

Both retrospectively and, recently, prospectively1, the prognostic value of SLN has been confirmed and SLN histological status is therefore an independent factor, just as tumor thickness. This way, in patients with 1.2 mm or higher Breslow melanomas, survival is lower, and disease-free interval is shorter in patients with metastatic SLN when compared with those whose SLN is negative for metastasis.

On the other hand, SLN therapeutic value is poor, i.e., the possibility for lymphatic mapping and SLNB to prevent neoplastic progression in patients with metastatic SLN is questionable. In the MSLT-1 trial, survival was not better in patients undergoing SLN biopsy compared with those who remained under observation, and disease-free interval was only 7% better in intermediate melanomas and 10% in thick melanomas, i.e., patients undergoing lymphatic mapping and SLNB have a lower relapse index at the mapped zone compared with those who remain under observation.

In patients with metastasis-positive SLN, the number, as well as the size and site of metastasis are prognostic factors for recurrence and survival.

Patients with thin melanomas of less than 1 mm Breslow thickness have very low possibilities of metastasis to the SLN. 5.2% on average, and lymphatic mapping with SLNB is therefore not indicated. However, there are subgroups where the procedure should be considered. Probably the factor that most accurately predicts the presence positive SLN in melanomas with less than 1 mm thickness is tumor thickness: mean SLN metastasis in patients with less than 0.75 mm Breslow thickness is 2.9%, and in those with 0.75 mm to 1 mm it is 7.1%.

Other factors that influence and increase the probability of metastatic SLN in patients with thin melanomas include mitotic index higher than 1 mitosis/mm2, lymphovascular invasion, ulceration (infrequent in thin melanomas), tumor lymphocytic infiltration and regression, with all these being factors that have to be taken into account when deciding between lymphatic mapping with SLNB or surveillance. For some authors, the possibility of metastatic SLN in the presence of at least one of the above mentioned factors is as high as 18%.

Unlike intermediate melanomas, the prognosis is not changed by SLN histological status in patients with melanomas with less than 1 mm Breslow thickness.

In patients with metastasis to the SLN, worldwide consensus is to perform complementary lymphadenectomy of the area regardless of the type of metastasis, although factors such as the number of metastatic SLNs, the presence of parenchymal and non-subcapsular metastases, ulceration and tumor thickness are currently known to be directly proportionally associated with the possibility of metastatic non-sentinel lymph nodes. However, metastatic SLN-related complementary lymphadenectomy current value lies in that an average of 21% of these patients will have metastatic lymph nodes at dissection, which allows for them to be stratified by recurrence risk; i.e., those with metastasis in non-sentinel lymph nodes have worse survival and disease-free interval than those with metastases restricted to the SLN and eventually might be candidates for adjuvant therapies or clinical trials.

In summary, lymphatic mapping with SLNB has been instituted as the standard approach for lymph node staging in patients with cutaneous melanoma with Breslow thickness of more than 1 mm. If there is no possibility to perform it, there are two attitudes that can be adopted: to keep the lymph node zone under surveillance or to refer the patient to centers or groups that do it with adherence to international standards (this attitude is preferred).

Lymphatic mapping and SLNB is not indicated in stage 0 or less than 0.75-mm thick melanomas or in those thin melanomas between 0.75 and 1-mm thickness, except if in the patient other risk factors coexist, although these are not yet adequately established. The key to attain success is adhering to the recommended technique since the diagnostic process.

Finally, evidence shows that in patients with metastatic SLN the behavior to be followed is to complete the lymphadenectomy of the lymph node area undergoing mapping.

REFERENCES

ORIGINAL ARTICLE

Effectiveness of Nutritional Intervention in Reduction of Gastrointestinal Toxicity during External Beam Radiotherapy in Women with Gynecological Tumors

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Received for publication: 14 December 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract  Objective: To assess if nutritional intervention with a diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) decreases acute gastrointestinal toxicity induced by pelvic external beam radiotherapy in patients with gynecologic tumors. Material and methods: Single-center, randomized, prospective clinical trial comparing patients on a low-FODMAP diet vs. standard Mexican diet, designed to detect an 80% decrease in Grade 1-2 acute gastrointestinal toxicity in the standard diet group to 25% of Grade 1-2 acute gastrointestinal toxicity in the low-FODMAP diet group. Results: Thirteen patients were recruited per group, with higher gastrointestinal toxicity grade 1-2 (85 vs. 77%) and 3 (23 vs. 0%) being reported in the standard diet group with regard to the low-FODMAP diet (p = 0.16). The low-FODMAP diet group had a lower end of treatment symptom score in the cervical cancer patient quality of life questionnaire (1.41 vs. 1.85; p = 0.01) and lower ECOG mean deterioration (0.61 [SD ± 0.5] vs. 0.23 [SD ± 0.43]; p = 0.049). Excellent adherence to the diet was shown by 85% of patients. No factors associated with the presence of grade 3 gastrointestinal toxicity were found. Conclusion: Implementation of a low-FODMAP diet during pelvic external beam radiation therapy is a low-cost and high-adherence measure that reduces end-of-treatment performance status and symptom deterioration in patients with cervical cancer. (creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

Uterine cervix and uterine body malignant tumors represent in women the fourth and sixth place in incidence, and the fourth and fourteenth place in cancer-related mortality worldwide. In Mexico, cervical cancer is the second most common cancer in women in terms of incidence and mortality, and uterine body cancer occupies the ninth and thirteenth place with regard to these parameters. In addition, these figures are expected to increase by 2020.2,3

Treatment with external beam radiation therapy (EBRT) or teletherapy is used in 60-71% of women with cervical cancer and in 38-45% of those with uterine body tumors sometime during the course of the disease. The main adverse effect of this therapy is gastrointestinal (GI) toxicity, which occurs at mild-to-moderate grades in 70-90% of patients and severe grades (3-4) in around 3%, with incidence and severity increasing with commonly present factors such as concomitant use of chemotherapy, which doubles the risk for grade ≥ 3 GI toxicity.4

Women who experience acute GI toxicity during pelvic EBRT suffer a negative impact on abdominal symptoms, which also affects their nutritional status and quality of life. In addition, treatment prolongation or discontinuation due to these symptoms increases the risk for suboptimal results in disease control, life expectancy, and quality of life.5

Multiple interventions related to modification of treatment technical aspects have been developed to decrease EBRT-associated GI toxicity over the past few decades; however, it remains the main adverse effect in these patients, and these measures cannot be employed in all centers owing to the technical and economic requirements they involve.6,7

With regard to different dietary interventions that have been assessed, their effectiveness has not been conclusively corroborated in spite of being a readily accessible and easy to implement option, which makes their use feasible in all healthcare centers, either alone or in combination with other measures for the reduction of this toxicity.8

Based on the lack of conclusive evidence on GI toxicity reduction with nutritional interventions using the modification of a single dietary element in radiotherapy treated patients, the advantage of the low fructose, fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet lies in its intervention on multiple factors that influence radiation-induced enteropathy such as bowel motility regulation, lactose and osmotic agents restriction, and bacterial flora modification, together with an improvement observed in abdominal symptoms of patients with inflammatory bowel disease, which has similarities with radiation-induced damage in its pathogenesis, and reported high compliance.9,10

This is why assessment of this type of nutritional intervention is necessary in EBRT-treated patients in order to improve treatment tolerance and in this way positively influence patients’ oncologic outcomes and quality of life.

The main purpose of this trial was to assess if nutritional intervention by means of a FODMAP diet decrease pelvic EBRT-induced acute GI toxicity in patients with gynecologic tumors when compared with a standard Mexican diet.

MATERIAL AND METHODS

Patient selection and eligibility criteria

Patients diagnosed with cervical cancer or endometrial cancer at the Northeastern National Medical Center UMAE 25 of the Mexican Institute of Social Security in Monterrey, Nuevo León, were included. To be considered eligible, patients had to be between 18 and 70 years old at the moment of entering the study, have histopathological corroboration of diagnosis, performance status according to the Eastern Cooperative Oncology Group (ECOG) of 0-2, have adequate kidney, liver and bone marrow function, not being pregnant or in the postpartum period, and be candidates for radical or adjuvant treatment with EBRT with or without concomitant chemotherapy. Patients who had received prior treatment with pelvic radiotherapy, or with inflammatory bowel disease, active severe comorbidity, or active collagen disease, or those with distant metastasis according to disease-extension studies with chest X-ray and abdominopelvic CT scan were excluded.

The study protocol was approved by the Research and Ethics in Health Research Local Committee and informed consent was obtained from the patient for her participation in the study according to institutional guidelines.

Surgery

The patients treated with surgery of the primary tumor who were candidates for adjuvant therapy were treated with hysterectomy plus bilateral salpingooophorectomy with or without lymphadenectomy.

Chemotherapy

In patients who were candidates for concomitant chemotherapy, cisplatin was used at a dose of 40 mg/m² on radiotherapy days 1, 8, 15, 22, and 29 and in case of contraindication for this drug, carboplatin was used at dose of an area under the curve (AUC) of 1.5 according to Calvert’s formula.

Radiotherapy

All patients received EBRT with tridimensional conformal technique, at a dose of 50 Gy in 2 Gy fractions or 50.4 Gy in 1.8 Gy fractions.

Diet

The types of diet to be assigned comprised a diet low in fructose, oligosaccharides, disaccharides, monosaccharides, olyols and polyols (FODMAP) specified by means of an alimentary guideline. The assessment of adherence to this diet was made by means of weekly self-assessment with a Likert-type scale with adherence values ≥ 75% of the time, 50-75% of the time, 25-50% of the time, and < 25% of the time. The other diet was a standard Mexican diet according to recommendations of the Mexican Official Standard NOM-043-SSA2-2012, Basic health services. Promotion and education for alimentary health. Criteria to offer guidance.
Safety

At the beginning of EBRT, symptoms, weight, performance status (PS) and quality of life (QoL) were assessed by applying the European Organization for Research and Treatment of Cancer (EORTC) QLQ C-30\textsuperscript{28,29} questionnaire to all patients, as well as the specific modules for cervical (CX-24)\textsuperscript{30} or endometrial cancer (EN-24)\textsuperscript{31}, according to the primary tumor. Subsequently, the degree of GI toxicity was assessed weekly according to the National Cancer Institute (NCI) v4.03 scale, and medical and/or hospital management was provided when it was required.

At the end of the EBRT treatment, patients’ QoL, weight, and GI toxicity were newly assessed.

Study design

A single-center, prospective, randomized clinical trial was carried out, with patients being assigned to the FODMAP diet or standard Mexican Official Standard (NOM – Norma Oficial Mexicana) diet groups by means of randomization tables.

The study was designed to detect a decrease from 80% of grade 1-2 acute GI toxicity in the standard diet group to

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics by assigned diet</th>
</tr>
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<tr>
<td></td>
<td>FODMAP n (%)</td>
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<tr>
<td>Mean age</td>
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</tr>
<tr>
<td>Primary tumor</td>
<td></td>
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<tr>
<td>Cervix</td>
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<tr>
<td>I</td>
<td>1 (7)</td>
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<tr>
<td>II</td>
<td>5 (38)</td>
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<td>III</td>
<td>4 (31)</td>
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<td>Endometrium</td>
<td>3 (23)</td>
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<td>I</td>
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<td>II</td>
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<td>III</td>
<td>3 (23)</td>
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<td>Primary tumor previous surgery</td>
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</tr>
<tr>
<td>Yes</td>
<td>4 (31)</td>
</tr>
<tr>
<td>No</td>
<td>9 (69)</td>
</tr>
<tr>
<td>Concomitant chemotherapy</td>
<td></td>
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<td>Yes</td>
<td>9 (69)</td>
</tr>
<tr>
<td>No</td>
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</tr>
<tr>
<td>Radiotherapy dose</td>
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<tr>
<td>50.4 Gy/28 fr.</td>
<td>8 (62)</td>
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<tr>
<td>50 Gy/25 fr.</td>
<td>5 (38)</td>
</tr>
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<td>Volume of bowel portion receiving 45 Gy &gt; 195 cc</td>
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<td>13 (100)</td>
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<td>Volume of colon receiving 40 Gy &gt; 60%</td>
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<td>13 (100)</td>
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<tr>
<td>No</td>
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<tr>
<td>Follow-up</td>
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<tr>
<td>5 weeks</td>
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<tr>
<td>6 weeks</td>
<td>5 (38)</td>
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<tr>
<td>&gt; 6 weeks</td>
<td>4 (31)</td>
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</table>
25% of grade 1-2 acute GI toxicity in FODMAP-diet patients, with an alpha-value of 0.005 and statistical power of 80%. Thirteen patients were planned to be recruited per group, for a total of 26 patients.

RESULTS

Twenty-six patients who agreed to participate in the study were recruited from August to October 2016 at the Northeastern National Medical Center. The characteristics of the patients assigned to each type of diet are presented in Table 1.

Toxicity

The results on the highest degree of GI toxicity experienced by the patients according to the assigned diet type are presented in Table 2.

Table 3 shows the incidence according to the type and grade of toxicity experienced by the patients according to

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade</th>
<th>FODMAP n (%)</th>
<th>NOM n (%)</th>
<th>p</th>
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<tr>
<td>Bloating</td>
<td>0</td>
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<td>11 (86)</td>
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<td>0</td>
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<td>8 (62)</td>
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</tr>
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<td></td>
<td>4</td>
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<td></td>
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<tr>
<td>Nausea</td>
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<td>2 (15)</td>
<td>0.83</td>
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<td>Vomiting</td>
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<td>Diarrhea</td>
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<td>Constipation</td>
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Table 2. Incidence of gastrointestinal toxicity according to assigned diet; \( p = 0.16 \)

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<th>Toxicity</th>
<th>FODMAP n (%)</th>
<th>NOM n (%)</th>
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</tr>
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<td>1, 2</td>
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<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 4. Adherence to the FODMAP diet

<table>
<thead>
<tr>
<th>Compliance</th>
<th>FODMAP n (%)</th>
<th>NOM n (%)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>11 (85)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>2 (15)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Non-compliance</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Table 5. Weight loss and ECOG at the end of external beam radiotherapy

<table>
<thead>
<tr>
<th>FODMAP</th>
<th>NOM</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss</td>
<td>2.43 kg (SD ± 2.33)</td>
<td>3.12 kg (SD ± 3.17)</td>
</tr>
<tr>
<td>Final ECOG</td>
<td>1.3 (SD ± 0.63)</td>
<td>1.3 (SD ± 0.63)</td>
</tr>
<tr>
<td>ECOG decrease</td>
<td>0.23 (SD ± 0.43)</td>
<td>0.61 (SD ± 0.5)</td>
</tr>
</tbody>
</table>

Table 6. Initial and final quality of life scores and score changes

<table>
<thead>
<tr>
<th>QLQ C-30</th>
<th>Initial</th>
<th>Final</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>1.58</td>
<td>1.58</td>
<td>0.00</td>
</tr>
<tr>
<td>Role</td>
<td>2.00</td>
<td>1.88</td>
<td>0.12</td>
</tr>
<tr>
<td>Emotional</td>
<td>1.86</td>
<td>1.57</td>
<td>0.29</td>
</tr>
<tr>
<td>Social</td>
<td>1.92</td>
<td>1.80</td>
<td>0.12</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2.15</td>
<td>2.40</td>
<td>0.25</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>1.80</td>
<td>2.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Pain</td>
<td>1.96</td>
<td>1.92</td>
<td>0.08</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1.46</td>
<td>1.38</td>
<td>0.08</td>
</tr>
<tr>
<td>Insomnia</td>
<td>1.92</td>
<td>1.85</td>
<td>0.07</td>
</tr>
<tr>
<td>Appetite loss</td>
<td>2.15</td>
<td>2.15</td>
<td>0.00</td>
</tr>
<tr>
<td>Constipation</td>
<td>2.15</td>
<td>2.15</td>
<td>0.00</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1.08</td>
<td>2.15</td>
<td>1.07</td>
</tr>
<tr>
<td>Financial difficulties</td>
<td>2.46</td>
<td>2.54</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EN-24</th>
<th>Initial</th>
<th>Final</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual interest</td>
<td>2.33</td>
<td>1.33</td>
<td>-1.00</td>
</tr>
<tr>
<td>Sexual activity</td>
<td>2.00</td>
<td>1.33</td>
<td>-0.67</td>
</tr>
<tr>
<td>Lymphedema</td>
<td>1.33</td>
<td>1.33</td>
<td>0.00</td>
</tr>
<tr>
<td>Urologic symptoms</td>
<td>1.41</td>
<td>1.50</td>
<td>0.09</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>1.13</td>
<td>1.20</td>
<td>0.07</td>
</tr>
<tr>
<td>Body image</td>
<td>1.33</td>
<td>1.33</td>
<td>-0.33</td>
</tr>
<tr>
<td>Back/pelvis pain</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Numbness</td>
<td>1.33</td>
<td>1.33</td>
<td>0.00</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>1.00</td>
<td>1.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Hair loss</td>
<td>2.00</td>
<td>1.00</td>
<td>-1.00</td>
</tr>
<tr>
<td>Taste changes</td>
<td>2.00</td>
<td>2.00</td>
<td>-0.00</td>
</tr>
</tbody>
</table>
In both groups, the most common GI toxicity was nausea, followed by vomiting (54 vs. 46%) and diarrhea (62 vs. 69%); no grade 3 GI toxicity events occurred in the low-FODMAP diet group.

Adherence to the FODMAP diet

Excellent adherence to the diet was considered when a Likert-type diet follow-up score > 75% was obtained in 50% or more of assessed weeks; if this score was 50-75%, adherence was regarded as being regular, and below 50%, non-adherence was considered. Data on low-FODMAP diet adherence are reported in table 4.

The excellent adherence to this diet had no significant association with the prevention of GI toxicity development of any grade, with an odds-ratio (OR) of 0.93 (95% CI: 0.93-1.17; p = 0.96).

Weight loss and performance status

Table 5 reports weight loss during treatment and end-of-treatment ECOG performance status reduction, with greater performance status deterioration being reported in NOM-diet assigned patients with regard to the low-FODMAP diet.

Quality of life

Initial and final mean scores obtained in the general QoL EQ-RTC QLQ C-30 and endometrial cancer-specific EN-24 tests showed no significant differences; however, in the cervical cancer-specific test, a significant difference was observed, with lower presence of symptoms in the low-FODMAP diet-assigned patients’ final questionnaire. The results are presented in table 6.

Factors that influence gastrointestinal toxicity

No factors having a significant influence on the occurrence of grade 3 GI toxicity were observed in the univariate analysis (Table 7).

DISCUSSION

The incidence of GI toxicity in pelvic EBRT-treated patients has been previously reported, with values ranging from 70 to 90% for grades 1 and 2 and from 3 to 9% for grades 3 and 4, whereas in our study we observed a similar incidence of grade 1 and 2 toxicity, with 77 and 85% according to the low-FODMAP or NOM diet group assigned, respectively. On the other hand, the incidence of grade 3 GI toxicity was higher than that reported in the literature, with all the events of this grade occurring in the NOM-diet group, which corresponds to 11% of total patients and 23% of patients assigned to this diet group. In addition, the type of toxicities experienced by the patients is consistent with that observed in other studies, with main toxicities being nausea, vomiting, and diarrhea, all of them higher in the NOM-diet group.

The higher incidence of GI toxicity in the NOM-diet group was accompanied by greater mean weight loss in these patients (2.43 vs. 3.12 kg) and greater mean deterioration of ECOG performance status at treatment completion (0.61 [SD ± 0.5] vs. 0.23 [SD ± 0.43]), with the difference being statistically significant (p = 0.049). In addition, cervical cancer patients who were assigned to this group had more symptoms at treatment conclusion according to the specific QoL CX-24 questionnaire (1.41 vs. 1.85; p = 0.01).

Hence, in spite of not having achieved a significant decrease in GI toxicity with the low-FODMAP diet, its severity was lower in this group of patients, and this was accompanied by better outcomes in some aspects of QoL in cervical
cancer patients, as well as less weight loss in the course of treatment.

On the other hand, the excellent low-FODMAP diet-adherence rate (85%) places it as a tool that can be applied alone or in combination with other measures used to decrease GI toxicity that, in addition, by not requiring specific resources or infrastructure, can be implemented at any center.

CONCLUSION

In conclusion, implementation of a low-FODMAP diet during treatment with pelvic EBRT is a low-cost and high-adherence measure that decreases performance status deterioration and symptoms at treatment conclusion in patients with cervical cancer. Long-term patient follow-up is necessary in order to assess its impact on chronic toxicity, as well as a study with a larger number of patients designed for the reduction of severe toxicity (grade 3-4) during EBRT in order to establish its role in this scenario.

DECLARATION OF INTEREST

The authors declare not having any conflicts of interests.

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7. Andreyev HJN, et al. Does acute gastrointestinal toxicity during radical pelvic radiotherapy predict late gastrointestinal toxicity? A study using the IBDO and a Vaizey score, more sensitive measures of radiotherapy-induced toxicity than the RTDG or LENT SOM scales, Abstracts for the NCRI Cancer Conferences. 2008. [Accessed April 12, 2016].
Disparities in Breast Cancer Characteristics in Mexico

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Received for publication: 6 December 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract  Introduction: There are no data on breast cancer epidemiological characteristics of patients treated in the private medicine setting in Mexico. Methods: A retrospective study of women treated in a private tertiary health center in Mexico City from 2001 to 2016 was carried out, and a comparison was made with data of the National Institute of Cancer (InCan). Results: A total of 240 patients with a median age of 52 years were included. The most common histologic subtype was ductal carcinoma, with most cases corresponding to the luminal immunophenotype. Tumors were detected by radiologic screening in 34% of cases, and in 41.7%, tumors were diagnosed in situ or at stage I, in comparison with the InCan series, where 48% are stages III and IV (p < 0.01). Of the cases detected by self-palpation, half had lymph node metastases vs. 7% in the screening-detected cases (p < 0.001). In 60%, conservative surgery was performed, and in 13%, mastectomy with immediate reconstruction. Five-year disease-free survival is higher than 90%. Conclusions: Breast cancer epidemiology is consistent with InCan statistics. There is significant difference in clinical stages at presentation, which is attributed to disparity in access to screening. (creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

Breast cancer is one of the most common malignancies, with high impact on general mortality worldwide, and it represents a public health problem in Mexico. According to a recent analysis of GLOBOCAN statistics, the breast cancer-related mortality rate in Mexico has increased over the past few decades, in contrast with a decrease in the rate of mortality for this neoplasm in European countries. In Mexico, it is the most common malignant tumor in women since 2007, when it surpassed cervical cancer. According to GLOBOCAN 2012, the national rate of incidence is 35.35 x 100,000, which is higher than the 27.2 reported in 2008. Unlike developed countries where the diagnosis is made at early stages, in developing countries, most diagnoses are made at advanced stages, with a severe negative impact on the possibility of cure. One of the reasons for the delay in diagnosis is the consequence of difficulties in access to detection. In addition to late diagnosis, other breast cancer epidemiological particularities have been reported in our country, such as presentation at earlier stages than in developed countries, which entails a higher impact on national public health services since it primarily affects women at productive age. On the other hand, significant differences have been reported even within developed countries in both detection and treatment of breast cancer owing to sociodemographic and economic factors, with a direct impact on mortality. In developing countries, the gaps in detection and quality of care are broader than in developed countries, and Mexico is not an exception. There are no reports on epidemiological and clinical characteristics of breast cancer treated in the private medicine setting in our country. The purpose of the present work is to describe the epidemiological, clinical, and management characteristics of patients with breast cancer treated in the practice of one of the authors (HMF) in a tertiary care private hospital in Mexico City, and to compare these characteristics with those reported by tertiary care public centers in our country (National Institute of Cancer), with an emphasis on the importance of screening for early detection of this neoplasm.

MATERIAL AND METHODS

A retrospective study was carried out, which included all breast cancer-diagnosed patients (phyllodes tumors were included) treated by one of the authors (HMF) from January 1, 2001 through June 30, 2016 in a tertiary care private hospital of Mexico City. Patients treated for exclusively palliative purposes, or receiving first or second opinion care, but whose treatment was in charge of another institution or another physician, were excluded. Demographic variables, family hereditary history, and clinical and radiological characteristics were recorded, as well as histopathological and surgical variables. Descriptive statistics was used, as well as categorical variables comparison with the chi-square test and Pearson’s exact test, and mean comparison with Student’s t-test. Survival plots were constructed with the Kaplan-Meier method. All statistic calculations were performed using SPSS v20.0 software. Significance was established at a p-value < 0.05.

RESULTS

A total of 240 patients were included in the study period, with a mean age of 54.73 years (+14.09) and a median of 52 years (range: 28-99). Direct family history of breast and/or ovarian cancer was found in 26.7% of patients, with the highest number of affected relatives being two sisters and one daughter in one patient in whom BRCA1 gene deleterious mutation was corroborated. Tumor distribution according to laterality was even, with a slight predominance of the left side and, in 1.7% of cases, cancer occurred bilateral. The tumor detection method was radiological screening in 34.2%, physician in 4.2%, patient in 61.7%, and maternal grandmother in 6.25%.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean 54.73 ± 14.09; Median 52 years (28-99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history</td>
<td>26.7%</td>
</tr>
<tr>
<td>Sister</td>
<td>15.62%</td>
</tr>
<tr>
<td>Mother</td>
<td>25.0%</td>
</tr>
<tr>
<td>Daughter</td>
<td>6.25%</td>
</tr>
<tr>
<td>Maternal aunt</td>
<td>15.62%</td>
</tr>
<tr>
<td>Maternal grandmother</td>
<td>6.25%</td>
</tr>
<tr>
<td>Paternal grandmother</td>
<td>6.25%</td>
</tr>
<tr>
<td>Detection method</td>
<td></td>
</tr>
<tr>
<td>Screening</td>
<td>34.2%</td>
</tr>
<tr>
<td>Physician</td>
<td>4.2%</td>
</tr>
<tr>
<td>Patient</td>
<td>61.7%</td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>1.7%</td>
</tr>
<tr>
<td>Right</td>
<td>43.3%</td>
</tr>
<tr>
<td>Left</td>
<td>55.0%</td>
</tr>
<tr>
<td>Tumor size*</td>
<td>Mean 2.31 cm (+ 1.5 cm); Median 1.57 (0.5-11 cm)</td>
</tr>
<tr>
<td>Clinical stage (AJCC)*</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>18.3%</td>
</tr>
<tr>
<td>II</td>
<td>35.8%</td>
</tr>
<tr>
<td>IIA</td>
<td>28.3%</td>
</tr>
<tr>
<td>IIB</td>
<td>9.2%</td>
</tr>
<tr>
<td>IIIA</td>
<td>1.7%</td>
</tr>
<tr>
<td>IIIB</td>
<td>1.7%</td>
</tr>
<tr>
<td>IIC</td>
<td>1.7%</td>
</tr>
<tr>
<td>IV</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

*Invasive cancers. †Phyllodes tumors excluded. AJCC: American Joint Committee on Cancer.
Table 2. Histopathologic characteristics (n = 240)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of tumor</td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal</td>
<td>59.16%</td>
</tr>
<tr>
<td>Ductal in situ</td>
<td>15.83%</td>
</tr>
<tr>
<td>In situ with micro-invasion</td>
<td>2.5%</td>
</tr>
<tr>
<td>Infiltrating lobular</td>
<td>10.0%</td>
</tr>
<tr>
<td>Mucinous</td>
<td>2.5%</td>
</tr>
<tr>
<td>Apocrine</td>
<td>0.8%</td>
</tr>
<tr>
<td>Tubular intracystic</td>
<td>0.8%</td>
</tr>
<tr>
<td>Mixed</td>
<td>0.8%</td>
</tr>
<tr>
<td>Benign phyllodes</td>
<td>3.3%</td>
</tr>
<tr>
<td>Malignant phyllodes</td>
<td>0.8%</td>
</tr>
<tr>
<td>Grade*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10.0%</td>
</tr>
<tr>
<td>2</td>
<td>58.3%</td>
</tr>
<tr>
<td>3</td>
<td>26.7%</td>
</tr>
<tr>
<td>Molecular type†</td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>44.3%</td>
</tr>
<tr>
<td>Luminal B</td>
<td>35.05%</td>
</tr>
<tr>
<td>Pure HER</td>
<td>7.2%</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>12.4%</td>
</tr>
<tr>
<td>Unidentified</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

*Excluding phyllodes. †Only invasive cancers (n = 194).

histological grade was 2 (58.3%). Most patients with invasive cancers (n = 194) were positive for estrogen (81.4%) and progesterone receptors (75.3%), with a minority of HER2 over-expression (19.6%); in eight cases (4.2%), HER2 status is unknown. Ki-67 was determined in 176 cases (90.7%), with a median of 20 (2-90%). The predominant molecular type was luminal A (44.3%) (Table 2).

With regard to treatment, 12.5% of patients received neoadjuvant chemotherapy. Conservative surgery was performed in 60% of patients, and in 5% no resection surgery was performed due to either metastatic disease or the patient being of extremely advanced age. The type of surgery is shown in table 3. Post-surgery metastatic disease to axillary lymph nodes was reported in 43.3% of invasive cancer cases. Post-surgery histopathologic stage is shown in table 3. Postoperatively, 94.4% of patients treated with conservative surgery received adjuvant radiotherapy according to international guidelines, whereas 7.5% of mastectomy treated patients also received radiotherapy, either due to tumor size being > 5 cm, or presence of > 3 positive axillary lymph nodes. All patients with positive hormone receptors received hormone blockade, predominantly with letrozole in postmenopausal (89.9%) and tamoxifen in premenopausal and 10% of postmenopausal women. Of the group of patients with invasive cancers (n = 194), 46.4% received chemotherapy either adjuvant or as treatment for metastatic disease (2.5%), while 10.3% received trastuzumab owing to HER2 over-expression.

With a median follow-up of 36 months (range: 1-172), 14 recurrences occurred (7.2% of patients with invasive cancers), with four being local, four regional, two systemic, and four regional and systemic simultaneously. Disease-free survival was 93%, and overall survival was 96%.

An analysis of tumor characteristics was carried out in invasive cancers among those detected by screening and detected by the patient. Those detected by medical examination were included in the latter group, since they represent a very low proportion (4.2%). Smaller tumor size, absence of lymph node involvement, and lower American Joint Committee on Cancer (AJCC) clinical stage were found to be statistically significant factors in favor of the group of detection by screening (Table 4). No differences were found in age or type of surgery (mastectomy vs. conservative) according to the detection method. Another finding was a significant difference in tumor size according to histological grade, with mean tumor size in grade 2 tumors being 1.97 + 0.97 cm vs. 3.26 + 2.31 cm in grade 3 tumors (p = 0.0001).

On the other hand, a comparison of data of patients attended in a private setting was made with the breast cancer experience in the Seguro Popular era (2007-2013) using data on 3,109 cases published by Mohar A. et al. of the National Institute of Cancer (InCan) of Mexico City. According to the published data, median age was 50.9 years, with no difference with 52 years in our study. In addition, more than 80% corresponded to canicular carcinoma, without significant difference with 77.5% in our series, when infiltrating canicular carcinoma, carcinoma in situ, and ductal carcinoma in situ with micro-invasion are combined. With regard to immunophenotype, in both series luminal is predominant, although a significant difference was documented: 79.35% in our series vs. 58.1% in the InCan series (p < 0.05). Similarly, pure HER subtype was less common in our series than...
Disparities in breast cancer characteristics in Mexico

94

in the InCan series (7.2 vs. 21.9%; p < 0.05), with the same being observed for triple-negative status (12.4 vs. 17.2%), although in this case the difference was not statistically significant. The main difference between both series lies in tumor stage at presentation. Although the InCan series does not specify the method for tumor detection (screening vs. patient or doctor detection), stages at presentation are significantly more advanced than in our series. In this way, stage zero or carcinomas in situ accounted only for 1.9% of cases in the InCan series vs. 14.2% in our series (p < 0.05); no significant differences were found for stage I, with 21.6 vs. 27.5% in the InCan and the present series, respectively, but in stage II, cases were more common in our series (41.7 vs. 29.2% in the InCan series), and stages III and IV were significantly less frequent in our series (10.8 vs. 34.5% and 3.8 vs. 13.3%, respectively; p < 0.05).

**DISCUSSION**

Breast cancer is the leading malignant neoplasm in women in Mexico, and as in other countries of Latin America, it often appears at advanced stages with high associated mortality. There are very limited data on epidemiological, clinical, histopathological, and surgical characteristics of breast cancer managed in private medicine settings where, by definition, greater access to both screening and best available treatments is generally possible. Most series in our country come from large public health institutions, such as the National Institute of Cancer. In the present series we found that patient age does not differ from that of women attended to in public institutions, and this is corroborated with observations already published in other series that have established that, on average, breast cancer onset in our country occurs up to one decade earlier than in the USA or Europe. The infiltrating canalicular histologic type is not different from that reported by national and international series; however, the immunophenotype was significantly different, in particular with a lower proportion of the most aggressive subtypes, namely, pure HER2 and triple-negative, although in the latter the difference was not statistically significant with regard to the InCan series (12 vs. 17%). However, in another series of the same institution of 2,074 Hispanic women treated from 1998 to 2008, the prevalence of triple-negative breast cancer was 23.1%, mainly associated with young age, premenopausal status, high histological grade, and more advanced disease. The lower frequency of aggressive cancers in our series is also reflected in the lower frequency of high-grade tumors in our series compared with the InCan series (26.7 vs. 46.8%).

The main difference between our series and the InCan series and other national and Latin American public hospitals is the stage of the disease at presentation. In one study conducted among 138 Mexican oncologists, 58% of new breast cancer cases were reported to be diagnosed at stages III and IV (48% in the InCan series and 13.3% in ours). One possible explanation for the difference in stages at presentation is not only the disparity in health services availability, specifically open population screening, but, at least in part, since this was a surgical series, there is reference bias with a tendency to occur with the surgeon at earlier stages than with the medical oncologist or a national health institution.

Another significant difference is that in the InCan referred study, 63% of patients underwent mastectomy, in comparison with 60% of conservative surgery in our series, whereas 13.3% of patients, although undergoing mastectomy, were immediately reconstructed. The main reason for presentation at advanced stages is the limited access to screening by the general population, which is in clear contrast with the population attended to in private medicine settings, where 34% of cases are detected by this method. However, there is still a majority that, even with access to health services, is still detected by the patient, generally at advanced stages. The main effect of screening is a reduction in the frequency of advanced cancer cases. In a Swedish study, a reduction of more than 20% in advanced cancers translated into a 28% reduction in breast cancer-related

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**Table 4. Demographic and histopathologic characteristics according to detection method**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Screening</th>
<th>Patient/physician</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>53.24 ± 11.32 years</td>
<td>55.51 ± 15.34 years</td>
</tr>
<tr>
<td>Tumor size</td>
<td>1.19 ± 0.40 cm</td>
<td>2.80 ± 1.63 cm</td>
</tr>
<tr>
<td>Positive lymph nodes</td>
<td>7.3%</td>
<td>48.71%</td>
</tr>
<tr>
<td>Conservative surgery</td>
<td>75.6%</td>
<td>60.56%</td>
</tr>
<tr>
<td>Stage</td>
<td>(34.1%)</td>
<td>(65.9%)</td>
</tr>
<tr>
<td>0</td>
<td>38.5%</td>
<td>1.3%</td>
</tr>
<tr>
<td>I</td>
<td>46.2%</td>
<td>19.0%</td>
</tr>
<tr>
<td>II</td>
<td>10.2%</td>
<td>58.0%</td>
</tr>
<tr>
<td>III</td>
<td>2.5%</td>
<td>15.2%</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

---

$p = 0.07$

$p = 0.0001$

$p = 0.0001$

$p = 0.105$

$p = 0.0001$
mortality in women invited to screening\textsuperscript{11}. In our series, it is clear that in screening-detected women, 84.7\% had early stages (stages 0 and I), whereas in 77\% of women whose neoplasm was detected by self-palpation or physical examination by the physician, it was at advanced stages (stages II, III, IV). The proposed mechanisms by means of which screening reduces breast cancer-related mortality are the detection of smaller tumors, negative lymph nodes, and lower tumor grade\textsuperscript{12}. In our series, when the comparison of screening-diagnosed patients vs. patients whose neoplasm was detected by self-palpation was made, significant differences were found in tumor size (mean 1.19 vs. 2.80 cm; \(p = 0.0001\)), positive axillary lymph nodes (7.3 vs. 48.7\%; \(p = 0.0001\)) and tumor size according to the grade: mean tumor size in grade 2 tumors was 1.97 + 0.97 cm vs. 3.26 + 2.31 cm in grade 3 tumors (\(p = 0.0001\)). Evidently, detection by self-palpation is not useful for early stage diagnosis, since when this occurs, the tumor measures an average of 2.8 cm and nearly half of patients will have metastasis to axillary lymph nodes.

The main strength of this series is that it represents the practice of a single surgeon (HMF) in the same institution, without variability in both diagnosis and treatment criteria. The differences in stages at diagnosis are essentially due to disparities in health services availability, a highly prevalent situation in Latin America and other developing countries, but also already reported in the USA\textsuperscript{15,16}, Europe\textsuperscript{6,17} and other countries of the world. In fact, an analysis of GLOBOCAN statistics has been conducted, demonstrating that breast cancer-related mortality is directly associated with the Human Development Index\textsuperscript{18}, with one of the reasons being late detection, aggravated by unavailability of best treatments. The main weakness of the present series is the sample size, which may not be representative of nation-wide private practice, which warrants the performance of prospective studies with a representative sample of this population.

In conclusion, in a sample of patients treated in a tertiary care hospital with access to all diagnostic and therapeutic resources, breast cancer onset occurs at the same age as at the national level, with a lower proportion of aggressive phenotype tumors and, especially, with diagnosis at earlier stages of the disease, with the breast being able to be preserved in a significant proportion of patients, and with an excellent long-term survival. Inequity of health service access is a situation that must be resolved in government organizations.

**DECLARATION OF INTEREST**

The authors declare not having any conflicts of interests.

**REFERENCES**

Impact of a Protocol for the Prevention and Care of Oral Mucositis in Pediatric Patients Diagnosed with Cancer

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Received for publication: 30 October 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract

Introduction: Oral mucositis (OM) is a common complication associated with cancer treatment, and it can range from mild to severe degree. Up to 80% of children undergoing chemotherapy will experience some degree of OM, which can get to increase mortality by up to 40% in severe cases. Although OM incidence differs according to cancer type and treatment regimen, children with hematological malignancies experience higher incidence and prevalence than children diagnosed with solid tumors. According to the above, OM is likely to represent a major cause for common protocol-indicated dose reduction and, hence, for treatment delay. Therefore, this work analyzes approaches that been used to impact on OM prevention and opportune treatment, in addition to contributing to yet-unpublished national statistics.

Material and Methods: This was a retrospective, descriptive study of 157 cancer-diagnosed pediatric patients attended to during the period from September 2014 to June 2016 and who were at risk for experiencing an OM event during their treatment stage. Results: OM occurrence was 21.6% and annual prevalence was 2.6% out of a total of 1731 assessments. Conclusions: Our results are promising, and expanding the sample size, as well the perspective of collaboration with other institutions is suggested in order to promote a standardized oral care protocol for children diagnosed with cancer.
INTRODUCTION

Oncologic problems are characterized by cell proliferation deregulation and increase, as well as apoptosis decrease. Therefore, conventional antineoplastic treatments are targeted to block this proliferation. However, chemotherapy and radiotherapy actions are non-specific, and in the context of antineoplastic treatments it is therefore common to observe harm to tissues that in physiological conditions have high cell-replication rates, since they act on cell multiplicative phase. Among these mechanisms, toxicity to oral and gastrointestinal mucosa stands out, which produces lesions such as mucositis.

OM is an acute and severe toxic inflammatory reaction that affects the entire gastrointestinal (GI) tract from the oral-labial to the anogenital mucosa, secondary to toxicity of antineoplastic treatments such as radiotherapy, chemotherapy or conditioning for hematopoietic stem cell transplantation (HSCT) used in hematological malignancies. This inflammation can progress until confluent ulcers are formed, thus decreasing patient quality of life owing to pain, malnutrition, delay on treatment administration and increasing the risk of a life-threatening infection and increasing hospital stay and cost of treatment.

OM prevalence is 40% to 100%, depending on the type of cancer and the employed treatment. In adult population, it occurs in approximately in 40 to 76% of patients on treatment with standard-dose chemotherapy, as well as in 80 to 100% of patients undergoing HSCT conditioning, and in 100% of patients undergoing radiotherapy to the head and neck region, especially if the dose exceeds 4.000-6.000 cGy.

On the other hand, incidence of OM in children has increased in comparison with adult population; however, there are many variations in the literature, with estimates ranging from 52 to 80%.

Currently, the literature on OM in pediatric population is scarce in spite of the fact of the risk to experience this complication being high in comparison with adult population, since there are factors related to patient age that may contribute to its development, such as deficient oral hygiene practices and inadequate nutritional status.

Patients with OM usually experience a dry mouth sensation, difficulty to swallow, burning, tingling in the lips, pain, etc. One of the most serious complications is the development of infections (especially with herpes simplex virus or Candida albicans), with an increased risk in patients with prolonged neutropenia and which may threaten the patient’s life. Currently, there is no appropriate clinical prophylaxis or efficacious antidote for OM, and management is therefore mainly focused on symptom palliation and infection prevention.

There are different methods to assess and quantify the changes produced in the oral epithelium as a side effect of antineoplastic treatment. Among scale-based classifications, “general” mucositis assessment scales and “multiple-variable” OM assessment scales can normally be differentiated. The former are usually comprised by 4 or 5 variables and allow for overall status of the mouth to be associated with mucositis. In contrast, the second type of scales assesses different variables and their relationship with oral health status and function; the obtained scores are added, and a mucositis severity overall assessment is obtained.

In 1979, the World Health Organization (WHO) defined MO lesions status according to their severity by assessing tolerance to the oral route for food ingestion and being able to speak, with five grades, from 0 to IV, being established in patients. Meanwhile, the National Cancer Institute presented in 1998 an update of the Common Toxicity Criteria (CTC), where OM caused by radiation, chemotherapy and OM deriving from HSCT are differentiated, with OM being classified according to the zone of appearance (assessed areas: right and left jugal mucosa, hard and soft palate, lingual dorsum, lateral borders of the tongue, floor of the mouth). Of both classifications, the one that includes tolerance to the oral route, pain, oral cavity clinical characteristics and patient speech capability is the WHO scale and, therefore, it is the one that was chosen to be used in our hospital center for OM classification.

In addition to taking the MO classification into account, it is important for predisposing factors to be identified, including: a neglected oral cavity, dental pathology and high doses of certain antineoplastic agents (chemotherapy and radiotherapy). Oral cavity health status prior to the disease is one of the main conditioning factors for the development of mucositis, its degree, duration and evolution. Other factors that modulate OM symptoms are blood diseases with oral manifestations, cytostatic agents and radiotherapy, as well as immunodepression state or coexistence of other systemic diseases such as diabetes, renal failure or organ transplant. Mucositis severity can vary according to the type, dose, frequency and chronicity of the antineoplastic treatment received by the patient, although there are treatments that are particularly related to its occurrence. However, chemotherapy and radiotherapy actions are not specific, and in the context of antineoplastic treatments it is therefore common to observe damage to tissues that in physiologic conditions have high cell replication rates. Among them, oral and GI mucosa toxicity stands out.

According to a correct classification and identification of risks, treatment modalities can be non-pharmacological or with pharmacological agents such as lidocaine and morphine, which are used for the management of pain and as palliatives. Non-pharmacological treatments include measures of oral hygiene, mild mouthwashes, cryotherapy, etc. Recently, photobiomodulation (laser therapy) has been used in different aspects of dentistry, since it is a safe non-pharmacological method that can modulate several metabolic processes by means of energy absorption by macrophages.

Low-level laser therapy (LLLT) acts on mitochondrial respiration and leads to an increase in the production of Adenosine Triphosphate (ATP), which produces intracellular reactive oxygen species. These changes result in fibroblast proliferation, collagen synthesis, inflammatory response adjustment, and they also induce angiogenesis and tissue repair. There are different explanations for the reduction in pain caused by photobiomodulation, for example, inflammatory process modulation, excitation alteration, peripheral nerve sensory conduction and endogenous endorphin release stimulation. Therefore, LLLT is considered for preventive and palliative treatment of OM. LLLT with laser diodes of several wavelengths (630-680, 700-830 and 900 nm) has been reported to be an efficacious, simple and non-invasive technique in the treatment of OM, without toxicity.
being observed in clinical trials and, and lesions within the oral cavity have therefore been suggested to likely respond better to LLLT than skin wounds, since the oral region has higher blood irrigation\(^4\)\(^-\)\(^6\).

Having interdisciplinary support in hospital centers is of vital interest. Hence, we consider important assessing the OM care protocol in pediatric cancer patients with the purpose to improve quality of life and translate this into adequate clinical evolution.

**GENERAL PURPOSE**

To analyze the impact of implementing a standardized protocol of hospital oral care and the record of OM incidence in immunocompromised pediatric cancer patients undergoing treatment with chemotherapy, radiotherapy and/or hematopoietic stem cell transplantation (HSCT).

**Hypothesis**

Our dental care protocol in patients starting antineoplastic treatment reduces the prevalence of OM in comparison with the prevalence reported in the international literature.

**MATERIAL AND METHODS**

**Study group**

This was a cross-sectional, retrospective study. The sample consisted of records of pediatric population younger than 18 years diagnosed with cancer starting treatment in the outpatient or inpatient setting during the period from September 2014 to June 2016 and who were referred to the pediatric dentistry department for the first time and subsequently. After each primary assessment, and according to the specialist’s criterion, a preventive or therapeutic dental protocol with general measures and/or laser therapy was started (Table 1). For patients with oncologic assessment who started a protocol with high-dose chemotherapy with methotrexate or who underwent head and neck radiotherapy, the therapeutic protocol implemented in our center by the department of pediatric dentistry was directly initiated. Data were collected in an electronic database on order to analyze treated patients’ epidemiological characteristics, as well as OM prevalence and to assess the implemented dental protocols.

**Inclusion criteria**

Patients of the female or male gender, younger than 18 years of age, diagnosed with pediatric cancer, who received dental care in our hospital center.

**Exclusion criteria**

Patients of the female or male gender, younger than 18 years, not diagnosed with pediatric cancer, who received care in our hospital center.

**Elimination criteria**

Patients counter-referred to other hospital unit, healthy patients who were candidates to bone marrow donation.

**General description of the study**

This was a retrospective, descriptive study that included electronic medical file and registry log data from 157 patients diagnosed with pediatric cancer attended to during the period from September 2014 to June 2016. Data were obtained from registry logs of the hospital’s Departments of Pediatric Dentistry, Infectology, Clinical Laboratory and Pathology. Of the 157 patients attended to, 1731 records related to first-time and subsequent dental care were obtained, which were recorded in electronic spreadsheets and, subsequently, the OM relationship was analyzed by specific oncologic pathology (Grades I, II, III or IV according to WHO criteria). Subsequently, data were analyzed according to the occurrence of OM in the groups of higher frequency, and the preventive measures and/or dental treatment used were retrospectively assessed. Data were analyzed in the SPSS V21 Program, and the graphs in the GraphPad Prism 5 program.

**RESULTS**

**Epidemiological characteristics of the study population**

Of a total of 157 patients, a mean age of 7.9 (SD ± 5.1) years was recorded, with 20% being females and 80% males. Patients were classified in three groups according to the associated pediatric oncologic pathology based on the Seguro

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**Table 1. Protocol for oral mucositis prevention**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Therapeutics A</th>
<th>Therapeutics B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dental examination</td>
<td>Low-level laser therapy (LLLT)</td>
</tr>
<tr>
<td></td>
<td>Oral hygiene</td>
<td>– Beam: infrared.</td>
</tr>
<tr>
<td></td>
<td>Tooth brushing</td>
<td>– Output power: 300 mW.</td>
</tr>
<tr>
<td></td>
<td>Mild mouthwashes</td>
<td>– Wavelength: 980 nm.</td>
</tr>
<tr>
<td></td>
<td>Antiseptics</td>
<td>– Dose/Energy density: 18 J/cm².</td>
</tr>
<tr>
<td></td>
<td>Lip moisturizing</td>
<td>– Frequency: 8000 Hz.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Application: single point instead of scanning in motion for 8 continuous minutes or fractioned in two 4-minute episodes, according to patient cooperation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Duration:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a) Start 24 hours after high-dose methotrexate administration or at the beginning of radiotherapy at the head and neck level or at the start of HSCT conditioning thrice weekly.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Apply at the completion of chemotherapy with standard-dose methotrexate in a single session.</td>
</tr>
</tbody>
</table>

---

\[^4\]:

\[^5\]:

\[^6\]:
**Table 2. Epidemiological characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>157 patients.</td>
</tr>
<tr>
<td>Age (95% CI: 7.1-8.7)</td>
<td>Mean: 7.9 (SD ± 5.1)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20%</td>
</tr>
<tr>
<td>Male</td>
<td>80%</td>
</tr>
<tr>
<td>Oncologic diagnosis</td>
<td></td>
</tr>
<tr>
<td>Hematological malignancies</td>
<td>43%</td>
</tr>
<tr>
<td>ALL</td>
<td>30</td>
</tr>
<tr>
<td>HL</td>
<td>5</td>
</tr>
<tr>
<td>BL, NHL</td>
<td>5</td>
</tr>
<tr>
<td>LCH</td>
<td>3</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>39%</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>12</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>7</td>
</tr>
<tr>
<td>Non-rabdo. sarcoma</td>
<td>6</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>5</td>
</tr>
<tr>
<td>AML</td>
<td>4</td>
</tr>
<tr>
<td>Ovary, testicle, Wilms, thyroid tumor</td>
<td>3</td>
</tr>
<tr>
<td>Germ cell tumor, hepatoblastoma</td>
<td>2</td>
</tr>
<tr>
<td>CNS tumors</td>
<td>18%</td>
</tr>
<tr>
<td>Glioma</td>
<td>6</td>
</tr>
<tr>
<td>Ependymoma, medulloblastoma</td>
<td>5</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>5</td>
</tr>
<tr>
<td>Pineoblastoma, craniopharyngioma</td>
<td>1</td>
</tr>
<tr>
<td>Thalamic tumor</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 3. Oral health in pediatric patients diagnosed with cancer**

<table>
<thead>
<tr>
<th>Dental diagnosis according to first-time assessment by the Pediatric Dentistry Department</th>
<th>Oral cavity integrity (soft tissues)</th>
<th>Caries</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity integrity</td>
<td>48%</td>
<td>48%</td>
<td>4%</td>
</tr>
</tbody>
</table>

**Figure 1. Oral mucositis grades associated with oncologic pathologies.** The bars represent main associated pathologies occurrence by mucositis severity grade. Bars are shown in shades of gray according to severity grade. ALL: Acute Lymphoblastic Leukemia; CGL: Chronic Granulocytic Leukemia; EWG: Ewing Sarcoma; LBL: Lymphoblastic Lymphoma; NB: Neuroblastoma; NHL: Non-Hodgkin Lymphoma; OSC: Osteosarcoma; RB: Retinoblastoma.

**Figure 2. Oral mucositis-associated microorganisms.** The black bar shows the percentage of Candida sp occurrence; Herpes simplex occurrence is shown in gray.

**Popular (people's health insurance) National Program reference.** In order of frequency, hematological malignancies were the most frequent with 43%, followed by solid tumors in 39% and central nervous system (CNS) tumors in 18%. Further description is made in table 2.

Of the totality of patients with cancer diagnosis, first-time assessment by the pediatric dentistry department is described. Nearly half the patients (48%) had caries and non-complete oral cavity integrity (48%). Dental evaluations are detailed in table 3.

**Frequency of oral mucositis by oncologic condition**

Of the 157 patients attended to, 34 had at least one mucositis event, i.e., an occurrence rate of 21.6% over the study period. Annual prevalence of at least one OM event in one year within the analyzed period was 2.6% out of 1731 total assessments by the pediatric dentistry department. OM grades were assessed according to the WHO classification (Table 4). Ninety-four percent of recorded OMs corresponded to Grade I and Grade II, and only 4% to Grade III and 2% to Grade IV. OM and oncologic pathology diagnosis were associated according to the percentage of occurrence, with a higher number of recorded events corresponding to children with diagnoses of acute lymphoblastic leukemia and osteosarcoma (Fig. 1).

**Microorganisms associated with oral mucositis events**

Of the recorded OM events, samples were taken for basic morphological staining with the purpose to identify yeasts...
(KOH staining) or intracytoplasmic inclusions (Tzank), which help to infer the type of infection with *Candida* sp or with herpes simplex, respectively. Seventy-seven corresponded to morphological findings positive for related yeasts, of patients mostly diagnosed with leukemia, and 33% related to intracytoplasmic inclusions (Fig. 2).

**Association of dental treatment used and risk for OM**

Fischer’s exact test was used to analyze the relationship between OM grades in patients diagnosed with leukemia and osteosarcoma and the therapeutics used according to dental assessment (Table 5).

**Description of therapeutic protocols used by the department of pediatric dentistry**

Patients with first-time or subsequent dental evaluation, as well as patients at risk for the development of OM (leukemia and osteosarcoma), received general measures and/or low-level laser therapy. According to the diagnosed OM grade, the therapeutic protocol described in Table 6 was implemented.

---

**Table 4.** Antineoplastic therapy used and associated mucositis grades

<table>
<thead>
<tr>
<th>Grade I Mucositis</th>
<th>Grade II Mucositis</th>
<th>Grade III Mucositis</th>
<th>Grade IV Mucositis</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Very High Risk</td>
<td>17 MAPIE</td>
<td>33 MAP</td>
<td>50</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Risk</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Risk</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very High Risk</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*MAP: Methotrexate, Doxorubicin, Cisplatin. MAPIE: Methotrexate, Doxorubicin, Cisplatin, Etoposide, Ifosfamide. **For the leukemia protocol, patients receive antineoplastic therapy according to their treatment stage with: Methotrexate, AraC, Vincristine, Dexamethasone, etc.

**Table 5.** Association and risk for experiencing oral mucositis and dental therapeutics used

<table>
<thead>
<tr>
<th>Pathology</th>
<th>* p ≤0.05</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemias</td>
<td>No</td>
<td>0.6</td>
</tr>
<tr>
<td>Osteosarcomas</td>
<td>No</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*Fisher's exact test.

---

**Table 6.** Oral mucositis therapeutic protocol

<table>
<thead>
<tr>
<th>WHO Grade I oral mucositis</th>
<th>Tooth brushing</th>
<th>Mild mouthwashes</th>
<th>Antiseptics</th>
<th>Lip moisturizing</th>
<th>Low-level laser therapy</th>
<th>Ant-inflammatory / Analgesic / Topical anesthetic</th>
<th>Mucoprotector</th>
<th>Pain medicine</th>
<th>Infectology / Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Beam: infrared</td>
<td>Output power: 300 mW</td>
<td>Wavelength: 980 nm</td>
<td>Dose/Energy density: 18 J/cm²</td>
<td>Frequency: 8000 Hz</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO Grade II oral mucositis</td>
<td>Toth brushing</td>
<td>Mild mouthwashes</td>
<td>Antiseptics</td>
<td>Lip moisturizing</td>
<td>Low-level laser therapy</td>
<td>Ant-inflammatory / Analgesic / Topical anesthetic</td>
<td>Mucoprotector</td>
<td>Pain medicine</td>
<td>Infectology / Pathology</td>
</tr>
<tr>
<td>WHO Grade III and IV oral mucositis</td>
<td>Tooth brushing</td>
<td>Mild mouthwashes</td>
<td>Antiseptics</td>
<td>Lip moisturizing</td>
<td>Low-level laser therapy</td>
<td>Ant-inflammatory / Analgesic / Topical anesthetic</td>
<td>Mucoprotector</td>
<td>Pain medicine</td>
<td>Infectology / Pathology</td>
</tr>
</tbody>
</table>
DISCUSSION

Oral health remains a public health problem in Mexico. Our data are consistent with what the Ministry of Health’s last report reflects\(^{11}\), where 50% of children have some dental problem, especially caries. In addition to oral health status, cancer-diagnosed pediatric patients have to face a new organic challenge. For this reason, oral health should be linked within the oncologic support our patients receive.

As in many international reference centers\(^{12}\), implementation of both OM preventive and therapeutic protocols is being initiated; however, there are no definitive national guidelines for its treatment in pediatric population. Children have been shown to be at higher risk for developing OM than adults\(^{13}\), and poor control of this condition can have a negative outcome in children’s development.

With the protocol implemented in our center, a prevalence of about 22% is observed, which is less than half the prevalence reported in global literature\(^{14}\). Furthermore, more than 90% of oral mucositis cases had grade I and grade II presentations and were resolved according to MO therapeutic management protocols in less than 7 days.

An intact oral mucosa creates a physical barrier against pathogens and provides clearance of mucosa-colonizing microorganisms thanks to the detachment of epithelial cells. Once the mucosal barrier is ruptured, infection occurs, mainly with colonizing microorganisms such as Candida species, mainly albicans, Streptococcus of the viridans group and herpes simplex virus (HSV) infection reactivation. Our data on OM-associated microorganisms’ frequency are consistent with those reported in the literature, where most oral infections in patients are produced by Candida albicans, with the incidence in series of patients with leukemia who received chemotherapy being from 21.8% to 58%, and in series of patients with solid tumors, up to 70%. With regard to HSV infection, it occurs in 21% to 90% of seropositive patients who receive high-dose chemotherapy, especially in transplanted patients. This infection is associated with severe grades of mucositis\(^{15-17}\).

According to our observations, implementation of good oral hygiene and the use of a standardized oral care protocol for all OM-susceptible children is as important as the use of therapeutic laser, since the non-significant association between using or not low-level laser does not decrease the risk to suffer OM. In the international literature there is an important number of preventive oral health care protocols\(^{18-20}\); however, national reference reports are not yet available. Evidence on efficacious pharmacological treatments in the treatment of oral mucositis is insufficient, and it is limited to clinical trials in adults with drugs such as recombinant keratinocyte growth factor. A multimodal approach to oral cavity adequate care with general measures such as those proposed in our work (Therapeutics A and B), and interdisciplinary management between the departments of infectology, pain medicine, psycho-oncology and dentistry, may help to decrease oral mucositis duration and severity during antineoplastic treatment in pediatric patients by giving support and reducing delays in the management provided by the pediatric oncologist.

CONCLUSIONS

Oral care protocols can offer OM reduction in children undergoing antineoplastic therapy. Our results are promising, although interpreting them with a larger number of patients is necessary, as well as research in multi-institutional collaboration in order to define and promote the optimal oral care regimen in children diagnosed with pediatric cancer.

ACKNOWLEDGEMENTS

We would like to thank Dr. Isaac Urrutia Ballesteros, of the Department of Pain Medicine, for his contribution to analgesic management support within the mucositis care therapeutic protocols. We also express our gratitude to MS Gina de Gasper Estrada for her collaboration in data acquisition.

CONFLICT OF INTERESTS

The authors declare no conflicts of interests.

REFERENCES

ORIGINAL ARTICLE

Presence of Human Papillomavirus and Epstein-Barr Virus in Breast Cancer Biopsies as Potential Risk Factors

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Received for publication: 8 October 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract
Introduction: Breast cancer represents the leading cause of cancer-related death in the Venezuelan female population. Many risk factors favoring its appearance have been reported; however, human papillomavirus and Epstein-Barr virus have been associated in the past few decades as potential risk factors in the development of this malignancy. Objective: To detect the presence of human papillomavirus and Epstein-Barr virus in breast cancer biopsies in order to establish a possible link between infection with these viral agents and the development of this pathology. Methods: Fresh biopsies were collected from patients with breast cancer and patients with breast benign pathology attending the Hospital Universitario de Caracas for surgery. Human papillomavirus detection was made using the INNO-LIPA® HPV Genotyping Extra commercial kit (Innogenetics), and Epstein-Barr virus genome was detected with Epstein-Barr Virus BMLF1 commercial kit (Maxim Biotech, Inc.). Results: 63.6 and 13.6% of breast cancer cases were positive for human papillomavirus and Epstein-Barr virus DNA, respectively, whereas the benign pathology samples had 4.5% positivity for each one of the viruses; 42.90% of breast cancer samples had mixed infection with low and high oncogenic risk human papillomavirus genotypes. Conclusion: We can suggest that human papillomavirus and Epstein-Barr virus are important risk factors for breast cancer; however, studies allowing for the role of these viruses in the development of the disease to be elucidated are required.

KEYWORDS
Breast cancer; Human papillomavirus; HPV; Epstein-Barr virus; EBV; Risk factors

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doi:10.24875/j.gamo.17000018
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INTRODUCTION

Breast cancer is the second most common cancer in the world. In 2012, the International Agency for Research on Cancer (IARC) estimated 1.6 million new cases of breast cancer, accounting for 25% of all types of cancer diagnosed that year. In Venezuela, according to the People’s Power Ministry for Health (MPPS – Ministerio de Poder Popular para la Salud) Mortality Yearbook, breast cancer was the leading cause of oncological death in the female population in 2012, with 2,067 deaths being reported, and this pathology turning into an alarming public health problem and that, although it can affect males, current male mortality figures in the country do not exceed 10 annual cases.

The probabilities for developing breast cancer increase or decrease according to the number of concurring risk factors, which include the environment, genes, and lifestyle. Currently, there are well known risk factors associated with breast cancer; however, in most women, identifying a particular factor related to the development of the disease is not possible. In the past few decades, the action of a group of tumor-producing viruses has been considered to be an important factor in the development of cancer in both experimental and human models. The human papillomavirus (HPV) is the etiologic agent of cervical cancer, and the Epstein-Barr virus (EBV) is mainly associated with nasopharyngeal carcinoma and Burkitts lymphoma. These two viruses have been frequently associated with the development of breast cancer owing to their oncogenic role, either by the expression of viral genes that are similar to cell genes, or by the expression of proteins that are able to alter the cell cycle.

Human papilloma virus is a sexually transmitted virus and member of the Papillomaviridae family; its genome is composed of double-stranded, circular DNA of approximately 8,000 bp. This virus has special affinity for epithelial cells, and infects uterine cervix stratified squamous epithelium basement membrane cells; however, expression and replication of its genes depends on normal epithelium differentiation. This dependence of the virus on host cells, together with the expression of viral oncoproteins, drive the cell to a proliferative state without cell lysis occurring. The E6 and E7 oncoproteins are able to interact with p53 and pRb cell proteins, respectively, thus affecting their functions on cell-cycle regulation and driving to permanent cell immortalization. In addition, HPV genome integration interrupts and eliminates E2 open reading frame, with expression of this viral gene being lost. This way, integration of the viral genome and concomitant loss of E2 expression could be an important step in the carcinogenic process resulting from the altered expression of viral genes E6 and E7, given that the E2 protein acts as viral replication negative control. Based on this affinity of HPV for epithelial cells and its important capacity to lead to the development of cancer, numerous studies have investigated the presence of HPV in breast tissue epithelial cells, with some works having reported little or no evidence, while others have reported important infection figures in breast cancer patients, additionally proposing possible routes for HPV infection in breast tissue.

In turn, EBV is an enveloped virus, member of the Herpesviridae family, which is constituted by a linear, double-stranded DNA genome of approximately 172,000 bp. It is a virus with great affinity for B-cells and oropharyngeal epithelial cells, which are main route of infection with the virus, with two types of replication cycles having been able to be established: a lytic cycle, and another of latency in the host cell. Although EBV is frequently associated with human lymphoid neoplasms in immunosuppressed patients, several studies have demonstrated that there is also a possible association between EBV and the development of malignancy epithelial cells such as breast tissue, mainly based on some observations that support this hypothesis: (i) high incidence of male breast cancer in EBV-endemic Mediterranean countries; (ii) development of EBV-associated lymphomas at the level of the breasts; and (iii) morphological similarities between breast medullary carcinomas and nasopharyngeal carcinomas. The most specific evidence of an association of EBV with breast cancer has been the identification of EBV gene sequences in breast tumors. On the other hand, the expression of EBV latent protein expression has also been assessed in breast cancer cell lines in vitro and in vivo, as well as the oncogenic capacity of its viral proteins. However, some studies have failed to find any relationship between EBV and breast cancer.

These evidences allow for a possible relationship between breast cancer and infection, either with EBV or HPV, to be proposed, based on which these viruses can be regarded as risk factors in the development of this disease that causes many deaths in the female population worldwide. Based on this, the purpose of this work was to assess the presence of HPV and EBV in fresh biopsies of Venezuelan female patients with breast cancer as possible risk factors associated with this pathology.

MATERIALS AND METHODS

Patients

From April 2014 through May 2015, 44 Venezuelan patients attending the outpatient clinic of the Caracas University Hospital (HUC - Hospital Universitario de Caracas) Gynecology Department Breast Pathology Unit (UPM – Unidad de Patología Mamaria) were prospectively assessed. Each patient was invited to participate in the study after being briefed on the study design and protocol, with each one signing a HUC Bioethics Committee-approved informed consent form. Patient selection was made under the following selection criteria: patients diagnosed with stage 0, I, and II breast carcinoma, and those patients diagnosed with stage III and IV breast carcinoma who, for intrinsic reasons, did not receive neoadjuvant therapy, as well as patients diagnosed with benign breast pathology were included. Patients diagnosed with autoimmune disease or any other cancer not related to the primary tumor, pregnant patients, patients receiving neoadjuvant therapy, and those not accepting to participate in the study were excluded.

Genetic material extraction and quality assessment

Genetic material extraction was carried out with the Pure Link™ Genomic DNA commercial kit (Invitrogen), following
the manufacturer’s specifications. The DNA quality of all samples was assessed following the BIOMED-2 protocol by Van Dongen, et al. The reaction mixture was prepared using 0.1 µl of dNTP (100 mM), 2 µl of each primer (100 µM), 6.5 µl 10X buffer, 2 µl MgCl₂ (50 mM), 0.6 µl Taq polymerase, and 28.8 µl nuclease-free H₂O, for a final volume of 50 µl. Amplification conditions were seven minutes at 95 °C, 30 30-second cycles at 45 °C, 40 seconds at 60 °C, and 40 seconds at 70 °C, and a final amplification of 15 seconds at 70 °C.

**Human papilloma genome detection and typing**

Detection and genotyping of HPV 28 genotypes in breast cancer biopsies was carried out with the INNO-LIPA® HPV Genotyping Extra kit (Innogenetics), following the manufacturer’s recommendations. This test involves an assay with immobilized probes in nitrocellulose stripes, based on a reverse hybridization principle, designed to identify 28 different HPV genotypes by means of the detection of HPV genome L1 region specific sequences. This assay uses a series of SPF10 primers for higher amplification sensitivity of most more clinically relevant HPV genotypes.

**Epstein-Barr virus genome detection**

Epstein-Barr virus detection was made with the Epstein-Barr virus BMLF1 commercial kit (Maxim Biotech, Inc.), following the manufacturer’s specifications. The reaction mixture conditions were the following: 40 µl of master mix (Buffer, dNTP, MgCl₂), 0.2 µl Taq polymerase, and 10 µl of sample DNA were used to obtain a final volume of 50.2 µl. Amplification conditions were one minute at 96 °C, 35 one-minute cycles at 94 °C, one minute at 58 °C, one minute at 72 °C, and a 10-minute final amplification at 72 °C. Only those samples where a 265 bp band was observed were regarded as positive.

**Agarose gel electrophoresis**

The PCR amplification products were visualized by means of agarose gel electrophoresis at 2% with Invitrogen 10X TBE 1X stock buffer (1.0 mM base Tris, 0.9 mM borate and 0.01 mM EDTA, pH 8.0) and were stained with SYBR® SAFE (Invitrogen). Photographic registration was carried out with a ChemiDoc™ imaging system (Bio Rad).

**Table 1. Clinical characteristics of patients diagnosed with breast cancer and benign pathology**

<table>
<thead>
<tr>
<th></th>
<th>Breast cancer</th>
<th>Benign pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean</td>
<td>58 years (range 30-86)</td>
<td>29.4 years (range 14-50)</td>
</tr>
<tr>
<td>Menarche mean</td>
<td>12.5 years (range 9-17)</td>
<td>12.5 years (range 9-17)</td>
</tr>
<tr>
<td>Pregnancies mean</td>
<td>3.14 pregnancies (range 0-9)</td>
<td>1.32 pregnancies (range 0-6)</td>
</tr>
<tr>
<td>Partners mean</td>
<td>1.85 partners (range 1-4)</td>
<td>1.73 partners (range 0-5)</td>
</tr>
<tr>
<td>Use of OC</td>
<td>45.5%</td>
<td>72.7%</td>
</tr>
<tr>
<td>HPV presence</td>
<td>63.6%</td>
<td>4.5%</td>
</tr>
<tr>
<td>EBV presence</td>
<td>13.6%</td>
<td>4.5%</td>
</tr>
</tbody>
</table>

EBV: Epstein-Barr virus; HPV: human papilloma virus; OC: oral contraceptives.

**Statistical analysis**

Descriptive analyses were performed using central tendency and dispersion measures (mean, median, standard deviation) for continuous variables, and frequency analysis and contingency tables for discrete variables. The chi-square test was used to assess dependence of variables with the Microsoft Excel Office 2010 program. P-values ≤ 0.05 were considered to be statistically significant.

**RESULTS**

A total of 22 breast cancer patients and 22 benign breast pathology patients were included. Clinical characteristics of the study population are shown in table 1, where mean age, mean menarche, mean full-term pregnancies, mean number of partners, and use of oral contraceptives (OC) are indicated. Of total breast cancer samples, 63.6% corresponded to patients with stage II tumors, followed by stage I with 18.2%. With regard to histopathological diagnosis, collected samples of breast cancer tumor corresponded to ductal carcinoma in situ (DCIS), infiltrating ductal carcinoma, infiltrating lobular carcinoma, and infiltrating papillary carcinoma, with infiltrating ductal carcinoma being the most common in the group with 68.2%.

Once the genetic material was extracted from the study biopsies, its quality could be corroborated by identifying five constitutive expression genes in order to ensure the genome was in optimal conditions. All samples amplified the target genes, since the collected specimens were fresh biopsies preserved at -80 °C, without the need to be embedded in paraffin, thus avoiding possible DNA fragmentation during the processing of the sample to prepare paraffin blocks, which complicates viral DNA detection.

Subsequently, detection and genotyping was carried out, with 14 out of 22 (63.6%) breast cancer samples being found to be positive for HPV DNA, whereas in healthy tissue samples, HPV DNA was only detected in one sample, accounting for 4.5% of all benign pathology evaluated patients (Table 1).

Then typing was performed and we found that among the 14 positive samples, the most common types were 6 and 11, (27.27% each), followed by type 16 (21.21%). With regard to the HPV-infected patient with benign pathology, the high oncogenic risk HPV-33 viral genotype was identified. Figure 1 shows a bar graph with the frequency of each one of the HPV genotypes found in the patients with breast cancer.
In addition, continuing with HPV classification according to the reported viral genotype, HPV types were grouped in this work according to their oncogenic risk associated with the development of several types of carcinoma. Figure 2 depicts the frequency of infection with the high- and low-risk genotypes found in both study groups. Of the breast cancer patients, 28.57% were observed to have single infections with low- or high-risk genotypes, and 42.90% of patients showed coinfection with both genotype groups. As for the HPV DNA-positive healthy tissue sample, it was found to be of high oncogenic risk.

Within the group of cases with cancer, the presence of EBV genome was detected in three of 22 assessed samples, which accounted for 13.6% of infection, and in the healthy tissue samples, this genome was detected in one sample, accounting for 4.5% of the total (Table 1).

Of the breast cancer patients, three were observed to have HPV and EBV coinfection, which accounted for 13.6% of all evaluated patients. In the case of the group with benign pathology, the only EBV DNA-positive sample was also found to be HPV DNA-positive, and it corresponded to a patient diagnosed with abscessed mastitis. However, no statistically significant relationship was found between HPV and EBV infection in the assessed breast cancer samples (p = 0.344).

**DISCUSSION**

Clinical data of the assessed breast cancer patients are consistent with those obtained in previous publications, where the age range of breast cancer-affected patients has been reported to be between 45 and 65 years in Venezuela and between 50 and 80 years in European and North American countries, whereas benign pathologies are associated with early female ages all over the world. The high incidence of benign lesions in young women has been proposed to be a result of the hormone load being higher at younger ages, where hormone stimulus during the reproductive cycle drives to an increase in cell mitotic activity, with the risk for developing benign pathologies on that site increasing if young patients have hormone imbalances, whereas in middle-aged and elderly patients, the presence of benign pathologies is proportionally lower with higher numbers of full-term pregnancies, which possibly gradually decreases the hormone load.

In this study, the presence of HPV was detected in 63.6% of breast cancer samples, while only 4.5% of benign pathology tissue biopsies were positive for this virus. This detection frequency is within the previously published worldwide range, with a prevalence ranging from 10-86% across studies, where the works by Akil, et al. in 2008 and Antonsson, et al. in 2011 detected 61.06 and 50% of HPV DNA-positive cases by means of multiplex PCR and assembly PCR, respectively.

In Latin America, the rate of HPV infection in breast cancer is low. Positivity has been reported to range between 8-40%. Therefore, our work is the first to report high frequency of HPV in malignant breast tissue of Venezuelan female patients. However, some studies have failed to detect HPV in breast tumor or normal tissue. This difference in published reports can be attributed to the number of assessed samples, differences in methodology, and sensitivity of the employed methods, such as the use of different primer kits, as well as the type of sample used.

When identification of HPV viral genotypes was made, we found that all 14 positive samples showed single and mixed infections with oncogenic high-risk genotypes 16, 18, 52, and 56, and oncogenic low-risk genotypes 6 and 11. In malignant tumors, the most common high-risk genotype was 16 (21.21%), and in the case of low-risk genotypes, the types found were 6 and 11 (27.27% each). In the patient with benign pathology infected with HPV, viral genotype 33, of oncogenic high-risk, was identified. These identified genotypes are consistent with those reported at the global level, where the most commonly detected oncogenic high-risk genotype is HPV-16 in between 20 and 90%.

When viral genotypes were grouped according to their oncogenic risk, we found that 28.57% of breast cancer patients had single infections with high- or low-risk genotypes, and 42.90% of HPV-positive patients showed coinfection with both genotype groups.

High-risk genotypes 16 and 18 are associated with an accelerated growth of cervical epithelial cells, since they tend to integrate into the genome and drive to uncontrolled cell proliferation owing to an inhibition of cell proteins p53 and pRb function. In this work we found that out of 57.1% of HPV 16 and/or 18-infected patients, 75% of them had elevated expression of cell proliferation Ki67 marker reported.
on medical records, whereas in the rest of the patients infected with other viral genotypes (6, 11, 52, 56), only one out of six patients had K67 over-expression reported, which suggests that the presence of high-risk genotypes (16 and 18) can drive to uncontrolled cell proliferation in breast tissue.

Ever since the presence of EBV was first reported, the number of studies focused on the oncogenic potential of this and other viruses has been progressively increasing. In this sense, new investigations have been carried out associating this virus with other types of lymphomas and several types of carcinoma, such as gastric and lung carcinoma and, recently, breast carcinoma. These associations are based on the type of cells EBV is able to infect, which include B-cells and epithelial cells. In addition, most these tumors are characterized by the presence of multiple viral genome extra-chromosomal copies and expression of EBV-encoded latent genes, which contributes to the malignant phenotype.

In this study, the presence of EBV was detected in 13.6% of Venezuelan female patients with breast cancer, whereas in the studied patients with benign pathology, EBV DNA was detected in only one patient (4.5%). This result is below the values reported in other studies conducted at a global level, which range from 21-55% of EBV DNA positivity, depending on the type of technique employed for viral DNA detection. In works using some type of PCR, as in our study, low infection rates were detected, as in the trial by Xue, et al. in 2003, who, using BZLF1 gene-specific reverse transcriptase PCR, detected viral DNA in 17% of breast cancer cases, and the work by Yahia, in 2014, who detected 11% of EBV DNA positivity in breast cancer cases using PCR with EBNA-1 gene-specific primers.

In Latin America, very few studies have assessed the presence of EBV in breast cancer, and have reported a rate of infection ranging from 6-31%. Differences found with regard to other studies may be due to EBV epidemiology variations according to the geographic region.

Therefore, with EBV being a co-factor in the development of several malignancies, including different carcinomas (nasopharyngeal carcinoma, gastric carcinoma), we can suggest that the presence of EBV in breast tissue tumors might be an important risk factor for breast cancer, owing to its oncogenic potential. However, it is important to take into consideration the genomic DNA load of the virus and the amount of assessed DNA in the samples, in order to avoid discrepancies in viral DNA detection.

With regard to coinfection with HPV and EBV in breast cancer, all three patients who had EBV DNA were found to also have HPV DNA detected, accounting for 13.6% of all assessed patients with breast carcinoma, with this coinfection rate being intermediate with regard to that reported in other studies at the global level, which ranges from 2.1 to 38%.

It is important to highlight that there are only few studies that have assessed the presence of this virus in South America. In Chile, Aguayo, et al. studied coinfection with EBV and HPV, and found 6.5% of EBV positivity in breast cancer samples.

To the best of our knowledge, this is the first study to assess the presence of both viruses in Venezuelan female patients with breast cancer. Even though there was no statistically significant relationship with regard to the presence of both viral agents, the biological behavior of viruses should not be disregarded.

CONCLUSIONS

The presence of HPV was detected in 63.6% of breast cancer patients, whereas EBV was detected in 13.6%. Even when there were no statistically significant differences, we can suggest that HPV and EBV might act as important risk factors in breast cancer pathogenesis, owing to their oncogenic characteristics. Our finding is mainly based on viral genome detection with the PCR and reverse hybridization technique, and confirmatory tests would therefore be required. In addition, further investigations are needed to determine the role of HPV and EBV in breast cancer etiology or progression, including viral load assessment for both agents and determination of HPV viral integration to the host genome, since this is known to be an important step in the development of carcinogenesis that affects important viral replication checkpoints. In addition, increasing the number of samples is necessary, which will allow for a clearer tendency to be observed with regard to the relationship between the presence of these viruses and the development of breast cancer.

DECLARATION OF INTEREST

The authors declare not having any conflicts of interests.

The authors thank the funding granted by research projects PEII no. 2012001201 and FONACIT G2005000408.

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ORIGINAL ARTICLE

Interleukin-6 Induces Epithelial-Mesenchymal Transition in Breast Cancer Cells

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Received for publication: 25 October 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract  Background: Breast cancer is the leading cause of cancer-related death in Mexico, with most deaths being related to locally advanced or metastatic disease at diagnosis. Epithelial-mesenchymal transition (EMT) is one of the steps that are indispensable for metastasis. Different factors trigger EMT, like TGF-β, EGF and interleukin 6 (IL-6), among others. EMT is characterized by E-cadherin expression loss and N-cadherin and vimentin expression. In this study, we investigated the role of IL-6 on EMT induction. Methods: MBCDF and MBCD17 primary breast cancer cell cultures were used. E-cadherin expression was measured using Western Blot. Cells were stimulated with IL-6 to induce EMT. STAT3 activation was measured using phospho-specific antibodies, and E-cadherin expression was measured as EMT marker. Results: MBCDF and MBCD17 primary breast cancer cell cultures stimulation with IL-6 induced STAT3-Tyr705 phosphorylation without its total levels being altered; in addition, IL-6 cell-stimulation was shown to induce EMT, as evidenced by E-cadherin loss. Conclusions: The results of the present work suggest that IL-6 induces EMT in primary breast cancer cell cultures through STAT3 phosphorylation.
INTRODUCTION

Globally, breast cancer accounts for 25% of all malignancies in women, which places it as the most common in this group. In Mexico, it is the leading cause of cancer-related death in women since 2006-12. In 2012, an increase in breast cancer incidence was reported, from 2% in 1980 to 5% being reported in 2010-13. In 2009, the Mexican epidemiological surveillance system published a nation-wide incidence of 15 cases per 100,000 population, with the highest incidences being noted in Distrito Federal (now Mexico City), Coahuila and Nuevo León, with 17, 18 and 14 cases per 100,000 population, respectively, while the state with the lowest documented incidence was Chiapas, with 1.5 cases per 100,000 population. With regard to these data, it should be noted that in Mexico there is no national cancer registry, and the reported figures are therefore only an estimate of breast cancer actual situation in the country.

Breast cancer molecular study has enabled to classify the disease in different subtypes, with the purpose to translate this information into targeted therapies and define prognostic groups. In the past few decades, this classification has undergone modifications that represent research advances and adaptations for global classification criteria, with a specific value of Ki67 being eliminated and clinical parameters and multi-parametric molecular markers being added, as main modifications. Hence, luminal tumors, characterized by hormone receptor expression and HER2 non-expression, which are subdivided in luminal A-type, such as those tumors where immunohistochemistry analysis reveals estrogen receptor (ER) and progesterone receptor (PR) high expression, a clearly low Ki67 determination, and tumors classified by size as T1 and T2 and involvement of 0 to 3 lymph nodes; if access to multi-parametric molecular markers determination is available (Oncotype DX®, MammaPrint®), the result should be a favorable risk assessment. This subtype accounts for 40% of breast cancer cases and is associated with favorable prognosis. Luminal B subtype is characterized by hormone receptor low expression, clearly high Ki67, nodular involvement higher than 3 lymph nodes, histological grade 3 (poorly differentiated tumor), extended lymph-vascular invasion and bulky tumors (T3); this type accounts for 20% of breast cancer cases and is associated with a mortality risk of 1.96 (95% CI: 1.08-3.54). HER2 overexpression (25% of tumors) or the lack of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression is defined as “triple-negative” (15 to 20% of breast cancer cases), these two groups considered of poor prognosis, with a mortality risk of 7.39 (95% CI: 1.72-31.77) and 12.41 (95% CI: 5.82-26.49), respectively. It should be noted that, in women younger than 40 years, the factors that more negatively influence on overall survival are lymph node infiltration or triple-negative molecular subtype-14.

The introduction of targeted therapies to specific molecules such as the epidermal growth factor receptor (Herceptin®, TDM-1, Pertuzumab®), have achieved objective tumor responses of up to 70% when combined with chemotherapy, and have improved overall survival. This is only effective for a selected group of patients; however, the disease has been observed to be able to progress over time and to acquire the capability to generate metastasis to other sites-15.

One of the first events in tumor cell dissemination is the loss of the epithelial phenotype through drastic changes in the cytoskeleton. These series of events are known as epithelial-mesenchymal transition (EMT), which is a process whereby epithelial cells undergo biochemical changes to transform into mesenchymal cells; intercellular bonds are lost and cells therefore become elongated and non-polarized, which allows them to move across the extracellular matrix, by means generating a new set of tumor cells with different polarity than the epithelium they originated in-16. EMT is not a process exclusive to tumor cells, but it has been described in 3 different biological scenarios: Type 1 EMT participates in embryo implantation, embryogenesis and organogenesis; type 2 EMT is associated with wound healing, tissue regeneration and fibrosis in organs such as the kidney; and type 3 EMT is involved in tumorigenesis, which accounts for the progression of a carcinoma in situ to an invasive one-17, and has also been associated with resistance to apoptosis and chemotherapeutic drugs-18. The role of EMT in cancer is already well established, and it is assumed to be an indispensable component for metastasis-19. Once tumor cells have reached the distant metastasis site, they return to the primary tumor phenotype through a process known as mesenchymal-epithelial transition (MET). Therefore, EMT steps appear to be reversible, thus indicating the existence of dynamic components in tumor progression-20.

Several markers implied in EMT have been described, and one of the most widely studied is E-cadherin expression decrease, which is a protein involved in cell-cell adhesion that lowered its levels to the minimum with an exchange for N-cadherin. There are other markers that are positively regulated in EMT, such as: vimentin, fibronectin, smooth muscle actin; transcription factors such as Snail, Twist, Slug and ZEB; in addition to growth factors such as platelet-derived growth factor (PDGF)12-15. Other cell-cell adhesion molecules such as claudin 3, 4, 7, α-catenin, γ-catenin and occludins are negatively regulated in cells with the mesenchymal phenotype13-15. EMT is a dynamic process and is activated by different stimuli of the tumor microenvironment, which include growth factors, tumor cell-stroma interactions and hypoxia-18. Signals that activate EMT include growth factors such as transforming-growth factor β (TGF-β), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), insulin-like growth factor 1 and 2 (IGF 1 and 2) and epidermal growth factor (EGF)-19.

In the past few years, the influence of tumor microenvironment has started to be taken into account in tumor development and behavior. Cytokines and growth factors secreted by cells surrounding tumors have been observed to act as crucial agents in tumor cells biological processes such as proliferation, migration, invasiveness and metastasis. Owing to this, systemic and tumor microenvironment markers, which are related to innate and acquired immune response have shown to play a crucial role in anti-tumor response-19. Tumor microenvironment is composed by different cell types, including tumor-associated fibroblasts, neuroendocrine cells, adipocytes, inflammatory, endothelial and lymphatic cells-19. All these cells types contribute to tumor development by secreting growth factors, metalloproteinases and cytokines that are necessary for tumor development.

One of the cytokines that has been implied in tumor progression is interleukin-6 (IL-6). This is a pleiotropic cytokine
that is produced by hematopoietic and epithelial cells. Since its identification in mononuclear cell cultures supernatant, its role in biological functions such as B cell differentiation and T cell proliferation has been described. In breast, kidney and prostate cancer, as well as in myeloma multiply, it has been correlated with poor prognostic and tumor aggressiveness. Recent studies characterize IL-6 as a VEGF positive regulator. IL-6 circulating levels have been found to be 10-fold higher in patients with breast cancer than in healthy women, with a correlation existing between higher levels of IL-6 and breast cancer more advanced stages. The study of IL-6 in breast cancer cells in vivo has yielded controversial results: on one hand, its implication in doxorubicin resistance and in the promotion of the motility required for metastasis have been demonstrated, and on the other, treatment with low-dose IL-6 for 6 days has been shown to inhibit ER-expressing cells proliferation in vitro via apoptosis activation by DNA fragmentation. IL-6 has also been implicated as an EMT promoter by inducing E-cadherin expression repression.

IL-6 signaling occurs through interaction with its receptor (IL-6R), and membrane-binding glycoprotein gp130, which is bound to JAK1,2. JAKs are in charge to phosphorylate (IL-6R), and membrane-binding glycoprotein gp130, which is bound to JAK1,2. JAKs are in charge to phosphorylate IL-6-stimulation assays were carried out in order to assess STAT3 phosphorylation by Western blot, using a phospho-specific antibody against pSTAT3 Tyr705 in MBCDF and MBCD17 cells; for this, 1 X 10⁶ cells were seeded in 60-mm culture plates, maintained in RPMI-1640 medium supplemented with 10% bovine fetal serum, antibiotic and antimicotic (Invitrogen Corporation, Camarillo, CA) at 37 °C in a moisturized atmosphere with 5% CO₂.

**Materials and Methods**

**Reagents**

Primary antibodies against E-cadherin, tubulin, pSTAT3 (Tyr705) and STAT3 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and E-cadherin was obtained from Cell Signaling Technology (Cambridge, MA). Anti-mouse or anti-rabbit secondary antibodies were acquired from Jackson Immunoresearch (West Grove, PA), Interleukin-6 (IL-6) was obtained from PeproTech (RockyHill, NJ).

**Cell culture**

MBCDF and MBCD17 primary breast cancer cells, which were derived from a biopsy of the specimen resulting from a mastectomy performed in a patient with breast cancer (protocol approved by the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán Ethics Committee, ref. 1549, BQ0-008-06/9-1). The cell cultures were maintained in RPMI-1640 medium supplemented with 10% bovine fetal serum, antibiotic and antimicotic (Invitrogen Corporation, Camarillo, CA) at 37 °C in a moisturized atmosphere with 5% CO₂.

**Cell stimulation**

IL-6-stimulation assays were carried out in order to assess STAT3 phosphorylation by Western blot, using a phospho-specific antibody against pSTAT3 Tyr705 in MBCDF and MBCD17 cells; for this, 1 X 10⁶ cells were seeded in 60-mm culture plates, maintained in RPMI-1640 medium supplemented with 10% bovine fetal serum. The cells were allowed to adhere overnight at 37 °C and 5% CO₂. Stimulation was applied with IL-6 1 ng/mL for the following time intervals: 0, 5, 15, 30 and 60 minutes.

For the EMT induction and reversion assays, MBCD17 epithelial cells, two experimental models were designed: for the first one, short times of EMT IL-6-mediated induction were used of 0, 4, 8, 12 and 24 h.

**Immunoblot assay (Western Blot)**

Stimulated cells were lysated with a lysis buffer containing: HEPES 50 mM (pH 7.4), EDTA 1 mM, NaCl 250 mM, 1% Nonidet, NaF 10 mM and 1 x protease inhibitors (Complete, EDTA-free, Roche). 25 µg total protein underwent denaturation polyacrylamide gel electrophoresis and were transferred to Immobilon-P PVDF membranes (Millipore Corp, Bedford, MA), which were blocked for 60 minutes in 5% skim milk in 0.05% PBS-Tween. Then, they were incubated with the respective antibodies overnight at 4 °C with agitation. Subsequently, the membranes were incubated with the anti-mouse or anti-rabbit-HRP antibodies, as appropriate, for 45 minutes. The signal was visualized by chemoluminescence using the Super Signal West Pico kit (Thermo, Rockford, IL) and was finally exposed to a Kodak radiographic film.

**Results**

IL-6 induces STAT3 phosphorylation in breast cancer cells

It is well established that IL-6 signals through STAT3 activation. To demonstrate that IL-6 was able to induce STAT3 activation in the primary breast cancer cell cultures, we stimulated the MBCDF and MBCD17 cells with 5 ng/mL of IL-6 for...
different time intervals. STAT3 activation was measured as STAT3 Tyr705 residue phosphorylation using phospho-specific antibodies. The results demonstrate that IL-6 induces STAT3 phosphorylation. Phosphorylation of STAT3 had an activation peak in MBCDF cells at between 15 and 30 min, whereas in MBCD17 cells, pSTAT3-Tyr705 activation peak occurred at 15 minutes (Fig. 1). These results confirm that, in these cultures, IL-6 signaling is mediated by STAT3 activation.

IL-6 induces epithelial-mesenchymal transition

Once we demonstrated that IL-6 induces STAT3 phosphorylation in primary breast cancer cell cultures, we investigated whether IL-6 stimulation elicits a decrease in E-cadherin expression as an EMT marker. IL-6 was found to induce a slight drop in E-cadherin expression from 2 h, which becomes more pronounced from 12 h and onwards. An anti-tubulin antibody was used as loading control. These results suggest that IL-6 induces rapid changes in E-cadherin expression, as a marker indicating that the tumor cell enters in EMT process (Fig. 2).

DISCUSSION

EMT is one of the most critical steps in the development of metastasis, and the description of the molecular mechanisms of which becomes activate and how it can be inhibited or reverted are therefore highly relevant to the development of new treatment strategies. In this work, we present evidence that IL-6 induces EMT through E-cadherin decrease in primary breast cancer cells. These data suggest that IL-6 is an important cytokine within the tumor microenvironment, which participates in breast cancer process of metastasis.

One of the main causes of mortality in breast cancer patients is metastasis. The process of metastasis includes several steps that a transformed cell has to complete to migrate to a distant site. Crucial steps of this process include EMT, which is characterized by cell polarity loss and invasive properties acquisition. In order to be able to study metastatic cells properties, we developed an EMT in vitro model based on primary breast cancer cell cultures. EMT is a dynamical process, and it is activated by different stimuli of tumor microenvironment, including growth factors, tumor cell-stroma interactions and hypoxia. EMT-activating signals include growth factors such as transforming growth factor beta (TGF-β), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), insulin-like growth factors 1 and 2 (IGF 1 and 2) and epidermal growth factor (EGF). IL-6 is an inflammatory cytokine that has been associated with EMT. In our primary breast cancer cell culture model, we stimulated epithelial marker-bearing cells (MBCDF and MBCD17) with IL-6 trying to induce EMT, demonstrating that IL-6 induces a decrease in E-cadherin expression in hours through STAT3 phosphorylation. These results demonstrate that, in our in vitro model of primary breast cancer cell culture, treatment with IL-6 is able to induce EMT, as demonstrated by the loss of E-cadherin expression.

CONCLUSIONS

In summary, our work demonstrates that IL-6 is a potent EMT inductor through STAT3 activation in primary breast cancer cell cultures. The implications of this suggest that the presence of IL-6 in the tumor microenvironment confers high metastatic potential. These data support the development of new therapeutic strategies for breast cancer treatment, as IL-6 inhibition could be, as an attractive approach to intervene with EMT.

CONFLICT OF INTERESTS

The authors declare no conflicts of interests.

REFERENCES


Abstract  Epidermal growth factor receptor is preferably expressed in head and neck squamous cell carcinomas and is a promising therapeutic target. Cetuximab is the only epidermal growth factor receptor-targeted agent that has been approved for the treatment of squamous cell carcinoma. The 2006 FDA-approved indication refers to the use of cetuximab in combination with radiotherapy for the treatment of locoregional, advanced, unresectable head and neck squamous cell carcinoma, except for nasopharyngeal carcinoma. In 2011, the use of cetuximab in combination with platinum and 5-fluorouracil was approved as first-line treatment for recurrent and/or metastatic head and neck squamous cell carcinoma. In order to homogenize and arrive at a multidisciplinary, multi-institutional consensus based on scientific evidence, a meeting was held where the existing literature was reviewed and the role of cetuximab in the treatment of patients with head and neck squamous cell carcinoma was discussed. This work reviews current evidence-based indications for the use of cetuximab in the treatment of patients with head and neck squamous cell carcinoma. (creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

Cetuximab (CTX) is a chimeric anti-epidermal growth factor receptor (EGFR) IgG1 monoclonal antibody. Binding of the antibody to the receptor blocks endogenous ligands (epiregulin, amphiregulin, betacellulin, transforming growth factor, epidermal growth factor) binding and promotes internalization of the receptor, which leads to EGFR signaling cascade deregulation. The blockade inhibits cell proliferation, angiogenesis, and metastasis and restores apoptosis. At the extracellular level, it promotes immune system cytotoxic cells attack against EGFR-expressing tumor cells, due to the recognition of the IgG1 Fc region by natural killer cells. In combination with radiotherapy (RT), CTX inhibits DNA repair and tumor angiogenesis, while facilitating apoptosis, radio-sensitizing cells at G1 phase, and reducing radio-resistance of cells at S phase.

Head and neck squamous cell carcinoma (HNSCC) treatment of choice has been surgery followed by radiotherapy; however, at locoregionally advanced or inoperable stages, the association of chemotherapy (CT) with RT (CTRT) emerged in the 1990s as an alternative that offered better control than RT alone. Subsequent studies demonstrated that concomitant CTRT had superior outcomes when compared with RT alone, with overall survival (OS) improvement in patients with unresectable tumors, in patients at high risk or relapse after surgery, and as a non-surgical conservative option of choice for advanced laryngeal and pharyngeal tumors candidate for laryngectomy. However, toxicity emerged as a limitation for systematic administration in patients who are often fragile and with serious comorbidities. Knowledge of the receptors and signaling pathways involved in the genesis and progression of HNSCC led to the development of anti-EGFR monoclonal antibodies and tyrosine kinase-inhibiting molecules. Cetuximab, a monoclonal antibody, has been tested in associations with CT and RT, and has demonstrated activity with a favorable toxicity profile. The indication approved by the FDA in 2006 refers to the use of CTX in combination with RT for the treatment of locoregionally advanced, unresectable HNSCC, except for nasopharyngeal carcinoma, in disease persisting to other treatments, in organ-preservation attempts (larynx), and concomitantly with RT in locoregionally advanced disease. In 2011, the use of CTX was approved in combination with platinum and 5-fluorouracil (5-FU) as first-line treatment for recurrent and/or metastatic HNSCC. This work reviews current indications, possible applications of the drug in adherence to the country’s characteristics.

Prior to the meeting, the participants answered a 13-question questionnaire (Table 1). The questions were formulated based on a previous literature review. The participants quantified their answers according to a level of agreement/disagreement scale from 1-5 (where 1 is total disagreement and 5 is total agreement).

RESULTS

The results are presented in figure 1 according to table 1 questions.

DISCUSSION

Question 1: In locally advanced disease, do you consider that cetuximab addition to radiotherapy represents a clinical benefit for the patient?

Bonner, et al. conducted a phase III trial to assess CTX associated with RT in patients with locally advanced oropharyngeal, hypopharyngeal, and laryngeal squamous cell carcinoma (SCC), non-candidates for surgery. Patients were randomized to RT and concomitant CTX at weekly standard doses (n = 211) vs. RT without CTX (n = 213) for 6-7 weeks. Loco-regional control was 24.4 months in patients with CTX and 14.9 months without CTX (p = 0.005). After a 54-month mean follow-up, overall survival (OS) was 49.0 and 29.3 months with and without CTX, respectively (p = 0.03), progression-free survival (PFS) was 17.1 and 12.4 months with and without CTX, and, finally, CTX addition decreased the risk for progression (p = 0.006) and death by 26%. The study concluded that concomitant treatment with RT and CTX improves locoregional control and reduces mortality without increasing the most common RT-associated adverse events. Five-year OS was 45.6 and 36.4% in the groups with and without CTX, respectively. The OS was higher in patients who developed acneform rash (at least grade 2) compared
to those who developed grade 1 rash or did not experience it at all \( (p = 0.002) \). This was the first study to demonstrate the additive effects of CTX and RT by improving survival in advanced SCC versus RT alone. By the time the study was published, concomitant CTRT had become the standard of care for unresectable HNSCC. Dattatreya, et al. \(^6\) recorded 19 unresectable patients treated with CTX/RT, with Bonner’s scheme, and observed overall responses (OR) of 68.42% and two-year OS of 84%. Two years after the protocol was concluded, 13 patients remained free of progression. These results corroborated Bonner’s findings. Okano, et al. \(^7\) assessed CTX with concomitant RT boost in 22 patients with standard-dose CTX for seven weeks and RT at 1.8 Gy once daily for 3.6 weeks, followed by 1.8 Gy in the morning and 1.5 Gy at noon for 2.4 weeks. All patients completed at least 70% of the scheme. At eight weeks, OR was 82%. Table 2 compiles different results of studies with CTX in locally advanced disease. In summary, CTX addition to RT improves OS and locoregional control when compared with RT alone.

**Question 2:** In locally advanced disease, do you consider the efficacy of cetuximab concomitant with radiotherapy equivalent to chemo-radiotherapy efficacy?

There are no comparative studies between CTX/RT and high-dose CTRT. Lefebvre, et al. \(^13\) compared two groups post-induction QT, with 116 cases being analyzed. No significant differences were observed between both groups with regard to larynx preservation (95 vs. 93%), laryngeal function preservation (87 vs. 82%) and OS (92 vs. 89%). Levy, et al. \(^23\) made an indirect comparison in a meta-analysis that included studies of cisplatin plus RT vs. RT alone, and CTX plus RT vs. RT alone. The study failed to find evidence of superiority between CTRT and CTX/RT when locoregional control and OS outcomes were analyzed. Both treatments can be considered equally efficacious when administered together with RT. Treatment selection can be made based on the toxicity profile. In summary, CTX/RT offers the same possibility of organ preservation as CTRT; however, there are no studies comparing both treatments in locoregionally advanced disease.

**Question 3:** In locally advanced disease, do you consider there is a benefit when using cetuximab concomitantly with radiotherapy after induction chemotherapy?

Lefebvre, et al. \(^13\) compared the efficacy and safety of induction CT followed by CTRT or CTX/RT with the purpose of preserving the larynx. Previously untreated patients with stage III or IV larynx/hypolarynx cancer received three induction CT cycles with docetaxel 75 mg/m\(^2\) and cisplatin (CDDP), each on day 1, and 5-FU 750 mg/m\(^2\) on days 1-5. Patients with responses < 50% underwent laryngectomy. Patients with higher responses were randomized to standard RT (70 Gy) and CDDP (100 mg/m\(^2\)/day) on days 1, 22, and 43 of RT (group A) or to standard RT and CTX at standard...
doses during RT (group B). Three months later, organ preservation was assessed, with pharyngeal function and OS being evaluated 18 months later. Out of 153 initial cases, 116 were analyzed. No significant differences were observed between group A and B with regard to larynx preservation (95 vs. 93%), laryngeal function preservation (87 vs. 82%) and OS (92 vs. 89%), respectively. However, treatment tolerance was superior with CTX/RT, and rescue surgery was feasible only in patients treated with CTX/RT. CTRT acute toxicity generated more protocol changes in comparison with CTX/RT. In the CTRT group, 22.4% of patients developed chronic renal toxicity. With regard to treatment compliance, 42% of patients received all three CDDP planned cycles in group A, and 71% of patients received all seven CTX planned cycles in group B. The study corroborated the favorable toxicity profile of the CTX-scheme, as well as higher rates of compliance, and failed to demonstrate clinical superiority of CTRT over CTX/RT. Two additional studies explored the efficacy of induction CT followed by CTX/RT with CTX. Keil, et al. assessed 49 patients who received three induction CT cycles: docetaxel (75 mg/m²), CDDP (75 mg/m²) on day 1, and 5-FU (750 mg/m²/day) on days 1 through 5, followed by CTX/RT with CTX at standard weekly doses. Forty-four patients received RT plus CTX. At three months, complete response (CR) was observed in 33 patients. Two years later, 25 patients remained with CR. Two-year PFS was 59% and two-year OS was 63%. The most common adverse effects were radiodermatitis (30%), mucositis (27%), and non-febrile neutropenia (17%). Rampino, et al. also assessed two cycles of docetaxel, CDDP, and 5-FU followed by CTX/RT. In 36 stage III and IV patients, CR was 60.6% and partial response (PR) was 33.3%. Toxicity included febrile neutropenia (6%) during induction, and dermatitis (48%), mucositis (33%), and dysphagia (12%) during the CTX/RT phase. In summary, CTX/RT after induction CT in responders with organ-preservation attempt offers the same results as CTRT, with a better safety profile and higher treatment adherence. In addition, rescue surgery in patients failing after CTX/RT has higher rates of success and lower rates of complications than that in patients treated with concomitant CT and RT.

**Table 2. Cetuximab in locoregionally advanced head and neck squamous cell carcinoma**

<table>
<thead>
<tr>
<th>(n)</th>
<th>Neoadjuvance</th>
<th>Treatment</th>
<th>Clinical indicators</th>
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<tbody>
<tr>
<td></td>
<td>First-line</td>
<td>Adjuvance</td>
<td>OR</td>
<td>CR</td>
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</table>
| 47  | CTX+P+Ca     | RT or CTRT or SX | 19% | 77% | 91% | 3 y | 87% | 3 y | Kles, et al. 2010  
|     |              |           |      |     |    |    |     |     |     |
| 30  | CTX+P+C-5-FU | RT+C      | 53% |     | 84% | 2 y | 65% | 2 y | Adkins, et al. 2013  
|     |              |           |      |     |    |    |     |     |     |
| 22  | CTX+D+C+5-FU | RT+CTX    | 3/18 | 15/18 |     |     |     |     | Charalambakis et al. 2013  
|     |              |           |      |     |    |    |     |     |     |
| 74  | CTX+P+Ca     | RT+CTX+P+Ca | 78% | 3 y | 55% | 3 y |     |     | Wanebo, et al. 2014  
| 39  | CTX+D+C      | RT+CTX+C  | 74% | 3 y | 70% | 3 y |     |     | Argiris, et al. 2010  
| 211 | CTX          | RT+CTX    | 49 mo | 17.1 mo | 24.4 mo |     |     |     | Bonner, et al. 2006  
| 19  | RT+CTX       | 68.4%     |     | 84% | 2 y |     |     |     | Dattatreya, et al. 2011  
| 22  | RT+CTX       | 82%       |     |     |     |     |     |     | Okano, et al. 2013  
| 116 | TPF          | RT+CTX    | 89% |     |     |     |     |     | Lefebvre, et al. 2013  
| 49  | TPF          | RT+CTX    | 33/44 | 63% | 59% | 2 y |     |     | Keil, et al. 2013  
| 36  | TPF          | RT+CTX    | 60.6% | 33.3% |     |     |     |     | Rampino, et al. 2012  
| 91  | RT+CTX       | CTX       |     |     |     |     | 59% | 1 y | Mesia, et al. 2013  
| 20  | RT+CTX+G     | 100%      | 61.5% | 38.5% | 53 mo |     |     |     | Granados, et al. 2011  
| 60  | RT+CTX+C     | CTX       | 66.7% | 39% | 28% | 66% | 2 y | 47% | 2 y | Egloff, et al. 2014  
| 238 | RT+CTX+C     |          |     |     |     |     | 19.4 mo |     | Harari, et al. 2014  
|     | RT+CTX+D     |          |     |     |     |     |     |     |     |     |
| 45  | RT+CTX+C-5-FU| 71%       | 32.6 mo | 79% | 2 y | 66% | 2 y |     |     | Merlano, et al. 2011  
| 43  | RT+CTX+P+Ca  | 84%       | 59% | 3 y | 58% | 3 y | 72% | 3 y | Suntharalinga, et al. 2012  
| 33  | RT+CTX+5-FU+H| 86% | 2 y | 69% | 2 y | 83% | 2 y |     | Kao, et al. 2011  

5-FU: 5-Fluorouracil; C: cisplatin; Ca: carboplatin; CR: complete response; CTRT: chemo-radiotherapy; CTX: cetuximab; D: docetaxel; G: gemcitabine; H: hydroxyurea; LRC: locoregional control; mo: months; n: patient number; OR: overall response; OS: overall survival; P: paclitaxel; PFS: progression-free survival; PR: partial response; RT: radiotherapy; SX: surgery; y: years; TPF: taxane, platinum and fluorouracil.
Table 3. Cetuximab in recurrent/metastatic head and neck carcinoma

<table>
<thead>
<tr>
<th>(n)</th>
<th>Treatment</th>
<th>Clinical indicators</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>CR</td>
<td>PR</td>
</tr>
<tr>
<td>117 C+CTX</td>
<td>26</td>
<td>9.2 mo</td>
<td></td>
</tr>
<tr>
<td>96 C+CTX</td>
<td>10</td>
<td>6.1 mo</td>
<td></td>
</tr>
<tr>
<td>222 C or Ca+5-FU+CTX</td>
<td>36</td>
<td>10.1 mo</td>
<td></td>
</tr>
<tr>
<td>121 C+5-FU+CTX</td>
<td>24</td>
<td>11.0 mo</td>
<td></td>
</tr>
<tr>
<td>33 C+5-FU+CTX</td>
<td>36</td>
<td>14.1 mo</td>
<td></td>
</tr>
<tr>
<td>68 C+5-FU+CTX</td>
<td>56</td>
<td>12.6 mo</td>
<td></td>
</tr>
<tr>
<td>46 P+CTX</td>
<td>54 22 32</td>
<td>8.1 mo</td>
<td></td>
</tr>
<tr>
<td>22 P+CTX</td>
<td>55</td>
<td>9.1 mo</td>
<td></td>
</tr>
<tr>
<td>42 P+CTX</td>
<td>38</td>
<td>7.6 mo</td>
<td></td>
</tr>
<tr>
<td>84 CTX+D</td>
<td>11</td>
<td>6.7 mo</td>
<td></td>
</tr>
<tr>
<td>54 C+D+CTX</td>
<td>44</td>
<td>14 mo</td>
<td></td>
</tr>
<tr>
<td>73 RT+CTX</td>
<td>59.4</td>
<td>18 mo</td>
<td></td>
</tr>
<tr>
<td>18 RT+CTX</td>
<td>47</td>
<td>8.3 mo</td>
<td></td>
</tr>
<tr>
<td>70 RT+CTX</td>
<td></td>
<td>24.5 mo</td>
<td></td>
</tr>
<tr>
<td>60 RT+CTX</td>
<td>58.4</td>
<td>47.5% 1 y</td>
<td></td>
</tr>
<tr>
<td>50 RT+CTX</td>
<td></td>
<td>10 mo</td>
<td>33% 1 y</td>
</tr>
<tr>
<td>28 RT+CTX</td>
<td></td>
<td>64 1 y</td>
<td></td>
</tr>
</tbody>
</table>

5-FU: 5-Fluorouracil; C: cisplatin; Ca: carboplatin; CR: complete response; CTRT: chemo-radiotherapy; CTX: cetuximab; D: docetaxel; G: gemcitabine; H: hydroxyurea; LRC: locoregional control; mo: months; n: patient number; OR: overall response; OS: overall survival; P: paclitaxel; PFS: progression-free survival; PR: partial response; RT: radiotherapy; SX: surgery; y: years; TPF: taxane, platinum and fluorouracil.

**Question 4:** In recurrent/metastatic disease, do you consider the efficacy of cetuximab concomitant with chemotherapy to be equivalent to the efficacy of chemotherapy alone?

The European Society for Medical Oncology (ESMO) and National Comprehensive Cancer Network (NCCN) international guidelines support the use of CTX as first-line treatment in patients with persistent, recurrent/metastatic disease. The pivotal study was coordinated by Vermorken, et al. As a previous exercise in 2007,24 his group assessed the feasibility and safety of CTX monotherapy at usual doses for six weeks in patients progressing on platinum-based therapy (2-6 cycles). Out of 103 patients whose disease progressed, 53 received CTX with or without platinum. With monotherapy, response rate was 13%, disease control 46%, and time to progression was 70 days. With combined therapy, OR was zero, disease control occurred in 26%, and time to progression was 50 days; OS reached 178 days. This study showed that single-drug CTX is active and well tolerated. One year later, the same author published the phase III EXTREME trial. The study enrolled 422 patients with untreated recurrent/metastatic SCC; half of them received CDDP (100 mg/m²) on day 1, or carboplatin (AUC = 5) plus 5-FU (1000 mg/m²/day) during four days every three weeks for a maximum of six cycles. The remaining patients received the same CT plus CTX at standard doses for a maximum of six cycles. Patients with stable disease (SD) on treatment with CT plus CTX continued with CTX until progression or unacceptable toxicity. The study demonstrated that CTX addition to CDDP or carboplatin and 5-FU significantly improves response with regard to CT alone. Addition of CTX prolonged OS from 7.4 to 10.1 months (p = 0.04), PFS from 3.3 to 5.6 months (p < 0.001) and increased the response rate from 20 to 36% (p < 0.001). Finally, the addition of CTX decreased the risk of death by 20%. Treatment duration with CTX was 18 weeks. The CTX relative dose intensity (RDI) was higher than 80% in 84 and 82% of patients at first phase of therapy and at follow-up, respectively. This was the first randomized trial to demonstrate benefit when adding a new drug to CDDP-based therapy over CT alone, thus concluding that the addition of CTX to platinum and 5-FU-based CT increases OS as first-line in patients with recurrent/metastatic HNSCC. The triple combination of CTX, CDDP, and 5-FU has been repeatedly assessed. Yoshino, et al.26 assessed the CTX, CDDP, and 5-FU combination as first-line therapy in metastatic recurrent SCC, using CTX at standard weekly doses, CDDP at 100 mg/m² on day 1, and 5-FU at 1000 mg/m²/day on days 1 through 4, for a maximum of six cycles. The OR rate was 36%, disease control rate was 88%, and PFS and OS were 4.1 and 14.1 months, respectively. With the same protocol, Guo, et al.27 explored the addition of CTX to cisplatin and 5-FU-based CT in 68 Asian patients. The OR rate was 55.9%, including two complete responses. The OS was 12.6 months.
and PFS was 6.6 months. De Mello, et al. \(^{28}\) retrospectively assessed 121 patients who received CDDP plus 5-FU and CTX every three weeks for a maximum of six cycles. Patients with stable disease continued to receive CTX until progression or unacceptable toxicity. The addition of CTX led to an OS of 11 months and PFS of eight months. The disease control rate was 48.9% and OR rate was 23.91%. Table 3 compiles different results of trials with CTX in recurrent/metastatic disease. In summary, the highest response rate and the best control are obtained in patients receiving the

<table>
<thead>
<tr>
<th>Grade II</th>
<th>Grade III</th>
<th>Grade IV</th>
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<tbody>
<tr>
<td>Follow-up/Continuity*</td>
<td>Twice-weekly. Continue treatment.</td>
<td>Assess the need for daily follow-up. Frequent monitoring looking for signs of local or systemic infection. In case of reaction occurring with doses ≥ 50 Gy, consider brief interruption.</td>
</tr>
<tr>
<td>Management</td>
<td>1. Dry desquamation without scales: Corticosteroid-based creams or ointments for a limited period (1-2 weeks). In the presence of signs of infection, topical antiseptics and antibiotics. Consider them for prevention of more severe reactions.</td>
<td>1. Confluent moist desquamation without scales: Topical antiseptic. Consider topical corticosteroids daily application to reduce inflammation for a limited period (1-2 weeks). Topical antibiotics against <em>Staphylococcus aureus</em> if there are signs of infection. Consider systemic antibiotics in case of more severe infection. Eosin or zinc preparations on folds.</td>
</tr>
<tr>
<td>Management</td>
<td>2. Moist desquamation in folds: Topical antiseptic. Consider topical corticosteroids daily application to reduce inflammation for a limited period (1-2 weeks). Topical antibiotics against <em>Staphylococcus aureus</em> if there are signs of infection. Consider systemic antibiotics in case of more severe infection. Eosin or zinc preparations on folds.</td>
<td>2. Confluent moist desquamation with scales: Topical antiseptic. If the infection increases in intensity, consider the use of IV antibiotics in the absence of response to oral therapy. Consider debridement with hydrogels. Avoid trauma to prevent infections. If hydrocolloid dressings are used, dressing thickness should be considered in RT dosimetry. Hydrofiber dressings can be used after RT completion.</td>
</tr>
<tr>
<td>Management</td>
<td>3. Dry desquamation with isolated, non-hemorrhagic scales: Topical antiseptics. Consider topical corticosteroids daily application to reduce inflammation for a limited period (1-2 weeks). Topical antibiotics against <em>Staphylococcus aureus</em> if there are signs of infection. Consider systemic antibiotics in case of more severe infection. Eosin or zinc preparations on folds.</td>
<td></td>
</tr>
</tbody>
</table>

*In grade I, follow-up frequency is weekly, unless there is rapid progression.
Adapted from Bernier, et al. 2011\(^{44}\)*

Table 4. Management strategies for patients developing radiodermatitis while on treatment with cetuximab plus radiotherapy
CT/CTX association, with this association therefore being the treatment of choice in recurrent/metastatic disease.

**Question 5: In locally advanced and recurrent/metastatic disease, do you consider the cetuximab toxicity profile to be predictable and manageable?**

Although in CTX/RT-treated patients dermatitis intensity is higher and appears earlier in comparison to RT alone, resolution times are shorter, treatment compliance rates are higher, and skin sequels such as scars generally do not persist. In summary, currently available information allows concluding that bio-radiotherapy-associated dermatitis is a predictable, manageable, and reversible event, the correct management of which does not affect treatment continuity or clinical outcome. Tables 4 and 5 summarize considerations on management and patient education exposed by Russi, et al.\(^{43}\) and Bernier, et al.\(^{44}\).

**Question 6: In locally advanced disease, do you consider there are differences in chemo-radiotherapy vs. bio-radiotherapy toxicity profiles?**

Lefebvre, et al.\(^{13}\) compared the efficacy and safety of induction CT followed by CTRT or CTX/RT for larynx preservation. There were no differences in grade 3 or 4 mucositis between groups, and the CTX/RT group had more grade 3 and 4 skin reactions. The CTRT group showed more renal and hematologic toxicity and decreased functional activity leading to more protocol modifications owing to acute toxicity (57 vs. 34%). With regard to late toxicity (at least six months after treatment completion), CTRT led to more renal dysfunction (22.4 vs. 0%). Taberna, et al.\(^{45}\) identified CTRT as a risk factor for moderate (odds-ratio [OR]: 0.292; 95% confidence interval [CI]: 0.125-0.680; \(p = 0.004\)) and severe late toxicity (OR: 0.299; 95% CI: 0.0909-0.999; \(p = 0.05\)) in comparison with CTX/RT. In summary, patients treated with CTRT with CDDP show higher rates of chronic, severe and irreversible toxicity in comparison with patients treated with CTX/RT.

**Question 7: In recurrent/metastatic disease, do you consider that the cetuximab toxicity profile permits its use as long-term maintenance therapy?**

With the EXTREME scheme, the incidence of grade 3 or 4 adverse events was similar between the compared groups (CTX/CT and CT alone), except for skin reactions (9 vs. 1%), hypomagnesemia (5 vs. 1%) and sepsis (4 vs. 1%), with greater effects associated with CTX\(^{31}\). In summary, in recurrent and/or metastatic disease, CTX-produced toxicity is not maintenance-treatment limiting.
Question 8: In locally advanced disease, does cetuximab addition to radiotherapy negatively affect patients’ quality of life?

Curran, et al.46 assessed Bonner’s study patients’ quality of life using the European Organization for Research and Treatment of Cancer (EORTC) quality of life QLQ-C30 and head and neck QLQ-35 questionnaires at treatment initiation and at 1, 4, 8, and 12 months. Out of 424 patients, 213 received RT alone and 211 RT plus CTX. The CTX/RT improved locoregional control (p = 0.005) and OS (p = 0.03) vs. RT alone, with no significant differences in quality of life. In summary, CTX addition does not affect quality of life.

Question 9: In recurrent/metastatic disease, does cetuximab addition to chemotherapy negatively affect patient quality of life?

Mesía, et al.47 examined treatment impact on quality of life in patients participating in Vermorken’s study, according to EORTC criteria (QLQ-30 quality of life and QLQ-35 head and neck questionnaires). Out of 442 randomized patients, 291 completed the questionnaires. The study concluded that the addition of CTX to platinum and 5-FU-based CT does not worsen quality of life; in fact, better quality of life/health global status was demonstrated (p = 0.041), with no difference in the social functioning scale, and better control of pain and swallowing problems. In summary, cetuximab addition to chemotherapy not only does not decrease quality of life, but improves it by decreasing neoplasm-associated symptom intensity.

Question 10: In locally advanced disease, do you consider the rates of adherence to cetuximab concomitant with radiotherapy more favorable with regard to chemotherapy?

In the study of Bonner, et al.4, 90% of patients received all eight planned doses. Similarly, an important aspect to be highlighted in the study of Lefebvre, et al.13 is the compliance rates, where the percentage of patients who completed the initially planned dose was 43% in the group of patients with CTRT and 71% in the CTX/RT group. The protocol was modified due to acute toxicity in 57% of patients with CTRT and in 34% with CTX/RT. Another study with larynx-preservation purposes using neoadjuvant CT with docetaxel, CDDP and 5-FU followed by CTX/RT or CTRT, revealed higher rates of compliance with the use of CTX/RT (79.5%) vs. CTRT with CDDP (51.7%). In contrast, the number of patients receiving therapy with CDDP has been observed to decrease with increasing treatment duration: using a CTRT scheme (CDDP 100 mg/m² q3wk), compliance rates of 88, 66 and 49% were obtained at first, second, and third cycle of treatment, respectively. In summary, treatment adherence rates are higher with CTX than with chemotherapy.

Question 11: In recurrent/metastatic disease, do you consider that cetuximab addition to chemotherapy negatively affects treatment adherence rates?

In the EXTREME trial31, 84% of patients receiving CTX after initial loading dose recorded a relative dose intensity (RDI) of 80% or higher, and 82% of patients reported an equal RDI in the maintenance phase. Patients in the CTX group received a mean of five CT cycles, and patients in the CT alone group, four cycles. For 89% of patients in the CTX group and 86% in the CT alone group, RDI was 80% or higher. Similarly, the GORTEC study35 reported a RDI of 80% or higher in 84% of patients, and 79% of patients initiated the maintenance phase. During that phase, RDI for CTX was close to 100%; mean duration of the maintenance phase was 4.6 ± 4.5 months. One patient was treated with CTX during maintenance for a period longer than 22 months. In summary, adherence rates were similar to those for CT in both groups, suggesting that CTX addition did not affect standard treatment tolerance.

Question 12: Do you consider that patients treated with bio-radiotherapy have better chances for surgical rescue and less postoperative complications?

Lefebvre, et al.11 showed that rescue surgery was possible only in patients undergoing CTX/RT after induction CT. León, et al.50 recorded the clinical response and surgical complications after rescue surgery due to relapse after CTRT (n = 154) or CTX/RT (n = 33). The CTX/RT-treated patients had higher mean age and ECOG with regard to CTRT-treated patients; 37.2% of patients with CTRT and 61.5% with CTX/RT underwent rescue surgery. A multivariate analysis demonstrated that the surgical rescue-associated variable with more weight was initial treatment. The frequency of postoperative complications was higher among those who received CTRT (62.5%) in comparison with CTX/RT (12.5%). Five-year OS after rescue surgery was 26.0% for patients who received CTRT and 70.0% for those with CTX/RT. In summary, patients with recurrence after CTX/RT were better candidates for rescue surgery than those who had been treated with CT/RT, with a lower rate of postoperative complications and better OS.

Question 13: In locally advanced disease, do you consider concomitant use of cetuximab with radiotherapy to be cost-beneficial?

In locoregionally advanced carcinomas, the CTX/RT scheme is cost effective. Brown, et al.33 estimated CTX/RT cost-effectiveness in comparison with RT alone. Independent economic analyses were carried out in Belgium, France, Italy, Switzerland, and the UK, with the economic model being based on patient data extracted from Bonner’s study. Each country’s specific costs of care came from official sources. Panels of clinical experts estimated the resources and validated the assumptions employed to extrapolate costs and health outcomes. In the analysis, incremental cost per quality-adjusted life year (QALY) for patients receiving CTX/RT
vs. RT alone among all countries was €7,538 to 10,836. This cost-effectiveness analysis indicated that CTX addition offers a good value/price alternative compared to RT alone. Another pharmacoeconomic study supports the concomitant use of CTX with RT. To estimate the real-world incremental cost per QALY with the CTX/RT scheme vs. RT alone as first-line treatment, a Markov model was constructed with the following classifications: “alive without progression”, “alive with progression”, and “deceased”. One-month transition probabilities were estimated based on clinical trial data and retrospective real-world data from two Dutch head and neck cancer centers (2007-2010, n = 141). Incremental costs per gained QALY range from €14,624 to 38,543, and acceptability curves for different scenarios show probabilities ranging from 0.76 to 0.87, with CTX/RT being cost-effective in comparison with RT alone. In summary, current results show that CTX/RT combination treatment is a cost-effective treatment option for patients with locally advanced HNSCC.

**Note:** In different institutions of Mexico’s health sector, the heterogeneous availability of different therapies complicates both first-line and second-line treatment of patients with head and neck cancer. The largest experience has been obtained from patients with locally advanced, unresectable disease that are not amenable for CTRT, and from the rescue attempt of patients with persistent or metastatic disease, not candidates for other treatments. This is why inter-institutional opinion differs with regard to availability of therapies and optimal timing for their use. The present consensus was based on worldwide recommendations based on updated scientific evidence.

**CONCLUSIONS**

Cetuximab is an alternative in the therapeutics of cancer originating in the mucous membranes of the head and neck area. In locoregionally advanced disease, CTX associated with RT is superior to RT alone, without an important increase in toxicity and less morbidity than the CTRT association. The CTX/RT treatment offers similar OS and locoregional control to the CTRT association, with less toxicity and higher treatment adherence, and in case rescue surgery is required, it can be accomplished more often and with lower morbidity than in CTRT-treated patients.

In patients treated with organ-preservation attempts, especially in those with laryngeal cancer in whom total laryngectomy is indicated owing to the extension of the neoplasm, CTX/RT in those showing complete response to induction CT offers similar results as CTRT, with less toxicity, higher treatment adherence, and higher possibility of surgical rescue if required. Bio-radiotherapy is an option as initial treatment in patients in whom tumor recurrence can be expected (organ preservation, mainly oral cavity and hypopharynx), with the purpose to facilitate surgical rescue if required and reduce morbidity. In recurrent/metastatic disease, CTX addition to platinum-based therapy is the option that offers the largest number of responses, without therapy tolerance and patient quality of life being affected, which is why it is considered the treatment of choice.

**DECLARATION OF INTEREST**

The May 2, 2016 meeting was financed by Merck Serono.

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REVIEW ARTICLE

Perioperative Pain Management in Gynecologic Oncology Surgery

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Received for publication: 18 August 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract Postoperative pain is a normal reaction to surgical intervention; however, it has been shown to increase morbidity, thus compromising the quality of recovery, which entails a delay in hospital discharge, increased risk of wound infection, and respiratory or cardiovascular complications, with an increase in mortality. Pain syndromes observed in patients with gynecologic oncology pathology result from three primary etiologies: those directly occurring due to the tumor, those deriving from treatments focused on reducing the tumor and those syndromes that are entirely independent of cancer or its treatment. Postoperative pain optimal management requires understanding the pathophysiology of pain and knowing the methods to be able to assess it in each patient, as well as knowledge on the different options available to control it. The key points that have to be considered are: the type of patient, type of surgical procedure, skills of the surgeon and anesthesiologist, and support of the working team. Improving the treatment of postoperative pain requires a broader perspective, since most healthcare providers have been shown to focus only on the postoperative period; however, ideal management is that where interventions are carried out before, during, and after surgery. (creativecommons.org/licenses/by-nc-nd/4.0/).

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INTRODUCTION

Pain is the most serious and feared symptom in cancer patients. Data yielded by 52 reviewed articles showed that pain is rather common, with figures of 33% in cured patients, 59% in patients on cancer treatment, and 64% in patients with metastasis or at advanced stages of the disease being reached. More than one-third of patients with pain in this review classified their pain as being moderate-to-severe. The consequences of pain poor control are devastating and can include dysfunction, social isolation, and emotional and spiritual stress, as well as discontinuation of potentially curative treatment, with this having an impact on disease recurrence-free periods and patient survival.

ACUTE PAIN

Pain syndromes commonly associated with the cancer patient result from three primary etiologies: the first one is associated with the pain experienced by direct relationship with disease progression; the second results from the different treatment schemes, progression control, and debulking focused on controlling the disease and reducing its impact and including oncologic surgery procedures, neoadjuvant and adjuvant chemotherapy, radiotherapy, intraoperative radiotherapy and hormone therapy. Finally, there is the pain that occurs entirely independently of the neoplastic disease process and/or its treatment.

Acute pain is considered to be an immediate sensorial consequence of nociceptive system activation, an alarm signal triggered by the body’s protecting systems. Acute pain is generally caused by somatic or visceral tissue damage, and it develops with a time course closely following the repair and healing of the causative lesion. If there are no complications, acute pain disappears with the lesion that caused it.

The International Association for the Study of Pain (IASP), in Kopf and Batel’s revision, defines acute pain as recent-onset and limited-duration pain, and its treatment should be aimed at having a protective role and preventing peripheral nervous system excitability and damaged tissue hypersensitivity, with the purpose of avoiding the presence of chronic pain syndromes that were initially acute.

POSTOPERATIVE ACUTE PAIN

Postoperative acute pain is defined as secondary to a direct or indirect aggression produced during a surgical act, taking into consideration pain caused by the surgical technique, anesthetic technique, inadequate posture, muscle contraction, and bladder or intestinal distension. Its importance lies in its elevated frequency, its inadequate treatment, and its consequences on patient recovery and survival. Formerly it was regarded as a normal response to surgical intervention; however, the direct consequences of undertreatment have been currently defined, which results in direct repercussions on sympathetic activity with increased oxygen consumption, myocardial and tissue ischemia, fear, anxiety, pulmonary complications, decreased immune response, and more recently, presence of surgical procedure-related chronic pain syndromes or chronic postoperative pain.

Pain enduring more than one month after surgery and persisting for at least three months with additional neuropathic symptoms occurs in 10-50% of common procedures, and 2-10% of these patients will continue with chronic pain. Pain that is not adequately treated tends to reduce patient satisfaction and impact on morbidity and mortality. Acute pain that becomes untreatable and persistent is known as chronic postoperative pain, which can have a significant impact on patient quality of life and daily activities, including sleeping and mood disorders.

PERIOPERATIVE MANAGEMENT

Patients will be concerned about perioperative pain control, and preoperative assessment is therefore an important opportunity to discuss the plan to be used, as well as to explain what to expect of pain control, which never should be that “the patient will experience no pain”. In this assessment, cognitive and psychological aspects that play a significant role in postsurgical pain severity can be identified. There is evidence that factors such as anxiety, depression, and catastrophism are essential pieces in the perception of pain and in coping with the experience of pain. Once the management plan has been established, the medical team and nursing staff should be in close communication in order to identify and report possible adverse effects.

Preemptive analgesia is a method intended to prevent or attenuate central sensitization resulting from the painful stimulus and the inflammatory reaction developing following the lesion. The efficacy of this modality (epidural analgesia, local anesthetic infiltration into the wound, use of non-steroidal anti-inflammatory drugs [NSAID], ketamine, clonidine) has shown beneficial effects in postoperative pain control.

Pain associated with abdominal surgical procedures is multi-factorial and includes parietal components originating from the surgical site, and visceral components originating from intra-abdominal structures.

In those patients who will undergo abdominal procedures, the use of transversus abdominis plane (TAP) block should be considered, which is not necessary in laparoscopic procedures, since pain in these cases is mainly on port insertion sites, where local anesthetic wound infiltration has shown benefits.

These effects were demonstrated by Lowenstein, et al. when they carried out a randomized trial with placebo and lidocaine 1% infiltrated into the incision site prior to the surgical procedure (hysterectomy), where the outcome was a significant reduction of postoperative pain within the first few hours.

TRANSOPERATIVE MANAGEMENT

The response to surgical stress is characterized by neuroendocrine, metabolic and inflammatory changes, which
adversely affect patient recovery. With recent advances in anesthetic and surgical techniques, this degree of surgical stimulation has decreased. Anesthetic depth, multimodal approaches, and surgical invasion reduction have been shown to result in postoperative pain decrease.

**Surgical technique**

Minimally invasive surgery is currently accepted as an integral part of gynecologic oncology surgery, which has prompted the performance of more advanced procedures in oncologic surgeries, showing promising results with robotic surgery incorporation in comparison with conventional techniques. The use of minimally invasive surgery in gynecologic oncology surgery has increased since 2006, when randomized trials showed it is a feasible and safe technique, with results including a decrease in hospitalization days and less postoperative pain.

**Anesthetic technique**

Multiple studies on general surgery have shown that intraperitoneal local anesthetic administration can reduce postoperative pain and the need for postoperative opioids. The same results have been observed in gynecologic procedures for benign pathologies such as tubal ligation, cystectomy or endometriosis surgery. A study conducted by Rivard, et al. demonstrates pain control superiority after intraperitoneal administration of bupivacaine in hysterectomy and cancer staging surgery. Since some decades ago, multiple studies have been carried out comparing the anesthetic technique used during surgery to assess its effect on postoperative pain control. The anesthetic technique has been shown to impact on postoperative pain evolution and control.

The use of anesthetics and subsequent epidural analgesia has shown pain improvement in comparison with other techniques such as patient controlled analgesia (PCA), and has therefore been accepted by numerous gynecologic oncology groups. There are meta-analyses supporting the use of epidural analgesia, since it shows superiority especially in those patients who underwent laparotomy or thoracotomy.

Catro, et al. demonstrated that those patients who underwent hysterectomy and were anesthetized with neuroaxial technique, specifically with spinal anesthesia, showed a significant improvement in the quality of recovery, and lower levels of pain reported during the first 48 hours after surgery, with less side effects. If we add the results of Collins, et al. to this technique by adding intrathecal opioid together with local anesthetic, postoperative pain is further reduced.

Recent reports suggest the use of neuroaxial techniques plus addition of regional blocks (TAP, peripheral nerve block, nervous plexuses block) in order to provide effective analgesia and being able to reduce opioid systemic effects; however, the results obtained by Siddiqui, et al. show that postoperative opioid decrease occurs only in the first 24 hours.

With the spinal and epidural techniques, a neuroaxial block of the nociceptive stimulus occurs. When using epidural anesthesia is decided, local anesthetic and opioid administration is recommended to be performed prior to the incision and to continue postoperatively; in addition to providing excellent analgesia, this has been also shown to facilitate mobilization and postoperative physical therapy, as well as lower incidence of postoperative ileus.

De León-Casasola, et al. demonstrated that in those patients undergoing cancer-related hysterectomy, intestinal function recovery was faster in those receiving patient-controlled epidural analgesia (PCEA) than in those who received PCA. In the study by Rivard, et al. in all patients who underwent laparotomy with vertical abdominal incision with suspected gynecologic malignancy, PCEA was shown to be associated with a decrease in pain scale levels and postoperative opioid total use, with postoperative ileum occurring in 13.4% of patients. According to meta-analyses results, epidural analgesia provides superior postoperative pain control in comparison with systemic opioid administration, in addition to an important decrease in adverse effects.

Surgical wound infiltration with local anesthetics has a rapid onset of action, and with vasoconstrictor addition the effect can be prolonged. The selection of the anesthetic to be used depends on the extension of the area that is wanted to be covered and the desired effect duration. Many studies have assessed the use of liposomal bupivacaine, demonstrating that its effect lasts up to 72 hours postoperatively.

**Intravenous drugs**

The use of intravenous lidocaine in up to 100 mg boluses and subsequent continuous administration at 2-3 mg/hour has been shown to have analgesic effects and anti-inflammatory properties, as well as to reduce opioid requirements.

Beta-blockers have been used to minimize sympathetic responses both at the moment of endotracheal intubation and induced by the surgical stimulus, which decreases inhaled analgesics and opioid requirements.

Alpha-2 agonists such as clonidine and dexmedetomidine have analgesic properties and have been shown to reduce postoperative pain and opioid consumption, with this effect being superior with dexmedetomidine; however, it cannot be used in all patients, especially in those with heart blocks or ventricular dysfunction, owing to the cardiovascular changes it produces.

**POSTOPERATIVE MANAGEMENT**

Intraoperative management is usually addressed by anesthesiologists, whereas postoperative pain is traditionally managed by surgeons, although this tendency is changing into a common perioperative management by the anesthetic-surgical team.

Since the PCA system introduction in the 1980s, postoperative pain daily management has extensively been optimized. This PCA provides adequate pain control, great satisfaction, and less adverse effects compared with administration on an as-needed basis. When patients experience pain, they self-administer the medication and, once the pain is reduced, they stop self-administering the medication. The initial dose is that which is required to reduce pain to 4/10 in the numeric rating scale (NRS), or to a respiratory rate of 12 breaths/minute or less, after which, the PCA pump is programmed establishing the bolus doses, maximum number of boluses in one hour and the closure interval, which limits how close consecutive doses can be. PCA is usually used.
with morphine or hydromorphone; fentanyl is not recommended unless the patient is under continuous monitoring as, for example, in the ICU. There are different routes of administration, including transdermal, epidural, in peripheral nerves, and the most widely studied is the intravenous route. Numerous studies have demonstrated epidural PCA superiority in comparison with intravenous administration. Postoperative beneficial effect is more significant in high-risk patients or in those who undergo major procedures. Peripheral nerve PCA results in increased postoperative analgesia and satisfaction, especially in limb surgery. In the study by Rivar, et al., the use of TAP with PCA was shown to reduce opioid medication, but only within the first 24 hours post-surgery, without difference in pain control in the ensuing days, which is consistent with previous studies in gynecologic oncologic surgery and that also concluded that the PCEA approach should be the method of choice in patients undergoing gynecologic oncology surgery with abdominal approach and vertical incision.

Opioids

To ensure opioid adequate use in postoperative pain management, education of both medical staff and patients is required. Opioids remain the mainstay in the control of postoperative pain; however, they have been associated with side effects such as nausea, vomiting, dizziness and constipation. They offer the convenience of different routes of administration, including intravenous, intramuscular, oral, transdermal or transmucosal, and provide fast and effective analgesia in patients with moderate-to-severe pain.

Morphine is the prototype of these drugs; it has a slow onset of action and intermediate action duration of approximately five hours, with a two-hour half-life. Its metabolites are excreted by the kidney, and its adverse effects are therefore prolonged in patients with renal failure.

Hydromorphone is a semi-synthetic opioid that is four to six-fold more potent than morphine; its onset of action is faster, but action duration is shorter; it has lower incidence of itching and sedation than morphine.

Fentanyl is a semi-synthetic opioid that is 50 to 80-fold more potent than morphine. It has a fast onset of action of 5-7 minutes, with a short duration of action of approximately one hour. In case immediate analgesia is required, the IV route is preferred. Although there are fentanyl-releasing patches, their use is not recommended for the management of immediate postoperative pain because changes in drug release vary with the patient body temperature, the required time to reach ideal plasma concentrations is very slow, and no quick dose adjustments can be made.

Oxycodone is a potent opioid agonist that is metabolized by the liver. It is more effective than morphine in the management of visceral pain.

Tramadol is an effective analgesic in mild-to-moderate pain and for neuropathic pain owing to its mechanism of action as a mu-agonist and serotonin and noradrenaline re-uptake inhibitor. The risk of respiratory depression is lower compared with other opioids.

Buprenorphine is an opioid receptor partial agonist, and has an analgesia ceiling effect in animal models, as well as in humans for respiratory depression. It is a safe drug in patients with renal failure, which makes it an attractive option for postoperative pain management in comparison with other opioids.

Non-opioid analgesics

Paracetamol is a medication that can be an effective component in multimodal analgesia. It can be administered orally, rectally, and parenterally. It significantly reduces pain intensity and spares opioid use after abdominal surgery. Its analgesic effect is 30% lower than NSAIDs, but with less adverse effects. It can be used in combination with NSAIDs and opioids. The main concern is liver toxicity, which is more common in geriatric patients and in those with chronic alcohol consumption.

The NSAIDs such as ibuprofen, ketorolac, naproxen, and COX-2 inhibitors are effective in painful states and possess a broad spectrum of anti-inflammatory and anti-pyretic effects. The NSAIDs increase the risk of gastrointestinal and postoperative bleeding, decrease renal function, and produce an imbalance in wound healing, as well as anastomosis weakening; therefore, their use should be guided by the type of surgery and agreement between surgeon and anesthesiologist.

Ketorolac is widely used during the postoperative period as short-term treatment for acute pain, and is used in combination with opioids for moderate-to-severe postoperative pain; it reduces opioid requirements and, therefore, their adverse effects.

COX-2 inhibitors also reduce postoperative pain, with lower risk of platelet dysfunction and bleeding than NSAIDs, but have been associated with cardiovascular risk in the perioperative period. The risk for renal adverse effects of NSAIDs and COX-2 inhibitors is increasing in patients with previous renal failure, hypovolemia, hypotension, and use of other nephrotoxic agents.

When in postoperative pain there is evidence of neuropathic pain, the indication of antidepressants (amitriptyline, nortriptyline, duloxetine) and antiepileptic drugs (gabapentin, pregabalin) can relieve pain secondary to nervous lesion and different types of neuropathy. Antiepileptic drugs such as gabapentin have been used to suppress both neuropathic and postoperative pain in breast surgery and hysterectomy.

Corticosteroids have been used as adjuvants to decrease opioid consumption and help to reduce postoperative pain. The most widely used and preferred is dexamethasone because it also has shown beneficial effect in the decrease of postoperative nausea and vomiting.

Perioperative management with ketamine at sub-anesthetic doses has been shown to decrease opioid requirements and pain intensity. At these low doses (0.2 mg/kg) in the postoperative period, it has not shown hallucinations or cognitive imbalance.

Special considerations

The number of patients undergoing ambulatory surgery has considerably grown in the past few years, owing to the advance in surgical and anesthetic techniques. However, procedure complexity has also been increasingly growing, and inadequate pain control has been demonstrated in these procedures, which causes hospital stays and readmissions to
increase. The incidence of moderate-to-severe pain remains at 25-35%, owing to the fact that postoperative analgesia is based exclusively on medications such as paracetamol and NSAIDs, with their previously mentioned limitations.

Exacerbation in chronic pain

Patients with chronic pain conditions require a specific plan for postoperative pain management, particularly those taking large doses of analgesics. Postoperative pain management can be difficult in opioid-tolerant patients, since initial assessment and therapeutic approaches are usually inadequate. These patients will usually require higher doses, and contacting the pain-specialist physician is therefore suggested for adequate management. Patients taking analgesics should continue doing so as usual until before the surgery, except for NSAIDs or COX-2 inhibitors, which must be preoperatively withdrawn. If patches are used, they should be removed during surgery and in the postoperative period. Transition of all chronically taken medications should be made to be intravenously administered.

CONCLUSIONS

Perioperative pain control is one of the main challenges, not only for the anesthesiologist, but for all the healthcare personnel involved in the care of the patient undergoing any type of surgical intervention.

Women’s oncologic conditions are alarmingly on the rise; however, implementation of opportune detection programs has offered the possibility to establish an early diagnosis in many cases. This implies the performance of surgical procedures that, in spite of allowing for the survival and disease-free period to be significantly increased in this group of patients, entail a larger challenge since improving the quality of life becomes necessary, including, of course, the management of both acute and chronic pain as a priority. Avoidance of the painful stimulus appearance is well known to have short, medium, and long-term implications, and knowing the mechanisms participating in the generation of pain is therefore necessary in order to adopt the required strategies that allow for adequate control to be achieved, and in this way have an impact on patient prognosis and quality of life. Thus, modifying our approach from a mere anesthetic act into a series of perioperative systematized pain-control strategies becomes essential.

DECLARATION OF INTEREST

The authors declare not having any conflicts of interests relevant to this manuscript.

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CLINICAL CASE

Dedifferentiated Parosteal Osteosarcoma of the Ulnar Diaphysis

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Received for publication: 4 June 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract
Introduction: Dedifferentiated parosteal osteosarcoma is a variant where a high-grade osteosarcoma coexists with a parosteal osteosarcoma. Clinical Case: A 20-year-old female patient presented with a 6-month history of right forearm pain and functional limitation, with no apparent cause. X-rays revealed a right ulnar diaphysis tumor lesion. On physical examination, there was pain on palpation at right ulna diaphysis and pronosupination limitation. Chest computed axial tomography showed metastatic disease to the left lung upper lobe. An incisional biopsy of the right ulna tumor reported a dedifferentiated parosteal osteosarcoma. Neoadjuvant chemotherapy with cisplatin and doxorubicin management was therefore started until 3 cycles were completed. Surgical treatment involved right ulna diaphysis intercalary resection plus microvascularized autologous right fibula diaphysis graft reconstruction and graft stabilization with a 3.5-mm DCP plate and a one-third tubular plate. In the same procedure, pulmonary metastasectomy was carried out by thoracoscopy. Postsurgical histopathological examination reported 100% necrosis. Currently, the patients is asymptomatic and with no evidence of tumor activity. Conclusion: Dedifferentiated parosteal osteosarcoma is a rare condition, but it should be suspected as differential diagnosis when a parosteal osteosarcoma is found. The fact that this condition can generate metastasis owing to its dedifferentiated pattern should be taken into account. It is important to plan a surgical treatment that enables appropriate functional reconstruction, always taking into account the oncologic principle. (creativecommons.org/licenses/by-nc-nd/4.0/).

KEYWORDS
Dedifferentiated parosteal osteosarcoma; Intercalary resection; Microvascularized autologous graft
INTRODUCTION

Surface osteosarcomas comprise a varied group of malignant bone tumors with different degrees of malignancy. Within this group, dedifferentiated parosteal osteosarcoma is a variant where a high-grade osteosarcoma coexists with a parosteal or low-grade osteosarcoma either at the same time (synchronously) or as a recurrence (metachronically).

Typical parosteal osteosarcoma is a superficial lesion formed by low-grade fibroblasts that produce laminar bone. Usually, it occurs at between 20 and 40 years of age, with most common location being the distal femur posterior region.

There are only a few cases of dedifferentiated parosteal osteosarcoma published in the literature. Clinicopathological findings, diagnosis, treatment and evolution of patients with this rare osteosarcoma have not been clearly defined.

CLINICAL CASE

A 20-year-old female patient presented with a 6-month history of right forearm pain that limited its mobility, with no apparent cause. On physical examination she had tenderness at the right ulna diaphysis and pronosupination limitation owing to pain. Forearm radiographic study showed (Fig. 1) a cortical bone-dependent right middle-third ulnar diaphysis tumor with extension to soft tissues, radiologically consistent with parosteal osteosarcoma.

Chest CAT revealed multiple pulmonary nodules, probably related to deposits secondary to the known primary lesion, with the largest located at left lung base periphery, of 8 mm in size (Fig. 2). Middle third ulna diaphysis tumor incisional biopsy histopathological report was: dedifferentiated parosteal osteosarcoma of the right ulna diaphysis, Enneking III, AJCC IVA (Fig. 3).

The patient was started on neoadjuvant chemotherapy with cisplatin and doxorubicin until 3 cycles were completed. After neoadjuvant therapy, she underwent intercalary
Resection of the right ulna diaphysis plus microvascular autologous right fibula shaft reconstruction graft and graft stabilization by placing a 3.5-mm DCP plate and a one-third tubular plate (Figs. 4 and 5). Subsequently, the thoracic surgery department carried out pulmonary metastasectomy by means of pulmonary wedge resection by thoracoscopy. Histopathology report indicated negative resection margins and 100% necrosis (Huvos grade IV), with metastasectomy product without evidence of viable neoplastic cells (complete response). Currently, at 12 months’ follow-up, the patient is asymptomatic, with no evidence of local or distant tumor activity, and tolerating forearm and hand movement (Fig. 6).

DISCUSSION

Dedifferentiated parosteal osteosarcoma is a rare condition, but it should be considered when a parosteal osteosarcoma is found. In addition, the fact that this condition can generate metastasis owing to its dedifferentiated pattern should be taken into account.

Dedifferentiation in parosteal osteosarcoma is commonly reported in disease recurrences. Wold et al. described 11 cases of parosteal osteosarcoma dedifferentiation. In 10 of these cases, dedifferentiation was reported at first, second or third recurrence, whereas the coexistence of low and high-grade zones was only reported in one case7.

The presented case illustrates the importance of considering the possibility of dedifferentiated areas coexisting in parosteal osteosarcoma, as well as the possibility of these zones generating metastasis.

Several authors suggest that parosteal osteosarcoma should be considered a different entity to high-grade superficial osteosarcoma, since clinical evolution and mortality are different, with lower mortality rate for dedifferentiated osteosarcoma6,8,9.

Invasion to the medullary canal is considered to be a prognostic factor for survival and disease-free interval in patients with dedifferentiated osteosarcoma6,10.

Currently, survival rates of 80% are reported in patients with dedifferentiated parosteal osteosarcoma, with neoadjuvant chemotherapy plus surgical treatment with wide margins being associated6,8,10.

There is a strong correlation between prognosis and tumor necrosis volume in patients treated with neoadjuvant chemotherapy and surgery, with this percentage being assessed with Huvos classification11, and with an excellent response being observed in this case (100% necrosis) after neoadjuvant treatment. In addition, it is important to consider that tumor debulking in response to neoadjuvant chemotherapy makes lesion excision much easier.

Reconstruction with vascularized fibula autologous graft enables better and faster integration of the graft in the...
recipient zone without causing donor area functional limitation. Taylor reported the first free vascularized fibular graft transfer in 1975.

Weiland et al. have described the use of vascularized bone grafts for the treatment of several conditions, such as tumor resection, post-trauma bone defects and congenital pseudoarthrosis. The role of this technique in long bone tumor reconstruction has been tried to be defined; it has been proposed that it might be indicated in defects larger than 6 cm after tumor resection, and this technique enables the transfer of up to 24 cm of bone, with an 8-cm vascular pedicle on average.

In conclusion, dedifferentiated parosteal osteosarcoma is an osteosarcoma predominantly located on bone surface where a low-grade parosteal component is found associated with a highgrade sarcomatous component. Dedifferentiated paraostal osteosarcoma behavior is more aggressive than that of conventional parosteal osteosarcoma, and the presence of metastatic disease should be suspected in the presence of this pathology. It is important to plan a surgical treatment that enables appropriate functional reconstruction always taking into account the oncologic principle.

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CLINICAL CASE

Sarcomatous Degeneration of an Arteriovenous Malformation

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Received for publication: 30 April 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract

Introduction: The prevalence of vascular malformations is estimated at 1.5% in the general population. Sarcomas are a rare group of mesenchymal-origin malignant tumors that accounts for less than 1% of all tumors in adults. We present one case of sarcomatous degeneration in an arteriovenous malformation of 50 years’ evolution.

Material: A 60-year-old male patient was admitted to our department presenting with soft tissue infection and hindfoot bleeding in the context of an arteriovenous malformation of 50 years’ evolution and unidentified etiology. The patient had bilateral distal pulses. A 5 x 6 cm ulcerated and over-infected hindfoot lesion, with mamelonated borders that collapsed on digital pressure, was observed at the right lower limb.

Methods: Diagnostic arteriography revealed a vascular formation with angiomatous nidus on the medial surface of the talotibiofibular joint, arising from the posterior tibial artery. The arteriovenous malformation was embolized, with total exclusion thereof at final control. In spite of medical treatment and dressing, bad evolution persisted, with over-infection and appearance of new mamelonated formations, and infracondylar amputation was therefore decided. Results: Anatomic pathology revealed a malignant mesenchymal neoplasm suggestive undifferentiated pleomorphic sarcoma with giant cells (malignant fibrous histocytoma of the giant cell type). The patient passed away at 3 months of diagnosis due to tumor disease dissemination.

Conclusion: Sarcomatous degeneration is a rare complication of arteriovenous malformations and, since clinical suspicion is low, the diagnosis is delayed, which entails poor results on the short-term.

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doi:10.24875/GAMO.17000052
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INTRODUCTION

The prevalence of vascular malformations is estimated at 1.5% in the general population. Abnormal communication between the arterial and the venous systems are characteristic of arteriovenous malformations (AVM). Sarcomas are a rare group of mesenchymal-origin malignant tumors that account for less than 1% of all tumors in adults. We present a case of sarcomatous degeneration in an arteriovenous malformation of 50 years’ evolution.

MATERIAL

A 69-year-old male patient was admitted to the Department of Angiology and Vascular Surgery with complaints of soft tissue infection and hindfoot bleeding in the context of an AVM of 50 years’ evolution and unknown etiology. The patient was a former smoker and had a personal history of high blood pressure and urothelial bladder cancer treated with transurethral resection.

On examination he had pedal and posterior tibial pulse on both lower limbs. A 5 x 6 cm ulcerated and over-infected hindfoot lesion, with mamelonated borders that collapsed on digital pressure and with sporadic capillary bleeding, was observed at the right lower limb (Fig. 1A).

METHODS

The patient was treated with intravenous antibiotic therapy according to the antibiogram and local dressing. A diagnostic arteriography revealed an increase in blood flow velocity in the right lower limb arterial system with regard to the contralateral limb, and a vascular formation with angiomatous nidus was observed on the medial surface of the talotibiofibular joint with main vascular efferent tributary of the posterior tibial artery.

Under rachianesthesia and 6F right femoral access, the posterior tibial artery was canalized. Intraoperative arteriography revealed the presence of an AVM dependent on the posterior tibial artery (Fig. 2A). The arteriovenous malformation was embolized with Glubran 2 acrylic glue (GEM, Viareggio, Italy), with AVM total exclusion at final control (Fig. 2B). Subsequently, surgical debridement of the ulcer was performed, with a sample of the borders being taken for histopathology examination. After surgical intervention, the lesion was improved and the patient was therefore discharged. At one month of the intervention, the patient was readmitted with the same clinical picture. A second debridement of the wound was performed, with complete excision thereof. In spite of antibiotic treatment and local compressive dressings, the surgical bed showed poor evolution, with infection persistence and appearance of new mamelonated structures, and intracondylar amputation was therefore decided.

RESULTS

Histopathology examination revealed an infiltrating malignant mesenchymal neoplasm ulcerating the epidermis, extensively necrotizing and reaching subcutaneous and striate skeletal muscle planes. Tumor cells expressed vimentin and, locally, soft muscle expressed actin; giant cells expressed CD68, and a proliferative index of 40% (Ki-67) was observed. A histopathological diagnosis of malignant mesenchymal neoplasm suggestive of undifferentiated pleomorphic sarcoma with giant cells (malignant fibrous histiocytoma of the giant cell type) was established.

The patient was referred to the oncology department, and two months after histopathological diagnosis, he was admitted to the internal medicine department presenting with symptoms of progressive dyspnea, which had started two weeks prior. Chest X-ray revealed multiple masses with a “cannonball” pattern. These findings were corroborated by a chest CAT scan showing pulmonary metastases (Fig. 1B). The patient died at 3 months of diagnosis due to tumor disease progression.

DISCUSSION

After gastrointestinal stromal tumor (GIST), most common mesenchymal tumors in adults are undifferentiated sarco-
mas, such as the one we described, followed by liposarcoma and leiomyosarcoma.

AVMs constitute a therapeutic challenge owing to their impact on the cardiovascular system and subsequent hemodynamic alterations. AVM clinical manifestations are dependent on their location and may cause congestive heart failure, venous hypertension and venous or arterial insufficiency and, as a consequence of the latter two, skin ulceration and even gangrene. Development of symptoms is caused by increased shunting that results in arterial steal and venous hypertension, both of which reduce tissue perfusion, which leads to pain, ulceration and bleeding. There are four indications for surgical treatment: bleeding, venous hypertension-derived complications, lesion located in an anatomical region that threatens patient’s life (e.g., close to the airway), or that affects vital functions (e.g., hearing, sight). Pain, functional impairment and recurrent infection, among others, are included among relative indications. AVM treatment is intended to produce the closure of all arteriovenous communications by means of conventional surgery or endovascular techniques. Currently, transarterial embolization is the first therapeutic choice.

Mesenchymal tumors occur as painless, slowly growing tumors; when the tumor reaches an important size, pain or compression-associated symptoms, such as edema or paresthesias on the limb can appear. Forty-six percent of sarcomas are anatomically found on the lower limbs (buttocks, groin and lower limb). Although their etiology is unknown, there are predisposing factors for the occurrence of sarcomas, such as genetics: Li Fraumeni, type I neurofibromatosis, treatment with radio- or chemotherapy, chronic lymphedema (post-surgical, filariasis, etc.) and chronic irritation. It is possible that in the presented case the persistence of the arteriovenous fistula for 50 years may have played a role in the development of sarcoma.

CONCLUSION

Sarcomatous degeneration is a rare complication of arteriovenous malformations, and given that clinical suspicion is low, the diagnosis is often delayed, which entails poor results on the short-term.

REFERENCES

CLINICAL CASE

Magnesium Deficiency in a Patient on Chemotherapy-Radiotherapy Treatment for Cervical Cancer: Case Report and Review

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Received for publication: 1 October 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract  Introduction: Magnesium deficiency is a common entity in cancer patients owing to cisplatin-containing chemotherapeutic schemes. Combination with radiotherapy in certain tumors where the gastrointestinal tract is part of the treatment field can elicit this entity. Clinical case: The case is presented of a 35-year-old woman diagnosed with uterine cervix cancer on treatment with concomitant chemotherapy plus radiotherapy. Owing to the tumor extension and lymph node involvement, the radiation treatment field was broad, with important doses at the level of the small intestine. During treatment, the patient had important diarrhea due to magnesium deficit. Results: Testing for hypomagnesemia is not common in clinical practice, but it should be contemplated in patients on treatment with cisplatin-containing chemotherapeutic schemes and extended-field pelvic radiotherapy. Discussion: Knowing this entity and providing adequate treatment can prevent quality of life deterioration in affected subjects and possible early treatment discontinuation due to poor tolerance. (creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

Magnesium deficiency is a known side effect in patients on chemotherapeutic treatment with cisplatin. Hypomagnesemia is defined as serum level of the ion lower than 1.8 mg/dl (1.5 mEq/l), and its relationship with the use of cisplatin is dose-dependent, with its frequency increasing with each administered cycle\(^ {\text{[5,6]}}\). It is one of the more common hydro-electrolytic disorders, but its clinical manifestations are unspecific. Clinical signs and symptoms are basically asthenia, confusion, irritability, convulsions, and neuromuscular or cardiac alterations\(^ {\text{[7]}}\). Its diagnosis is difficult since determination of serum magnesium is not common practice. In addition, there is close relationship between magnesium levels and phosphorus and potassium levels, which makes it hard to distinguish whether clinical symptoms are due to one or another ion.

Uterine cervix cancer treatment is based, except for very early stages, on the combination of chemotherapy and radiotherapy. The use of weekly cisplatin at a 40 mg/m\(^2\) dose, together with pelvic radiotherapy at doses up to 46-50 Gy, followed by brachytherapy, is the most widely used scheme. Surgery alone is reserved for stages IA and IB1. If tumor stage is advanced, the radiotherapeutic field can be broader, reaching in the upper limit the iliac bifurcation or the para-aortic lymph nodes, which significantly increases the possibility of radiation enteritis and, hence, malabsorption problems.

Treatment is based on correction of the underlying process whenever this is possible. If deficiency occurs in early stages of oncological treatment, magnesium salts can be administered by the oral route. If there is vital compromise, vials of magnesium sulfate 15% diluted in dextrose 5% solution are administered in 20 minutes, continuing on the ensuing days until the deficiency is corrected. Bolus administration should be avoided owing to possible cardiac complications that may derive from it\(^ {\text{[5,6]}}\).

CLINICAL CASE

The case is presented of a 35-year-old woman who had experienced menstrual cycle-unrelated bleeding and hypogastric pain for more than one year. In view of the described clinical symptoms, the patient attended the Gynecology Department. On physical examination she had normal external genitals and vagina, with cervix completely lateralized to the left. On bimanual examination, rigidity was found on the upper third of the left anterolateral vaginal wall, with supravaginal cervical tumor growth. On digital rectal examination, normal right parametrium was found, with the left one not being assessable due to the tumor lesion growth. The cervix touched the anterior side of the rectum. A gynecological ultrasonography showed a 5.2 x 3.4 cm cervical lesion with abundant vascularization and deep invasion without parametrical involvement.

Studies to find the extension of disease were carried out, with pelvic magnetic resonance imaging reporting a 5.5 x 4.5 x 4.0 cm cervical mass, with presence of a 1.2 cm right iliac chain adenopathy without parametrical, vaginal, ureteral, bladder, or rectal invasion. A positron emission tomography-computed tomography scan revealed hyper-uptake (SUVmax 16.55) on cervical region with 5.5 cm diameter and at the right iliac chain (SUVmax 12.30). Biopsy of the lesion was taken, which yielded the result of adenosquamous carcinoma of the cervix.

The case of this patient was presented to the multidisciplinary committee for gynecological tumors of our hospital and, in view of the anatomopathological and radiological result, FIGO IIIB, treatment with chemotherapy and radiotherapy was decided on. The patient was started on chemotherapeutic treatment with weekly cisplatin at 40 mg/m\(^2\) in combination with radiotherapy. The radiotherapeutic planning target volume reached up to the L4-L5 vertebrae at its upper limit. During the third chemotherapy cycle, and with a radiotherapy dose of 22 Gy of 50 planned, the patient experienced nausea, vomiting, and intense diarrhea that did not improve in spite of loperamide administration and dietary changes. Together with this there was a 7 kg weight loss in 20 days and significant asthenia. In addition, her relatives reported she was more irascible and had memory losses.

Blood count and chemistry was obtained, with K\(^+\) values of 3.4 mEq/l and Mg\(^{++}\) 1.3 mg/dl standing out. Tablets with 404.85 mg of magnesium lactate every eight hours for five days were prescribed. The patient started noticing improvement of her gastrointestinal and memory symptoms. New blood tests after treatment completion showed magnesium levels of 2.2 mg/dl.

DISCUSSION

The use of pelvic radiotherapy is one of the least known causes of magnesium deficiency, which is increased if the patient requires chemotherapy with cisplatin. There are several tumors with large pelvic fields of radiation therapy that can elicit radiation enteritis (uterine cervix, endometrium, prostate, rectum, or anal canal) and, therefore, nutrient malabsorption. Although at-risk organ-limiting doses are known, many times these cannot be complied with due to tumor extension or lymph node involvement.

In spite of being a known side effect in the literature (Table 1), there are few studies assessing the prophylactic use of magnesium with the use of cisplatin. Hunter, et al.\(^ {\text{7}}\) studied the beneficial effect of magnesium sulfate supplementation in more than 200 patients on cisplatin and radiotherapy combined treatment. In addition, they reported that patients with diabetes mellitus and/or hypertension were at higher risk of developing magnesium deficiency, owing to renal problems. The analysis by Evans, et al.\(^ {\text{8}}\) evaluated a sample of 28 patients with gastrointestinal cancer treated with chemotherapy with cisplatin, 5-fluorouracil and epirubicin, demonstrating statistically significant improvement with magnesium supplementation after the third cycle of cisplatin. There are more recent studies by Yamamoto, et al.\(^ {\text{9}}\) and the Iranian group clinical trial\(^ {\text{[5,10]}}\), which also report improvement with the prophylactic use of magnesium supplements in cancer patients who are to receive chemotherapy with cisplatin. The retrospective study by Kidera, et al.\(^ {\text{11}}\) analyzed the beneficial effect of magnesium supplements in different solid tumors, including lung cancer.

In everyday clinical practice, knowing magnesium serum values prior to initiating oncological treatment with cisplatin and pelvic radiotherapy may prevent situations
of deficiency that elicit clinical manifestations in patients on treatment. In our case, the patient’s quality of life had significantly deteriorated since the beginning of oncological treatment and there was doubt whether she would be able to complete it. Magnesium supplement administration produced an improvement of her clinical conditions, especially at the gastrointestinal level.

CONCLUSIONS

Magnesium deficit on treatment with cisplatin and radiotherapy with extended pelvic fields is a common entity in clinical practice that sometimes is not diagnosed, which results in important gastrointestinal clinical symptoms in affected patients. Routinely performing magnesium level controls and supplementing in case these are low is a beneficial solution for these subjects.

ACKNOWLEDGEMENTS

To the Hospital de Sant Joan d’Alacant Department of Radiotherapeutic Oncology.

DECLARATION OF INTEREST

The author declares not having any conflicts of interests.

REFERENCES

CLINICAL CASE

Corneal Intraepithelial Neoplasia as a Cause of Visual Acuity Decrease: a Low-Cost Approach

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Received for publication: 1 October 2016; accepted for publication: 25 February 2017
Available online: 4 August 2017

Abstract  Introduction: Corneal intraepithelial neoplasia is a dysplastic condition within the ocular surface squamous cell neoplasia spectrum. It is a rare condition that is seldom suspected, especially at its diffuse presentation. Once suspected, diagnosis and treatment are easy for a condition that if left untreated could be devastating. Clinical case: We present the case of a 63-year-old male patient presenting with a history of two weeks of right eye visual acuity decrease as only symptom. Right eye visual acuity was 20/40 and physical examination revealed a right cornea upper half geographic lesion with frosted appearance. After diagnostic rule out, epithelial scraping was decided, with low-grade corneal intraepithelial neoplasia reported by the pathology department. Management with mitomycin C 0.02% in two 2-week cycles with 3 topical applications per day, accompanied by fluorometholone 0.1% at the same dosage was then decided. Conclusions: The authors recommend the use of mitomycin C in developing countries. (creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

Conjunctival-corneal intraepithelial neoplasia (CCIN) is a non-invasive dysplastic condition that occurs in the conjunctival-corneal epithelium and that is also considered to be a carcinoma in situ. CCIN is part of the dysplastic spectrum of ocular surface squamous cell dysplasias. The squamous cell carcinoma lesion begins with dysplastic changes (carcinoma in situ that can be mild, moderate or severe), to finally cross the lamina propria and become an invasive squamous cell carcinoma (Table 1)1,2.

Of all tumors occurring in the ocular globe and its appendages, those of the ocular surface are the most common and, among these, neoplasias involving the epithelium account for one third to half the cases. When an epithelial tumor is diagnosed, 65% have not yet crossed the lamina propria. An incidence of corneal-conjunctival tumors of 0.13/100,000 has been reported, which is dependent on the geographic origin14. As in all tumors, changes in ocular surface squamous cell neoplas are due to a loss of affected cells life and division cycles control. Most important known risk factors are chronic exposure to ultraviolet rays, human papillomavirus (HPV) infection, p53 gene defective expression, male gender, age older than 60 years and human immunodeficiency virus (HIV) seropositivity1.

CCIN clinical presentation can occur in two forms: nodular or diffuse, with nodular cases being more common and showing lesions or masses that may have changes in normal coloration, erosions, bleeding, reddening and foreign body sensation; in diffuse cases, the patient can be asymptomatic, or if there is corneal diffuse invasion, with visual acuity (VA) decrease1.

Establishing a diagnosis in a suspected case of CCIN has to be done by means of histopathology, either first by epithelial scraping, or after tumor en bloc resection1. Traditionally, CCIN had been treated with tumor resection, but up to 56% have been shown to relapse, and treatment has therefore had radiation, cryotherapy or topical chemotherapeutics added. Recently, the use of topical chemotherapeutic agents (5-fluorouracil, mitomycin C or interferon) alone has been shown to have the same effectiveness than excision plus cryotherapy2.

In this work, we describe a case of corneal intraepithelial neoplasia presenting for the first time with a chief complaint of VA decrease, which was topically treated with mitomycin C (MMC).

CLINICAL CASE

A 63-year-old male patient presented to our private practice with a chief complaint of visual acuity decrease over the two previous weeks. The patient had no relevant personal or medical history other than the use bifocal glasses for hypermetropia and presbyopia. He also had no important hereditary or family history. At that time, he worked as an electrician installing telecommunication equipment for a private company. The patient referred right eye visual acuity decrease even with the use of glasses since two weeks prior, and that it had been progressive, without any other symptoms. On physical examination, right eye best-corrected visual acuity was found to be 20/40 (0.3 logMar) with +4.50 -2.25 x 97°, and +20/20 (0 logMar) with +3.25 .0.50 x 93° for the left eye, with best-corrected VA of 20/20 (0 logMar) in both eyes. Ocular movements and pupillary reflexes showed no abnormalities. Intraocular pressure was 14 mmHg for both eyes. On slit lamp examination, a right eye geographic lesion was observed at the corneal upper half, which included pupillary area with frosted appearance and dotting within. Slit lamp examination of the left eye was free of anomalies. Uncertain whether it was a corneal lesion caused by chemicals or a thermoelectric lesion the patient was unaware of, treatment was started with ciprofloxacin/dexamethasone 3 mg/1 mg/mL eye drops topically applied to the right eye thrice daily for 2 weeks, with indication for reassessment at the office two weeks after treatment was started.

When the patient attended two weeks later for reassessment, the lesion was observed to have spread to the right cornea lower pole and VA had not improved. Performing an epithelial scraping was then decided for histopathological assessment. Two weeks after the scraping, the pathology report indicated a low-grade corneal intraepithelial neoplasia (Fig. 1). Management was first started with mitomycin C 0.02% (0.02 mg/mL) eye drops thrice daily for 2 weeks and, finally, other 2 weeks with 3 applications per day (two complete courses), accompanied by fluorometholone 0.1% at the same dosage. Eight weeks after mitomycin C treatment had started, no corneal lesion was found and visual acuity had improved to 20/20 (0 logMar) in both eyes. One year after treatment completion, the patient had not experienced any new discomfort. He is currently on follow-up in order for any relapse to be early diagnosed (Fig. 2).

DISCUSSION

 Conjunctival-corneal intraepithelial neoplasia is a rare condition, and the diffuse presentation is even more infrequent. Data of VA decrease with an unclear explanation would probably drive the clinician to study other causes prior to directly considering a neoplasm. It is important underscoring that, although the study of the most common conditions is imperative and unavoidable, this type of pathologies should always be taken into account when making differential diagnoses.

Recently, topical treatment of CCIN has been shown to be as effective as surgery, and to have even better long-term outcomes if surgery is not combined with neoadjuvant treatment. In 2014, Nanji et al. did not find statistically
significant differences in terms of recurrence and complications between therapy with surgery or with interferon alpha 2B (IFNα-2B) for the management of corneal squamous cell carcinoma in a case-control study. In this study, recurrence in patients with surgery was 5%, whereas in the medically-treated group it was 3%. Since there are no important differences, non-invasive intervention might be preferable.

It is a huge step being able to avoid invasive treatments in patients and expose them to less risk, while preventing the advancement of a disease that without control might get to cause blindness or even death, in a simple and tolerable way for the patient. It could be considered that the next step is to find the most effective topical medication with the best cost/benefit. Currently, mitomycin C and interferon alpha 2B (IFNα-2B) have been suggested to probably be the most effective drugs to treat CCIN, although some claim that the advantage IFNα-2B has, is that it produces less adverse effects in comparison with MMC. The risks we expose our patients to when using MMC include pain, irritation, erosion, symblepharon and limbal stem cell deficiency, with these effects depending on the employed dose.

In a prospective study by Ballalai et al. with 23 patients observed for 24 months after the use of MMC for the treatment of CCIN, all cases were resolved, only one patient had recurrence, and 17.4% had corneal abrasion that was easily resolved with treatment. In developing countries, IFNα-2B is an expensive option that is not accessible for most population in general; however, our case bears witness that with appropriate assessment and the necessary care, MMC remains an efficacious, safe and convenient treatment for the patient.

CONCLUSIONS

Conjunctival-corneal intraepithelial neoplasia is a form of carcinoma in situ that belongs to the spectrum of squamous cell neoplasia of the eye surface. Although it is a rare condition, and its diffuse presentation is even more infrequent, it must be always taken into account in the differential diagnosis of our patients with visual acuity acute decrease. Currently, diagnosis and treatment can be minimally invasive. For diagnosis, epithelial scraping and histopathological assessment is sufficient, and treatment consists in topical
chemotherapeutic agents, with resection being only necessary in large tumors. The authors recommend the use of mitomycin C 0.02% in developing countries.

ACKNOWLEDGEMENTS

We thank Consultora en Investigación Biomédica Analimed for its cooperation in writing, organizing and submitting this work. We also thank the Universidad de Monterrey and Hospital Christus Muguerza Sur pathology departments, especially Dr. Óscar Antonio Ulloa Ortiz.

CONFLICT OF INTERESTS

This work was self-financed by the Centro de Oftalmología del Valle. The authors do not possess any personal or commercial interest on the material discussed in this article.

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