MMP-1 Levels as a Predictive Marker of Severity of Oral Mucositis among Head And Neck Cancer Patients undergoing Radiotherapy

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**Head and Neck Cancer**

identified by the area in which they begin:

- Oral cavity
- Salivary glands
- Paranasal sinuses
- Nasal cavity
- Pharynx
- Larynx (voicebox)
- Lymph nodes in the neck.

**Main causes:**

- Tobacco (smoked or chewed)
- Alcohol
- Human papilloma virus (HPV) infection

**Treatment options:**

- Surgery
- Radiation therapy
- Chemotherapy

Radiotherapy of head and neck malignancies

ORAL MUCOSITIS 85-100%

POOR NUTRITION

INFECTION

UNPLANNED BREAKS in Treatment

QUALITY of LIFE

Local Control and Survival

Matrix-metalloproteinases (MMP)

- are zinc-dependent endopeptidases

There is a correlation between MMP-2, -3, and -9 and oral mucositis.

*recommends further research* vis-à-vis *other relevant MMPs* and interventional therapies that target correlated MMPs *to prevent or reduce the severity of oral mucositis and to promote faster healing of oral mucositis lesions* (Al-Azri, 2013).

**MMP-1** (interstitial collagenase or collagenase-1)

: the only enzyme that has *the ability to initiate the breakdown* of the interstitial collagens such as Collagen type-1, Collagen type-2, and Collagen type-3.

(Ross, Pawlina. 2011.)
* Five-phase pathobiologic model of oral mucositis

- **0 Days**
  - Normal Epithelium

- **0-2 Days**
  - Phase I Initiation

- **2-10 Days**
  - Phases II & III Messaging, Signaling, & Amplification

- **10-15 Days**
  - Phase IV Ulceration (Mucositis)

- **14-21 Days**
  - Phase V Healing

- **Chemotherapy**
- **Radiotherapy**

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OBJECTIVES

GENERAL OBJECTIVE:

To determine the extent of **MMP-1 level increase** among head and neck squamous cell carcinoma (HNSCC) patients before and during radiation therapy.

SPECIFIC OBJECTIVES:

1. To determine **change in the levels of MMP-1** among head and neck carcinoma patients after 10 days of radiation therapy.

2. To determine the correlation between MMP-1 and the manifestation of radiation-induced oral mucositis.

3. To determine the correlation between **complete blood count (CBC) results** with levels of MMP-1 among head and neck carcinoma patients undergoing radiation therapy.
SCOPE AND LIMITATIONS

- Limited to 20 head and neck cancer patients receiving ionizing radiation

- Specific only to MMP-1

- Levels of MMP-1 will only be quantified **before and on the 10th day** of radiotherapy and chemotherapy.
Diagnosed with biopsy **proven malignancy of the head and neck, stages I-IVB** (i.e. lip and oral cavity, pharynx, larynx, nasal and paranasal sinuses)

Diagnosed early and advance head and neck cancer patients treated with **concurrent chemotherapy**.

Diagnosed early stage head and neck cancer patients treated with **radiation therapy alone**.

**Eastern Cooperative Oncology Group** performance of status ≤ 2.
Exclusion Criteria

- Recurrence of the disease.
- Patients diagnosed with metastatic disease during chemoradiotherapy planning.
- Active cancer in another part of the body.
D. Enzyme-Linked Immunosorbent Assay

**MMP-1 Human ELISA kit (Abcam)**

- Dilution of Samples: 5-fold
- Samples were tested in duplicates
- Based on colorimetric changes
- Stop solution: blue to yellow
- Absorbance: 450 nm
- Beer-Lambert’s law
D. Complete Blood Count

Day 0 and Day 10

- Hemoglobin
- Hematocrit
- RBC
- WBC
- Platelet
- Neutrophil
- Lymphocyte
- Eosinophil
- Basophil
- Monocyte

Sysmex Xn1000™ Hematology Analyzer
<table>
<thead>
<tr>
<th></th>
<th>[ 0 ]</th>
<th>[ 1 ]</th>
<th>[ 2 ]</th>
<th>[ 3 ]</th>
<th>[ 4 ]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SKIN</strong></td>
<td>No change over baseline</td>
<td>Follicular, faint or dull erythema/ epilation/dry desquamation/ decreased</td>
<td>Tender or bright erythema, patchy moist desquamation/ moderate edema</td>
<td>Confluent, moist desquamation other than skin folds, sticking edema</td>
<td>Ulceration, hemorrhage, necrosis</td>
</tr>
</tbody>
</table>

| **MUCOUS MEMBRANE** | No change over baseline | Injection/ may experience mild pain not requiring analgesic | Patchy mucositis which may produce an inflammatory serosanguinitis discharge/ may experience moderate pain requiring analgesia | Confluent fibrinous mucositis/ may include severe pain requiring narcotic | Ulceration, hemorrhage or necrosis |

| **SALIVARY GLAND** | No change over baseline | Slightly altered taste such as metallic taste/ these changes not reflected in alteration in baseline feeding behavior, such as increased use of liquids with meals | Moderate to complete dryness/ thick, sticky saliva/ markedly altered taste | _______ | Acute salivary gland necrosis |

| **PHARYNX & ESOPHAGUS** | No change over baseline | Mild dysphagia or odynophagia/ may require topical anesthetic or non-narcotic analgesics/ may require soft diet | Moderate dysphagia or odynophagia/ may require narcotic analgesics/ may require puree or liquid diet | Severe dysphagia or odynophagia with dehydration or weight loss (>15% from pretreatment baseline) requiring N-G feeding tube, I.V. fluids or hyperalimentation | Complete obstruction, ulceration, perforation, fistula |

| **LARYNX** | No change over baseline | Mild or intermittent hoarseness/cough not requiring antitussive/ erythema of mucosa | Persistent hoarseness but able to vocalize/ referred ear pain, sore throat, patchy fibrinous exudate or mild arytenoid edema not requiring narcotic/ cough | Whispered speech, throat pain or referred ear pain requiring narcotic/ confluent fibrinous exudate, marked edema/ hoarseness | Marked dyspnea, stridor or hemoptysis with tracheostomy or intubation necessary |
E. Radiation Therapy Oncology Group (RTOG) Grading

Patients with Oral Mucositis
F. Data and Statistical Analysis

**Paired T-Test**
- MMP-1 level before and during the 10th day radiation therapy

**Regression analysis**
- MMP-1 level for observable side effects of radiation

**Pearson Correlation test**
- Correlation between the CBC results and the levels of MMP-1
DEMOGRAPHIC PATIENT PROFILE

- Total of 20 individuals
- Males 85%
- Females 15%

Age Group Distribution

- 50-54: 25%
- 45-49: 20%
- 40-44: 15%
- 35-39: 5%
- 30-34: 5%
- 25-29: 5%
- 20-24: 5%
- 15-19: 5%
- 10-14: 5%
- 5-9: 5%
- 0-4: 5%

Pie Chart 4.1 Age Group Distribution
### Patient Profile

<table>
<thead>
<tr>
<th>Anatomic Site</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larynx</td>
<td>3</td>
</tr>
<tr>
<td>Nasopharyngeal</td>
<td>11</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>4</td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>20</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>1</td>
</tr>
<tr>
<td>Stage II</td>
<td>1</td>
</tr>
<tr>
<td><strong>Stage III:</strong></td>
<td><strong>8</strong></td>
</tr>
<tr>
<td>Stage IVA</td>
<td>6</td>
</tr>
<tr>
<td>Stage IVB</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>20</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With:</strong></td>
<td><strong>18</strong></td>
</tr>
<tr>
<td><strong>Without:</strong></td>
<td><strong>2</strong></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>20</strong></td>
</tr>
</tbody>
</table>
Graph 4.2. MMP-1 Trend of Day 0 and Day 10

Day 0 and Day 10 MMP-1 Concentration

- **CONCENTRATION 10**
- **CONCENTRATION 0**

Participant Concentration (pg/mL)
## RESULTS & DISCUSSION

Mean MMP-1 Levels of 20 patients 10 days after site-directed radiotherapy

<table>
<thead>
<tr>
<th></th>
<th>Concentration Day 0</th>
<th>Concentration Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>8031.08 ± 2672.34</td>
<td>8685.40 ± 3160.98</td>
</tr>
<tr>
<td><strong>Observations (n)</strong></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>T Stat</strong></td>
<td>-1.076241777</td>
<td></td>
</tr>
<tr>
<td><strong>p-value (two-tail)</strong></td>
<td>0.147647894</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2.1. T-test: Paired Two Sample for Means

**MEAN CONCENTRATION OF CONTROL GROUP:** 3080.026071 pg/mL
<table>
<thead>
<tr>
<th>Hematologic Parameter</th>
<th>Mean Value of Parameter on Day 0</th>
<th>Mean Value of Parameter on Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>105.534</td>
<td>109.45</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>12.7277</td>
<td>3.636111111111</td>
</tr>
<tr>
<td>RBC</td>
<td>4.619473684</td>
<td>4.055263158</td>
</tr>
<tr>
<td>WBC</td>
<td>8.983</td>
<td>6.5483333333</td>
</tr>
<tr>
<td>Platelet</td>
<td>280.95</td>
<td>293.944444444</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0.62</td>
<td>0.705263158</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.3189473688</td>
<td>0.245263158</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.0361111111</td>
<td>0.0211111111</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.0338888889</td>
<td>0.0155555556</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.5922222222</td>
<td>0.5266666667</td>
</tr>
</tbody>
</table>
## Table II. Pearson Correlation Value and p-value for CBC Parameters

<table>
<thead>
<tr>
<th>CBC parameters</th>
<th>Pearson Correlation Value</th>
<th>p-value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>-0.1029</td>
<td>0.6750</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.2152</td>
<td>0.3763</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>RBC</td>
<td>-0.2496</td>
<td>0.3027</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>WBC</td>
<td>0.4753</td>
<td><strong>0.0397</strong></td>
<td>Significant relationship</td>
</tr>
<tr>
<td>Platelet</td>
<td>0.5754</td>
<td><strong>0.0099</strong></td>
<td>Significant relationship</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0.2690</td>
<td>0.2655</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>-0.3638</td>
<td>0.1258</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.2478</td>
<td>0.3549</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.4686</td>
<td>0.0672</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Monocyte</td>
<td>-0.0059</td>
<td>0.9827</td>
<td>No significant relationship</td>
</tr>
</tbody>
</table>

*Relationship is significant if p-value is less than 0.05.*
Statistical Analysis on the Levels of MMP-1 with the Results of RTOG Grading System, specific for radiation-induced mucositis

Table 4.3. Regression Model of MMP-1 vs. RTOG

<table>
<thead>
<tr>
<th></th>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>12587.32853</td>
<td>1724.484</td>
<td>7.299185</td>
<td>8.81554E-07</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>-1903.37817</td>
<td>783.0479</td>
<td>-2.43073</td>
<td>0.025747092</td>
</tr>
</tbody>
</table>
CONCLUSION

• MMP-1 values exhibited an increasing trend from day 0 to day 10, but the recorded increase was not statistically significant (p > 0.05).

• Significant correlation between WBC and Platelet counts and MMP-1 levels on the 10th day of radiotherapy.

• There was a significant relationship between MMP-1 level (10th day) and the mean results of RTOG Analysis (Oral mucositis).

(24.71% MMP-1)
(75.29% other factors)
CONCLUSION

MMP-1 has the potential to be a predictive marker to estimate the severity of oral mucositis for head and neck cancer patients undergoing radiotherapy.

FUTURE DIRECTIONS: Interventional therapies that target correlated MMPs to prevent or reduce the severity of OM and to promote faster healing of OM lesions.
RECOMMENDATIONS

• **Larger** sample size

• Target population should be more **specific**

• Conduct with **longer time frame**, with the observation of MMP-1 levels at **more intervals**

• Explore **causality relationship** between MMP-1 levels and the parameters that were found to have a significant correlation (WBC and Platelet count)
THANK YOU!

Puerto Princesa Subterranean River (Underground River), UNESCO World Heritage Site, New 7 Wonders of Nature