Micronuclei Evaluation of Reduction in Neoadjuvant Chemotherapy Related Acute Toxicity in Locally Advanced Lung Cancer: An Indian Experience


Abstract

Background: Lung cancer is the most common cancer in the world accounting for 17.6% of cancers worldwide. The AAR in Indian population varies from 0.98-15.55. The aim of the present study was to analyze a reduction in neoadjuvant chemotherapy related acute toxicity in locally advanced lung cancer (stage IIIA and III B) using Wobe Mugos E and its evaluation using micronuclei as a cytogenetic marker. Micronuclei, which are cytoplasmic fragments of DNA, have been used as a biological dosimeter to assess DNA damage.

Material and Methods: Forty patients of locally advanced NSCLC were randomized into two study groups between 2001-2003. One group received neoadjuvant chemotherapy using Cisplatin and Etoposide. The other group received neoadjuvant chemotherapy using Cisplatin and Etoposide along with Wobe Mugos E which is a proteolytic enzyme preparation. A study of micronuclei frequency was done pre and post chemotherapy in both groups.

Results: Thirty eight patients were available for final evaluation. Anemia was the most common hematological toxicity observed. Nausea and vomiting were the most common non-hematological toxicity seen. Wobe Mugos E was found to reduce the incidence of leucopenia (p = 0.005), nausea (p=0.004), vomiting (p= 0.003), sensory neuropathy (p = 0.032) and treatment related depression (p= 0.005). A reduction in micronuclei was seen in patients on Wobe Mugos E. (p =0.01).

Conclusion: Neo-adjuvant chemotherapy related acute toxicity is a major problem in patients with advanced lung cancer. A reduction in micronuclei frequency shows Wobe Mugos E to be effective in reducing chemotherapy related acute toxicity.

INTRODUCTION

In India cancer of the lung continues to be major challenge for the treating physician. It is the leading site of cancer in males in three urban cancer registries of India. In males the AAR ranges from 1.36-15.55. Females show a lower incidence rate with AAR ranging from 0.98- 4.40. Cases of lung cancer have been recorded by cancer registries even in younger 20–24 year age group but peak age specific rates are seen in seventh and eighth decade of life. Non small cell lung cancer accounts for 75 – 80% of all neoplasm of lung. At time of diagnosis only 10 –20 % patients with lung cancer are candidates for surgical resection. As such a large number of patients with non-small cell lung cancer constitute the locally advanced cancer group and are potential candidates for systemic chemotherapy alone or along with radiotherapy. These patients are usually managed by neoadjuvant chemotherapy followed by radiotherapy and treatment maybe associated with a variety of side effects. One such Neoadjuvant regimen using Cisplatin and Etoposide has been used in various trials due to its synergistic action.

We have used Cisplatin and Etoposide despite availability of newer agents because of its proven efficacy and also because it is economical for our patients.

Treatment related toxicities is a major concern as it often leads to interrupted treatments and poor quality of life for patients. Reduction of treatment related toxicities in such patients is an area of active clinical research with immense potential benefits. Proteolytic enzymes like Wobe Mugos E (containing Papain 100mg, Chymotrypsin 40 mg, Trypsin 40mg) have been used for reducing treatment toxicities. Enzymes are known to act via binding to antiproteinases such as alpha 2 micro globulin and alpha 1 antitrypsin. Increase in levels of...
antiproteinases inactivates other proteinases like cathepsins, thereby modulating the tumor growth. Enzymes also influence cytokines like IL-1β, IL-6, IFN and TGF–β. A reduction in cytokine level can modulate tumor behavior.8

Mutagenic agents are known to cause chromosomal aberrations with generation of micronuclei. This fact has been exploited to study chromosomal damage in response to radiation exposure, chemotherapy drugs and other mutagens. Micronuclei are DNA fragments generated spontaneously as well as following exposure to carcinogens. These can be studied in human peripheral blood lymphocytes as shown by Countraman and Heddle in 1976. Fenech and Morley gave the cytokinesis block method for analyzing micronuclei in lymphocytes. Further a reduction in micronuclei has been shown to occur in cancer patients on antioxidants and chemo preventive agents. Kaul et al studied Wobe Mugos E in reducing acute sequel of radiation in head and neck cancers. They concluded that enzymes play an important role in reducing radiotherapy induced toxicities.

The aim of this present study was to study a reduction in Neo-adjuvant chemotherapy related acute toxicity using polypenzyme preparation Wobe Mugos E in Indian population. Micronucleus was used as a cytogenetic marker to quantify this reduction.

**MATERIAL AND METHODS**

Forty patients of Locally Advanced Non–small Cell Lung cancer were studied from 2001 to 2003. The patients were randomized into two study groups. Group A included 20 patients who received Neoadjuvant chemotherapy with Injection Cisplatin and Etoposide. Group B received same chemotherapy along with Wobe Mugos E Inj. CDDP was given in a dose of 100mg/m.sq in 3 divided doses for three days. Etoposide was used in a dose of 100mg/m.sq/day for three days. Wobe Mugos E was given from first day of chemotherapy on a daily basis till the end of the third cycle of chemotherapy. Dose of Wobe Mugos E used was 2 Tabs TID taken half an hour before meals with plenty of liquids. The patients were evaluated for Acute Toxicity of chemotherapy at weekly intervals. CTC version 2.0 was used for toxicity grading as well as grading parameters like anxiety, depression and neuropathy. At end of three cycles of chemotherapy response was evaluated by repeat CT scan of chest and abdomen using Response evaluation criteria in solid tumors (RECIST). Patients were then taken up for radiotherapy or further chemotherapy depending on response to treatment. Two patients showing radiological complete response underwent surgery.

A micronuclei analysis was done using peripheral blood lymphocytes before starting the 1st cycle and at the end of the 3rd cycle of chemotherapy. Micronucleus scoring was done as per Criteria of Heddle.

For statistical analysis data was arranged in SPSS format. Descriptive statistics i.e. frequency distribution and their percentage according to categories have been calculated. To see significance association chi – square test has been performed for each point of time. p value of 0.05 has been considered as statistical significance level. For micronuclei analysis student ‘t’ test at pre and post level has been performed. For overall response between groups Wilcoxon Rank Sum Test has been performed.

**OBSERVATION AND RESULTS**

The median age of presentation of patients in the study was 58.5 years. There were 30 male and 10 female patients. Fifteen patients suffered from NSCLC IIIA and twenty five patients had IIIB disease. Squamous cell carcinoma was the most common histology (75%). 75% patients were smokers with a mean duration of smoking of 15 years. Cough and chest pain were the most common presenting symptoms in 87.5% of patients. 38 patients completed three cycles of neoadjuvant chemotherapy and were available for evaluation. One patient defaulted after first cycle of chemotherapy while one patient died during treatment. Overall toxicity observed in the two groups is discussed below.

**Hematological toxicity** : Anemia was the most common hematological toxicity observed (81% of patients). At end of second cycle of chemotherapy Grade 1 anemia was observed in 39.4% of patients and 26.3% had grade 2 anemia. One patient developed Grade 3 anemia. After 3rd cycle of chemotherapy, 40% of patients developed grade 1 anemia while 27% had grade 2 anemia. 13.5% patients had Grade 3 anemia. There was no statistically significant difference between the two study arms.

After 2nd cycle 27% patients developed Grade 1 leucopenia while 18.9% patients had grade 2 leucopenia. At end of 3rd cycle Grade 1 leucopenia was seen in 38.8% of patients. 19.4% had grade 2 leucopenia. Grade 3 leucopenia was seen in 2 patients. The difference was stastically significant with patients on Wobe Mugos E showing lesser toxicity (p = 0.005).

Grade 1 thrombocytopenia was seen in 13.8% of patients and 11.11% developed Grade 2 thrombocytopenia after 2nd cycle. At end of 3rd cycle 44.44% patients had grade 1 thrombocytopenia while 3 patients had Grade 2 thrombocytopenia.

**Gastrointestinal toxicity** : Anorexia (Grade 1 and 2) was seen in 52.7% of patients at end of 3rd cycle.

Grade 1 nausea was seen in 36.8% of at end of first cycle of chemotherapy. At end of 2nd cycle 54% patients had Grade 1 nausea while 40.5% had grade 2 nausea. 36.11% patients had grade 2 nausea at end of 3rd cycle of chemotherapy Stastically significant decrease in nausea was seen in patients on Wobe Mugos E after third cycle (p=0.004).
At end of 1st cycle 47% of patients had Grade 1 and 2 vomiting. After 2nd cycle 48.6% had Grade 1 vomiting and Grade 2 vomiting was seen in 45% patients. After 3rd cycle 30.5% had grade 1 vomiting while 41.6% had grade 2 vomiting. A statistically significant difference was observed between the two groups (p=0.003) with lesser toxicity in enzyme therapy group.

**Neurological toxicity**: Grade 1 sensory neuropathy was seen in 33.3% of patients while 2 patients had Grade 2 after 3rd cycle of chemotherapy. Lesser toxicity was seen in patients on Wobe Mugos E (p=0.032).

Grade 1 or 2 anxiety was seen in 70% of patients while on treatment.

Grade 1 and 2 depression was seen in 21.6% of patients. 32.4% of patients had grade 1 depression after 2nd cycle of chemotherapy. At end of 3rd cycle Grade 1 depression was seen in 13% of patients while Grade 2 depression was seen in 41.6%. Patients on enzyme group had a lesser toxicity (p=0.005).

**Renal Toxicity**: Five patients developed Grade 1-2 increase in creatinine level. No renal failures were observed.

**Dermatological toxicity**: 88% of patients developed Grade 2 alopecia at end of 3rd cycle of chemotherapy.

**Constitutional toxicity**: Grade 1 fatigue was seen in 51.35% of patients while grade 2 was seen in 45.9% of patients.

54% experienced Grade 1 weight loss after first cycle while 21% had grade 2 weight loss after 2nd cycle of chemotherapy. At end of 3rd cycle 33.33% had Grade 1 weight loss while 63.8% developed a Grade weight loss. The difference was significant (p = 0.024) with lesser toxicity in enzyme arm.

**Febrile Neutropenias**: Five patients had febrile neutropenias with no mortality.

No hepatotoxicity was observed nor was any allergic reactions noted.

**Micronuclei Frequency**

The mean micronuclei frequency seen in post chemotherapy group was higher than in pre chemotherapy samples as shown in Table 2. Among the post chemotherapy samples patients on Enzyme therapy with Wobe Mugos E.

Had a lower mean micronuclei frequency (36.980) compared with chemotherapy alone group (44.8363) with a significant p value (p=0.019) Table 2.

**Overall response** was assessed using response evaluation criteria in solid tumors (RECIST). Two patients had complete response (5.26%). 14 patients had partial response (36.84%). Stable disease was observed in 11 patients (28.94%). Progressive disease was seen in 11 patients (28.94%). No statistically significant difference was seen between study groups.

### Table 1: Toxicity profile (N=38)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Group A (%)</th>
<th>Group B (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucopenia</td>
<td>38.8</td>
<td>16.6</td>
<td>0.005</td>
</tr>
<tr>
<td>Nausea</td>
<td>33.3</td>
<td>8.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Vomiting</td>
<td>50</td>
<td>22.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Sensory neuropathy</td>
<td>30.5</td>
<td>8.3</td>
<td>0.032</td>
</tr>
<tr>
<td>Depression</td>
<td>41.6</td>
<td>16</td>
<td>0.005</td>
</tr>
<tr>
<td>Weight loss</td>
<td>52.7</td>
<td>44.4</td>
<td>0.024</td>
</tr>
</tbody>
</table>

### Table 2: Pre and post chemotherapy micronuclei frequency (N=38)

<table>
<thead>
<tr>
<th></th>
<th>Group A Mean Micronuclei frequency</th>
<th>Group B Mean Micronuclei frequency</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre chemotherapy</td>
<td>11.3495</td>
<td>12.0340</td>
<td>0.019</td>
</tr>
<tr>
<td>Post chemotherapy</td>
<td>44.8365</td>
<td>36.9890</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Overall Response (N=38)

<table>
<thead>
<tr>
<th>Response</th>
<th>Group A No. of patients</th>
<th>Group B No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Partial Response</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Neoadjuvant chemotherapy is used in locally advanced non small cell lung cancer for causing regression of primary tumor before instituting definitive treatment. However chemotherapy is associated with considerable toxicity and consequent morbidity. Wobe Mugos E has shown to reduce chemotherapy side effects. In our study we observed anemia in 81% of patients making it the most common hematological toxicity observed. The frequency of hematological toxicity increased from from cycle one to cycle three. Grade 3 anemia was seen in 13.5% of patients after 3rd cycle of chemotherapy. Jean Pujol et al observed Grade 3-4 anemia in 18% of patients treated by cisplatin, etoposide and Ifosfamide. Leucopenia (Grade 1-3) was seen in 63.8% of our patients. Different studies have shown an incidence of leucopenia ranging from 54-77%. Wobe Mugos E has shown to decrease the incidence of leucopenia in our study (p = 0.005).

We observed thrombocytopenia in 44.4% patients after third cycle of treatment. 97.2% of patients on treatment experienced nausea after second cycle of chemotherapy. Majority of these were Grade 1 nausea. Almost all patients experienced vomiting during 2nd and 3rd cycles of chemotherapy. Grade 1 vomiting was seen 48.6% of patients while grade 2 vomiting was seen in 45% of patients after 2nd cycle of chemotherapy. An incidence of Grade 1 vomiting ranging from 21.4%-28%.
has been observed in different studies.27 Patients on Wobe Mugas E showed a decreased incidence of both nausea (p = 0.004) and vomiting (p = 0.003).

Sensory neuropathy was observed in 38 % of patients after third cycle of chemotherapy. Out of these 33.3% of patients had grade 1 sensory neuropathy. Patients on Wobe Mugas E showed a decreased incidence of sensory neuropathy (p=0.032).

Albain et al18 observed grade 1 neurological toxicity in 21.42 % of patients. Other studies have shown an incidence of 20.5% for sensory neuropathy.19 Five patients under study had a grade 1-2 increase in creatinine clearance. A 15 –23% incidence of grade 1 increase in creatinine clearance has been shown in various studies.16,17 No renal failure was observed in our study.41.6% of our patients had grade 2 depression after after 3rd cycle. A statistically significant difference was observed between treatment arms with patients on Wobe Mugas E showing lesser depression (p = .005). At completion of therapy 88 % patients had grade 2 alopecia .Five of our patients developed febrile neutropenia with no mortality. A reduction in weight loss was observed in the patients receiving Wobe Mugas E (p =0.024). Prakash et al have found oral enzyme therapy to reduce genitourinary symptoms, subcutaneous changes and vaginal reactions in patients receiving radiation therapy for advanced cancer cervix.20 Micronuclei generation has associated with exposure to various carcinogenic agents, radiation and chemotherapeutic agents. Spontaneous micronuclei frequency has also been studied in various groups not exposed to carcinogenic agents.21 A decrease in micronuclei frequency was observed in Wobe Mugas E arm (p = 0.019). These results are comparable with various studies using other chemo preventive agents.11,22,23 A reduction in micronuclei attests to the efficacy of Wobe Mugas E in reducing chemotherapy related toxicity.

Shepherd2 has observed that less than 10% patients achieve complete response to induction chemotherapy (range 0-23%).We observed a CR in 5.26% of patients. 36.84 % patients had a partial response.

Though the number of patients in this study is less still an interesting trend is seen which needs to be further explored.

**Conclusions**

Treatment related toxicities not only cause limitation in quality of life of patients but also cause interruptions of the proposed therapeutic regimen. The results of our study have revealed that Wobe Mugas E (proteolytic enzyme) is effective in reducing neoadjuvant chemotherapy related toxicity in locally advanced non small cell lung cancer. However due to limited sample size we suggest that a larger randomized trial may be called for since the results can have beneficial implications for patient’s quality of life.

**References**


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**Announcement**

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**Announcement**

Third Madras Diabetes Research Foundation (MDRF) – American Diabetes Association (ADA) Postgraduate Course on Diabetes, at Chennai, India, 6 - 8th October 2006.

The Third MDRF-ADA Postgraduate Course on Diabetes will be held from 6th to 8th October 2006 at Chennai, India. The meeting will be hosted by the Madras Diabetes Research Foundation, Chennai.

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