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Original Article

Oncology Section

Chemoresponse Assay in Head and Neck Cancer Patients: A Three-Year Follow Up

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ABSTRACT

Introduction: The majority of patients with advanced head and neck cancer receiving chemotherapy show partial response or frank resistance. Therefore, assessing the individuals' tumour reactivity to the eligible chemotherapeutic compounds carries the potential of personalizing the patient treatment and minimizing ineffective regimens which lead to excess toxicity and cost, treatment delays and possibly causing the tumour to be cross resistant to additional drugs.

Aim: To determine the effectiveness of a phenotypic chemoresponse assay in predicting response to chemotherapy in a retrospective series of head and neck cancer patients whose tumour specimens had been tested with ChemoFx assay (Precision Theraputic Inc.).

Materials and Methods: Twenty-two tumour specimens were submitted to Precision Theraputics Inc. for chemoresponse testing, all of which have been histologically confirmed as squamous cell carcinoma of the head and neck. Selection of treatment was at the discretion of the treating physician and the results of the assay were not used to determine the therapy. A portion of the patients' solid tumour was established in primary culture, then exposed to increasing doses of different chemotherapeutic agents. The resultant cell counts in the treated wells were used to indicate the tumours' response to the agent and based on the dose response score curve, the test was scored as "responsive," "intermediate response," or "non-responsive."

Results: Of the 22 tumour samples submitted, 16 (72.7%) showed adequate cell yield in cultures and subsequently underwent in vitro chemoresponse assays and are reported in this study. Of the 16 cases reviewed, 5 were excluded due to inadequate follow up. A predictable response assay was either a good response to chemotherapy in patients whose tumour specimens showed sensitivity to the chemotherapeutic agents or failure in patients whose tumours showed either intermediate response or non responsiveness to the chemotherapeutic agent/agents. Of the 11 patients reported in this study, nine showed a predictable chemoresponse assay (81.8% predictability of effective treatment). Three patients had a predictable good response and six who failed their chemotherapy regimen within six months of treatment and their chemoresponse assay showed an inadequate response to the chemotherapeutic agents they were treated with. At three years follow up, all patients who had a predictable poor response succumbed to their disease except one, whose test showed intermediate response.

Conclusion: While the current report has its limitation, we conclude, based on our findings, that chemoresponse assays may be useful adjuncts in the guiding the selection of chemotherapeutic agents in patients with head and neck cancer.

Keywords: Chemosensitivity, Chemotherapy, Individualization, Oncology

INTRODUCTION

The selection of a chemotherapy regimen is currently based on the clinical and histological features of the tumour and evidence from randomized clinical trials of different treatments applied to patients with the same diagnoses or the same primary subsites. Experience has demonstrated that histologically similar tumours and tumours that are similar in terms of their subsite locations in different individuals do not necessarily respond identically to a given agent or set of agents. Also, resistance to chemotherapy cannot be predicted by either clinical or histologic examinations, and the individual patient response to chemotherapy can only be judged after several cycles are administered. Moreover, using inadequate doses or regimens of chemotherapy carries the risk of inducing further chemoresistance in these patients [1,2].

With the increasing number of available anticancer agents, there is increasing pressure and need to select the most appropriate pharmaceutical. In addition to this availability, the understanding of the heterogeneity inherent to cancer cells is enabling the individualization of treatment plans for cancer patients.

Therefore, the quest to resolve an individual tumour's reactivity to the eligible chemotherapeutic compounds via chemosensitivity and resistance assays has been the focus of many studies in head and neck oncology over the past few decades [3,4].

Chemosensitivity testing is an ex vivo means of determining the cytotoxic and/or cytostatic or apoptosis inducing effects of anticancer drugs [5]. Chemosensitivity testing would allow for the individualization of the treatment plan for each patient. By identifying inactive drugs, patients can be spared the "one size fits all" approach of administering standard chemotherapy regimens, which ultimately leads to treatment delays, unnecessary morbidity and a waste of health care resources.

Many in vitro chemoresponse assays have been developed with the aim of acquiring information about a tumour's sensibility or resistance to cytostatic drugs [3,6]. Most of these assays have been limited by technical difficulties, requirements of large amounts of fresh tissue or a lack of clinical utility in predicting patient outcomes [4,7].

The ChemoFx assay is an assay that quantifies cellular effects via the direct visualization of cells following exposure to the anticancer agents to provide the medical oncologist with information regarding the tumour's sensitivity and resistance to the agents that have been tested [7].

The objective of this study was to determine the effectiveness of a phenotypic chemoresponse assay in predicting the responses to chemotherapy in a retrospective series of head and neck cancer patients whose tumour specimens had been tested with ChemoFx assays (Precision Therapeutics Inc.).

MATERIALS AND METHODS

A retrospective study was conducted on data of patients with specimens submitted to Precision Theraputics Inc. during 2011. At least three years had passed since the submission of these specimens that were included in the study.

Twenty-two tumour specimens from various head and neck subsites were submitted for chemoresponse testing. All patients included in this study provided written informed consent to allow research on their tumour specimens. Boston University Medical Center Institutional Review Board approval was obtained for the study. All tumour samples had been histologically confirmed as squamous cell carcinoma. Samples from recurrent tumours in patients who received prior chemotherapy or radiation therapy were excluded.

The selection of treatment was at the discretion of the treating physician, and the results of the assay were not used to determine the therapy. A small portion of the tumour was excised from the patients' tumour specimen which was then mechanically disaggregated and established in primary culture. Malignant epithelial cells were then allowed to migrate to form a monolayer. Those cultures were then exposed to increasing doses of different chemotherapeutic agents as shown in [Table/Fig-1]. Using automated cell counting software, the resultant cell count in the treated wells were compared with those in untreated control wells to generate a dose response curve for each therapeutic agent tested on the specimen of a given patient. We followed the method of Brower SL et al., to score the tumour's response to each of the ex vivo chemotherapeutic treatments as "Responsive", "Intermediately responsive", or "Non responsive" [8]. The in vitro studies and assessment of dose response curves were assessed by Precision Therapuetics Inc. who was blinded to the patient identity, chemotherapy agent used and its outcome.

RESULTS

Of the 22 tumour samples submitted, 16 (72.7%) produced adequate cell yields in culture, subsequently underwent in vitro chemoresponse assays and are reported in this study. Six cases were terminated either due to bacterial culture contamination or insufficient cell growth.

ChemoFx assays were used to categorize tumour specimens as responsive, intermediately responsive or non-responsive to a battery of chemotherapeutic agents (which can be specified by the submitting oncologist). Of the 16 cases reviewed, five were excluded due to inadequate follow up such as continuing chemotherapy at another institution.

The 11 cases reported in the study were initially classified as either exhibiting good responses or failures. Failure was defined by a persistent tumour or the progression of disease within six months following the initiation of chemotherapy. The patient in each group was then further subclassified as having a predictable chemoresponse assay or a non predictable assay. A predictable response assay entailed either a good response to chemotherapy in patients whose tumour specimens exhibited sensitivity to the chemotherapeutic agents or failure in patients whose tumours exhibited either an intermediate response or non responsiveness to the chemotherapeutic agent/agents. [Table/Fig-1] summarizes the patients' demographics, chemotherapy agents given and the assay predictability.

Of the 11 patients reported in this study, nine had predictable chemoresponse assays (81.8% predictability of effective treatment). Three patients had predictable good responses, and the six who failed their chemotherapy regimen within six months of treatment had chemoresponse assays that indicated inadequate responses to the chemotherapeutic agents they were treated with. At a three-year follow up, all patients who exhibited predictable poor responses had succumbed to their disease with the exception of one whose test indicated an intermediate response. Two patients, whose ChemoFx response were deemed unpredictable exhibited intermediate responses to the applied chemotherapeutic agents. One of these patients remained disease free throughout the follow up. The other developed extensive locoregional recurrence 18 months after the completion of therapy.

DISCUSSION

The role of chemotherapy in head and neck oncology has evolved extensively over the past few decades from a strictly palliative treatment to an important component in multimodal therapies. Chemotherapy has proven to have curative potential in head and neck cancer especially when combined with radiation treatment [9,10].

Initially, chemotherapy was deemed ineffective when the endpoint of survival was evaluated [11]. However, analyses of subsets of the data from the initial studies suggested that chemotherapy likely plays a role in the treatment of a subset of head and neck cancer patients. These findings were the basis for the development of organ preservation approaches [11,12]. Chemotherapy is frequently used to treat patients with advanced head and neck cancer. A significant portion of patients receives chemotherapy; however, these patients will only exhibit a partial response or frank resistance [13]. An ineffective regimen can result in excess toxicity and costs, may delay administration of a more effective treatment, and may cause the tumour to be cross resistant to additional drugs [14]. Chemoresponse has been demonstrated not only to be a predictor

Patient	Age (years)	Gender	Localization	Stage (AJCC)	TNM Classification	Treatment	Chemoresponse Assay Predictability
1	56	M	Oropharynx	Stage 3	T3N0M0	Surgery + CRT cisplatinum	Predictable good response
2	47	F	Mandible	Stage 3	T3N0M0	Surgery + CRT cisplatinum	Predictable good response
3	64	F	Tongue	Stage 4	T1N2bM0	Surgery + CRT cisplatinum	Predictable good response
4	64	F	Supraglottis	Stage 4	T4N2cM0	Surgery + CRT cisplatinum	Predictable poor response
5	54	F	Neck	Stage 4	TXN3M1	TPF	Predictable poor response
6	78	F	Tongue	Stage 4	T2N2bM0	Carboplatin/ Taxotere	Predictable poor response
7	48	M	Larynx	Stage 3	T3N0M0	Cisplatinum	Predictable poor response
8	55	M	Larynx	Stage 4	T4N2cM0	Cisplatinum	Predictable poor response
9	65	M	Larynx	Stage 4	T4N1M0	Taxol/ cisplatinum	Predictable poor response
10	69	F	Larynx	Stage 4	T4N2cM0	TPF	Unpredictable good response
11	62	M	Hypopharynx	Stage 4	T4N1M0	Surgery + CRT cisplatinum	Unpredictable good response

[Table/Fig-1]: Summary of the patients' demographics, tumour stage, chemotherapy agents given and the assay predictability.
CRT: Chemoresponsive, TPF: Docetaxel, cisplatin and fluouracil

of radiation responses but also the most important prognostic factor in patients undergoing treatment for advanced laryngopharyngeal cancer [15]. Ex-vivo testing of head and neck carcinoma specimens to the different cytotoxic drugs used has been described by Dollner R et al., and they concluded, the protocol may help provide clinically useful information regarding the individualization and choice of chemotherapy agents to be used [16].

The predictive value of the ChemoFx assay has been proven in ovarian cancer. Gallion H et al., found that the incorporation of the information from the ChemoFx assay into treatment plans seems to have the potential to improve the clinical outcomes of patients with ovarian cancer [17]. The role of chemosensitivity testing on survival has also been previously tested. Singh B et al., found that chemosensitivity, as determined by a histoculture drug response assay, seems to be a strong predictor of survival in patients with advanced head and neck cancer [18]. Moreover, from the health economics perspective, when assay directed therapy is adhered to in the treatment of recurrent ovarian cancer patients, the estimate savings are $15,571-24,772 [19]. ChemoFx can effectively assess the sensitivity to multiple agents using as little as 35 mg of tissue [7] (approximately two to three, 14-gauge core needle biopsies), which makes this assay a candidate for testing small amounts of tissue obtained via fine needle aspiration biopsies of neck masses, particularly those with unknown primaries.

In the current report, we demonstrated the potential clinical utility of the ChemoFx assay in adjuvant head and neck cancer therapies. Although adequate, the observed 72.7% cell yield in cultures is less than that found in other reports in the literature regarding successful tumour cell growth assessed with the ChemoFx assay, which had been reported to achieve cell yields as high as 83.9%, including from cultures of core needle biopsies [7]. It showed an 81.8% predictability of effective treatment in the tested group of patients, which was consistent on the long term follow up of those patients.

LIMITATION

The study is limited by its retrospective design and the limited number of patients included. Also, the in vitro studies were conducted by the Precision Therapeutics Inc. however they were blinded to both the identitfication of patients as well as the cytotoxic agents used and outcome of treatment. Therefore, further prospective studies and clinical trials are warranted.

CONCLUSION

Our findings, although preliminary, suggests a positive role for chemoresponse assays in guiding the selection of chemotherapeutic agents in head and neck cancer as the predictability of the effectiveness of some agents would allow better individualization and tailoring of the treatment.

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