Plasma EBV DNA concentration correlates with FDG PET in Nasopharyngeal Carcinoma treated with induction chemotherapy

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Background: While local control rate in locally advanced NPC (LA-NPC) has improved with concurrent Cisplatin and IMRT, development of distant metastases remains common in these patients and limits survival. The role of induction chemotherapy in the treatment of LA- NPC is still a matter of debate. The aim of this retrospective review is to evaluate the effect of induction chemotherapy and to correlate between EBV-DNA concentration and tumor response by PET-CT.

Methods : Patients with stage III-IVB , WHO II/III received induction chemotherapy with 2 cycles of TPF : Docetaxel: 75 mg/m2, Cisplatin: 75 mg /m2, 5Fluorouracil: 750 mg / m2 CI for 96 hours followed 3-4 weeks later by concurrent weekly Cisplatin (40 mg /m2) and IMRT (GTV:70 Gy over 35 fractions). EBV-DNA quantification was performed at baseline and repeated before each cycle of TPF. PET scans were performed at baseline and repeated before IMRT. The max standard uptake values (SUV) were recorded in the primary tumors. Metabolic response was defined as a decrease in maximum SUV of 35% or more.

Results: 20 patients with LA-NPC (75%Stage IVA/IVB) were reviewed. All but one completed therapy. Objective response, according to RECIST criteria: CR: 4; PR: 14 NE: 2. Median concentration of EBV-DNA was 11,300 copies / ml (range: 1,184 - 43,000). Post TPF, reduction of EBV-DNA copies by >50% was observed in 83% pts and 66% achieved complete biochemical response. In the FDG-avid tumor pts, the median SUV at baseline was 12 (range 10.5 - 17.4). Post TPF metabolic response was observed in 100% and was complete in 33%. All patients with complete biochemical response had also a complete metabolic response by PET. At 2-year loco-regional progression free rate is 95% and 2 year overall survival rate is 84%. No recurrence was seen in complete (biochemical / metabolic) responders.

Conclusion: A negative post induction FDG-PET and complete biochemical response after TPF are of significant value in LA-NPC and are useful determinant to predict outcome.

Introduction

Nasopharyngeal carcinoma is an endemic carcinoma associated with Epstein-Barr virus (EBV) infection. Radiotherapy is the primary treatment, but studies have supported the use of combined radiotherapy and chemotherapy for advanced cases. [1-4]

Incorporation of chemotherapy with standard RT has improved the therapeutic outcome of patients with locoregionally advanced NPC. This is supported by a meta-analysis of six randomized trials suggesting that when compared with RT alone, the addition of chemotherapy in any sequence increases disease-free survival by 35% at 2 to 4 years and overall survival by 20% at 3 to 4 years. A key question remains regarding the optimal sequencing of chemotherapy and radiotherapy. Many published randomized trials of concurrent chemoradiotherapy have demonstrated a progression-free (PFS) and/or overall survival (OS) benefit over radiotherapy alone. [5-6]

The recognition of the importance of Epstein-Barr virus (EBV) in the pathogenesis of NPC has stimulated a growing interest in EBV-based biomarkers for patients with this malignancy. [6]

EBV is present in cells from almost every primary and metastatic nasopharyngeal carcinoma, regardless of the degree of tumor differentiation or the geographic origin of the patient. [7]

Although initial studies based on qualitative measurement systems showed that plasma EBV DNA has a sensitivity of only 59–75% for diagnosis of NPC, its sensitivity in quantitative assays was as high as 90%. Such a diagnostic sensitivity appears to be at least as good as that of IgA-VCA. Of further interest is the observation that plasma EBV DNA was found to be rarely detectable in patients who had complete eradication of cancer. [8-12]

The early restaging by a single whole-body FDG-PET scan after the first or second course of induction chemotherapy is useful in detecting response to induction chemotherapy and in predicting therapy outcome for locoregionally advanced NPC patients. The non-major-responders may benefit from early PET restaging by switching to an alternative treatment to avoid the unnecessary side effect of chemotherapy or by fine-tuning the subsequent treatment modalities. [13]

The relationships between different 18F-FDG PET functional parameters and EBV DNA load has been described before in very few studies. [14]

In our study, we assessed the association of 18F-FDG PET functional parameters and EBV DNA load post induction chemotherapy for our patients with Nasopharyngeal Carcinoma (NPC).

Material and Methods

Twenty patients with locally advanced nasopharyngeal carcinoma with no distant metastasis who presented to the Oncology Centre at King Fahd Specialist Hospital, Dammam in Saudi Arabia were enrolled in this study during the period between July 2007 and April 2012. All patients received two cycles of TPF induction chemotherapy: Docetaxel: 75 mg/ m2, Cisplatin: 75 mg /m2 and continuous infusion of 5-Fluorouracil: 750 mg/ m2 for 96 hours followed 3-4 weeks later by concurrent weekly Cisplatin (40 mg/ m2) and IMRT (GTV:70 Gy over 35 fractions). All patients had a non-metastatic disease as proved by doing chest and abdominal CT scan, PET/CT scan and bone scan (when indicated).

Patient Monitoring and Follow up

Before treatment, patients underwent a complete history and physical examination, including evaluation of performance status, assessment for the presence of concurrent co-morbid conditions with estimation of body weight, height, vital signs and measurement of palpable or visual lesions. Laboratory studies included a complete blood count (CBC) with white blood cell differential count, biochemistry profile, kidney function test, liver function tests, hepatitis markers, random blood sugar and Epstein-Barr virus (EBV) DNA plasma level initially and post induction chemotherapy. Radiological examinations, including CT head and neck, chest, abdomen and pelvis, were required before starting treatment, at the end of induction chemotherapy, post concurrent chemoradiation and then periodically. Isotopic bone scan was not done routinely. FDG PET/CT was done at presentation and post induction chemotherapy. Patients should have dental clearance before initiation of radiation therapy.

Chemotherapy protocol

Neoadjuvant chemotherapy consisted of two cycles of TPF regimen: Docetaxel (75 mg/m^2 administered intravenously [IV] over 1 hour), Cisplatin (75 mg/ m^2 administered intravenously [IV] over 1 hour) and continuous infusion of 5-Fluorouracil (750 mg/ m^2 CI for 96 hours) given on days 1 and 21. During RT, cisplatin at a dose of 40 mg/m^2 IV infusion was administered weekly throughout radiation, given at approximately 60 minutes before receiving radiotherapy.

Radiation therapy technique

Intensity Modulated Radiation Therapy (IMRT) using Simultaneous Integrated Boost (SIB) was used in treating patients, three planning target volumes (PTVs) were created: PTV 70 Gy to the primary and involved nodes, PTV 60 Gy to the rest of nasopharynx, the oropharynx, posterior two thirds of the anterior maxillary sinuses and to non involved upper neck nodes, and finally PTV 54 Gy to non involved lower neck nodes.

Quantification of EBV DNA load

Plasma EBV DNA was measured by real-time quantitative PCR. DNA was extracted from plasma samples. Circulating EBV DNA concentrations were measured by a real-time quantitative PCR system that amplified a DNA segment in the *Bam*HI-W fragment region of the EBV genome. Results were expressed as DNA copies/ ml of plasma. The lower limit of reliable quantification was 5 copies/ assay, although occasionally 1 copy/assay was also detectable (the latter corresponding to 12 copies of EBV DNA/ ml of plasma).

Imaging with FDG PET/CT

All patients required to be fasting for at least 6 hours before 18F-FDG PET/CT. Imaging was performed with a PET/CT system consisting of a PET scanner and CT scanner. Before acquisition of PET images, helical CT was performed from the head to the proximal thigh. Emission scans from the head to the proximal thigh were acquired at 50–70 minutes after injection of 370 MBq of 18F-FDG. PET images were reconstructed with CT used for attenuation correction. The maximum standard uptake values (SUVmax) were obtained by drawing the regions of interest over the most intense slice of the primary tumor within the nasopharynx and the neck lymph nodes.

Results

From July 2007 to April 2012, twenty patients referred to Oncology Centre at King Fahd Specialist Hospital, Dammam in Saudi Arabia were enrolled in this study. This study was approved by the institutional review board of the hospital. We have previously reported the toxicity of induction chemotherapy and concurrent chemoraiotherapy.

The overall response rate to induction chemotherapy reached 90% with complete remission (CR) and partial remission (PR) rates of 30% and 60% respectively. Six weeks post concurrent chemoradiation therapy, 85% and 10% of patients were in complete remission and partial remission respectively, (Table 1).

Base line EBV-DNA ranged between 1,184 - 43,000 copies/ ml with a median of 11,300. Post induction chemotherapy with TPF 66% and 34% achieved complete and partial biochemical response, respectively. Reduction of EBV-DNA copies by more than 50% was seen in 83% of patients, (Table 2, 3).

For PET/CT the maximum standard uptake values (SUV) was seen in the primary tumor. SUV values ranged between 10.5 - 17.4 with a median of 12. Metabolic response was defined as a decrease in maximum SUV of 35% or more. All the patients showed metabolic responses which was variable. Complete and partial metabolic responses were seen in 33% and 67% of patients, respectively, (Table 2, 4).

All patients with complete biochemical response showed also complete metabolic response.

At 2 years loco-regional progression free survival was 95%. Overall survival rate at 2 years was 84%. No recurrences were seen in complete biochemical and metabolic responders. At last follow up seventeen patients (85%) were alive and free of disease.

DISCUSSION

Plasma EBV-DNA load is different from other peripheral blood markers in that it measures the tumor derived EBV genomic material rather than an antibody response to genomic or peptide components of the EBV. The use of real time PCR to detect EBV-DNA in plasma had been shown to be both sensitive (96%) and specific (93%). [11]

Many studies had confirmed the role of EBV-DNA as a prognostic marker in nasopharyngeal carcinoma. [15-16] Other trials had confirmed that the plasma level of EBV-DNA was undetectable in patients who had complete remission of their cancers. [12]

In our study, the baseline median concentration of plasma EBV-DNA was 11,300 copies/ ml. We have to take into consideration that the study population included only patients with locally advanced disease. Post induction chemotherapy 83% of patients showed >50% reduction of plasma EBV-DNA copies.

Currently, conventional imaging tools like CT and MRI are routinely used in staging NPC patients. A fused integrated morphological and functional imaging modality of positron emission tomography / computed tomography (PET/CT) is another possible useful new tool to be utilized in the assessment of NPC patients like in other oncological patients. [17]

Yen et al demonstrated that there is a significant correlation between the presence

of FDG hypermetabolism and the survival time of NPC patients [18]. Tumors having high FDG uptake are at greater risk of failure and should be considered for more aggressive multimodality therapy. [19]

The early restaging by a single whole-body FDG-PET scan after the first or second course of induction chemotherapy is useful in detecting response to induction chemotherapy and in predicting therapy outcome for locoregionally advanced NPC patients. The non-major-responders may benefit from early PET restaging by switching to an alternative treatment to avoid the unnecessary side effect of chemotherapy or by fine-tuning the subsequent treatment modalities. [13]

In our study, 33% of patients showed complete metabolic response as shown by PET/CT imaging which was done post 2 cycles of induction chemotherapy.

The relationships between different 18F-FDG PET functional parameters and EBV DNA load has been described before only in few trials. Makitie et al reported the first report on the correlation between EBV-DNA plasma levels and PET scan results in patients with NPC. [20] Tumor SUVmax, and nodal SUVmax were all significantly associated with plasma EBV DNA load. The highest degree of correlation was obtained between total lesion glycolysis (TLG) and EBV DNA load, suggesting a close association between this blood biomarker and activity as assessed by 18F-FDG PET. [12]

In our study, all patients who had complete biochemical response (EBV-DNA load) had also a complete metabolic response by PET/CT. No recurrences were seen in complete (biochemical / metabolic) responders.

In our study, 6 weeks post concurrent chemoradiation 85% of patients were in complete remission. Chan et al [21] reported complete remission rates of 100% in his study, while Paccagnella et al [22] and Yamouni et al [23] reported complete remission rates of 50% and 76.8%, respectively.

Conclusion

Our study encourages the use of PET/ CT and EBV-DNA load in the post therapy management of NPC patients. A negative post induction FDG-PET and complete biochemical response after TPF are of significant value in locally advanced NPC and are useful determinant to predict outcome.

Tables

Table 1: Treatment response

Mode of Assessment	6 Weeks Post Induction Chemotherapy		6 Weeks Post Concurrent CRT	
	No	%	No	%
CR	6	30	17	85
PR	12	60	2	10

Table 2: Baseline EBV-DNA and SUV max

Parameter	Range	Median
Plasma EBV-DNA concentration	1,184 - 43,000 copies/ ml	11,300 copies/ ml
SUV max	10.5 - 17.4	12

Table 3: Biochemical Response

Biochemical Response	%
Complete	66
Partial	34

Table 4: Metabolic Response

Metabolic Response	%
Complete	33
Partial	67

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST The authors indicated no potential conflicts of interest.

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