A case report: Early detection and monitoring of non-symptomatic recurrent metastasis of a nasopharyngeal carcinoma patient using the ultrasensitive realtime PCR assay of cell-free Epstein-Barr virus DNA

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Summary

As clinical signs and symptoms of nasopharyngeal carcinoma (NPC) are often nonspecific, it is difficult to detect in the early stage, leading to high risk of relapse or recurrent metastasis. Plasma cell-free DNA of Epstein-Barr virus (cf-EBV DNA) is markedly associated with the development of undifferentiated NPC, and its level prior and subsequent to treatment is reliable for the screening, diagnosis, monitoring and prognosis of NPC. The standardized and highly sensitive assay of cf-EBV DNA quantification has now been successfully established by our group, for the first time, in Vietnam for the detection of early NPC lesions with a sensitivity of 96.9% and a specificity of 98%. Here we present a case of NPC, which was diagnosed timely under the guidance of the sensitive assay of cf-EBV DNA. Even more importantly, recurrent bone metastasis without any clinical symptoms was detected early by the rise in cf-EBV DNA level.

Keywords: Nasopharyngeal carcinoma, Epstein-Barr virus.

1. Background

Nasopharyngeal carcinoma is endemic in Asia, and the undifferentiated nasopharyngeal carcinoma (WHO type III) is the most common subtype of NPC [1], [2]. Clinical signs and symptoms of NPC are often nonspecific, so it is difficult to detect, particularly, in the early stage [3], leading to high risk of relapse or recurrent metastasis. Plasma cell-free DNA of Epstein-Barr virus (cf-EBV DNA) is markedly associated with the development of undifferentiated NPC [4], and the level of cf-EBV DNA prior and subsequent to treatment is reliable for the screening, diagnosis, monitoring and prognosis of NPC [5]. The standardized and highly sensitive assay of cf-EBV DNA quantification has now been successfully established by our group, for the first time, in Vietnam for the detection of early NPC lesions with a sensitivity of 96.9% and a specificity of 98% [6]. Here we present a case

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of NPC, which was diagnosed timely under the guidance of the sensitive assay of cf-EBV DNA. Even more importantly, recurrent bone metastasis without any clinical symptoms was detected early by the rise in cf-EBV DNA level, which was later confirmed by PET/CT scan after almost three months.

2. Case presentation

A 46-year-old Vietnamese male, with a history of haemoptysis, was biospsied twice in early May 2017 in National Otorhinolaryngology of Vietnam, however, malignant lesions were not detected. Subsequently, the concentration of plasma cf-EBV DNA was determined to be 15,900copies/ml. With the guidance of such high concentration of cf-EBV DNA, the third biopsy was performed in the back of the nozzle, based on the MRI examination, and the cancerous lesion was confirmed by the end of May 2017. He was then diagnosed with undifferentiated nasopharyngeal carcinoma, with the stage of IVA (T4N2M0) and type III, according to the histological classification of tumours by WHO [7]. Computerized tomography (CT) scan of the chest, abdomen and pelvis, as well as, skeletal scintigraphy showed no evidence of primary malignancy or metastatic disease (Figure 1).

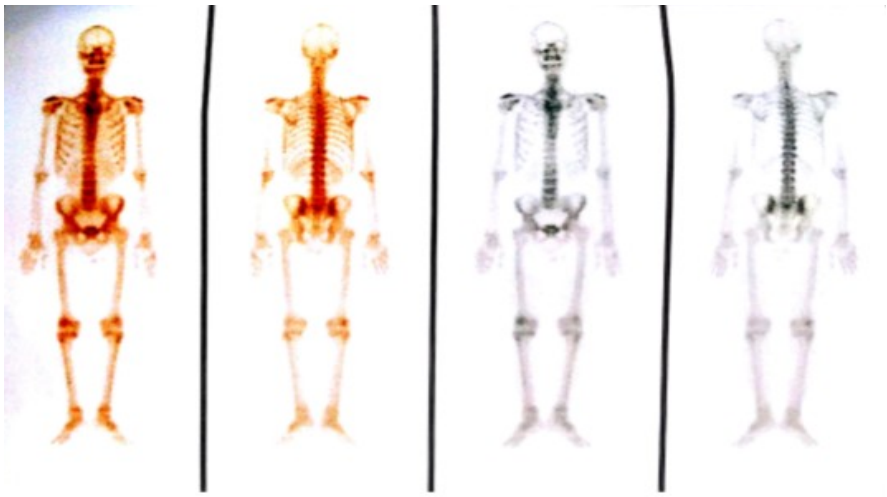

Figure 1. Skeletal scintigraphy before the start of treatment.

No evidence of metastasis was detected before the start of chemoradiotherapy.

The patient underwent the chemoradiotherapy process containing 3 cycles of cisplatin at a dose of 100mg/m^2 on the first day, 22nd day and 43rd day, which are followed by radiotherapy (RT), with the total dose of 70Gray (Gy). However, the last cycle of cisplatin was not given, because the patients could not tolerate. He was discharged from the hospital in early August 2017, after two months of the treatment process.

Follow-up examination one month later showed no evidence of recurrence, and the primary tumor has disappeared. However, he had detectable cf-EBV DNA with the level of 62 copies/ml. During the next 2.5 months, the level of cf-EBV DNA progressively rose from 62 copies/ml to 728 copies/ml after one month, and then finally up to 3502 copies/ml. By this time, patient complained of minor chest pains from the back spreading to the front, which was the only clinical symptom. The progressive rise of cf-EBV DNA suggests a possible recurrent disease, but no tumor was detected on the MRI of the head

and neck. Subsequently, the FDG-positron emission tomography (FDG-PET) scan was performed in September 2017, in order to search for evidence of distant metastasis. It turned out that there was discontinuous structure and decreased density at the transverse process in thoracic vertebrae of D6 with a FDG standard uptake value (SUV) of 12.2 (Figure 2).

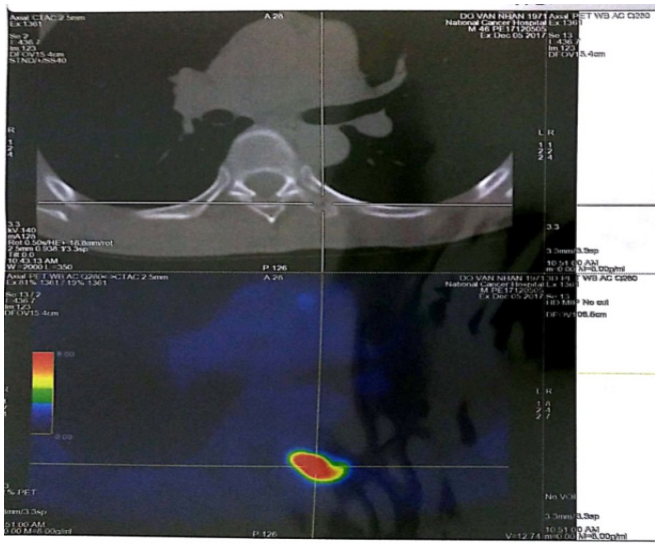

Figure 2. PET/CT scan at 3 months after the completion of chemoradiotherapy

Recurrent metastasis detected with discontinuous structure and decreased density of the transverse process in thoracic vertebrae of D6 with a FDG standard uptake value (SUV) of 12.2.

This PET/CT result confirmed skeletal recurrence metastasis from nasopharyngeal carcinoma, in accordance with the persistence of cf-EBV DNA, which had already been detected three month earlier. He was then treated with paclitaxel, cisplatin and biphosphonate for six cycles. In response to the chemotherapy, the level of cf-EBV DNA after the first cycle was 4015 copies/ml, and dropped to 960 copies/ml after the second cycle. He received good symptomatic relief, and continued to be treated following the initial treatment schedule.

3. Discussion

Nasopharyngeal carcinoma was closely associated with Epstein-Barr virus (EBV). The potential of cf-EBV DNA biomarker for early detection, prognosis and monitoring of treatment response has been reported in many high-impact international publications over the past 15 years [8]. The sensitivity of the cf-EBV DNA assay has been improved from 31% at the beginning, up to 98% nowadays . However, the sensitivity of this assay in Vietnam before 2017 was relatively poor with the detection limit of 300 copies/ml. This leads to high false negative rate and a moderate sensitivity of 64 - 68%, which is far below the international standard. Our group has successfully established the ultrasensitive cf-EBV DNA assay with the detection limit of 25 copies/ml and standardized this assay using the international panel of standards from Chinese University of HongKong. Our data showed that this assay was able to detect NPC with a sensitivity of 97% and specificity of 98% [6]. In this study, our sensitive assay for quantification of cf-EBV DNA has been utilized for detection and monitoring of residual disease of a NPC patient.

The patient was timely diagnosed with NPC by detectable cf-EBV DNA, even after two times of negative biopsy. In particular, high

concentration of cf-EBV DNA is the warning for the medical doctor to repeat biopsy, in order to make accurate diagnosis. Otherwise, the cancerous tissues had been missed and the patient would be diagnosed with later stage. Although biopsy has been the “gold standard” for diagnosis of NPC, the false negative of biopsy is not uncommon among NPC patients, probably due to the uncertainty of the cancerous tissue’s location within a tumor. By contrast, the cf-EBV DNA, which is a blood-based biomarker, was capable of detecting NPC, regardless of its locations.

Even though there isn’t any clinical manifestation at 1 month after the completion of treatment, cf-EBV DNA was still detectable, although its level was 250 times lower level than that at diagnosis. The existence and progressive rising of cf-EBV DNA level, which is 60 times increased within 2.5 months, was indicative of recurrence. In fact, the metastatic recurrence was confirmed by PET/CT scan after 3 months since the time cf-EBV DNA was first detected after regimen of treatment. Due to technical difficulty, the biopsy of the metastatic bone lesion was not available for histological analysis, however, the PET/CT imaging with high SUV (12.2) and fast rise of cf-EBV DNA level support the diagnosis of recurrent metastasis. He was then treated for recurrent metastasis of NPC and had a good response after two cycles of chemotherapy.

During the course of NPC diagnosis and early detection of recurrence metastases, the association for cf-EBV DNA level and NPC has been described. There was a high correlation between the level of cf-EBV DNA and the development of NPC. Before treatment, the cf-EBV DNA level was relatively high (15900 copies/ml), which was within the range of common levels among NPC patients detected at our institute. Among healthy individuals and recurrence - free NPC patients, the level of cf-EBV DNA is zero copies/ml. As the detection limit of our assay was 25 copies/ml [6], the low cf-EBV DNA level of 62 copies/ml was reliably detected at one month after the primary treatment. It appears that this low level of cf-EBV DNA derived from the secondary tumor starting to form, rather than primary tumor, which had possibly been fully treated. The cf-EBV DNA level continued to increase about 60 times/ 2.5 months (Figure 3) that was consistent with PET/CT result and showed the fast proliferation stage of the secondary tumour. The good correlation between cf-EBV DNA and PET/CT scan has been confirmed. After one month of treatment the decreasing cf-EBV DNA levels was consistent with his good clinical response to treatment regimen.

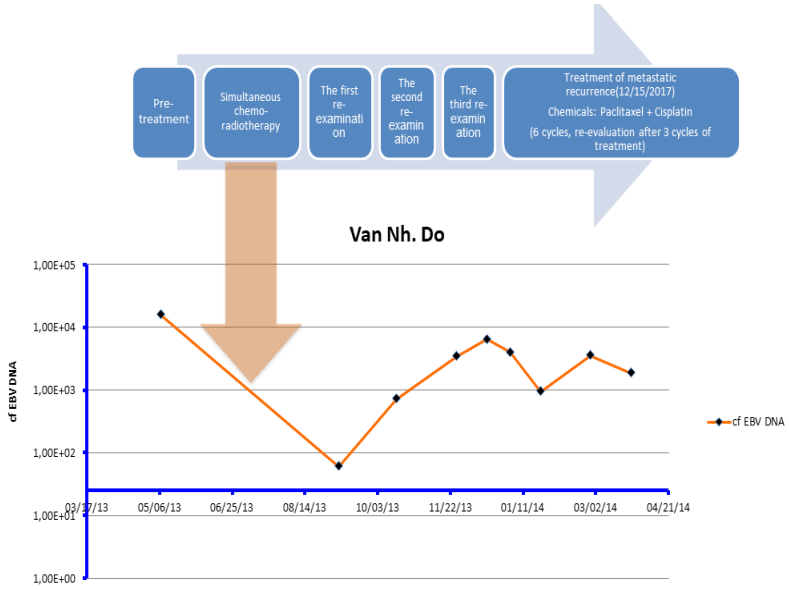

Figure 3. The change of cf-EBV DNA levels during and after chemoradiotherapy

The levels of cf-EBV DNA progressively increased about 60 times/2.5 months during the follow-up after primary treatment that suggested a possible non-symtomatic recurrence of NPC.

4. Conclusion

The cf-EBV DNA was detectable in the plasma of 96% of NPC patients in the study of the Chinese University of Hong Kong . Our cf-EBV DNA assay is the first assay reported in Vietnam with the sensitivity of 96.9% that ensure detection of NPC at the earlier stage. In addition, the assay specificity of 98% minimizes the false positive rate. This assay, which is a blood-based or “liquid biopsy” testing, is a noninvasive procedure. The convenience and high accuracy make this assay an excellent candidate for screening and early detection of NPC among high-risk populations. Last but not least, cf-EBV DNA has been shown, in this case study, as a valuable tool for monitoring the response to treatment of NPC patients, especially when the biopsy is not available or NPC patients with bone metastasis.

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