Ultrasound Evaluation of Carotid Atherosclerosis in Post-Radiotherapy Nasopharyngeal Carcinoma Patients, Type 2 Diabetes, and Healthy Controls

Introduction

Nasopharyngeal carcinoma (NPC) is a common head and neck cancer in Southern China, Southeast Asia, and the Middle East/North Africa [1]. Because NPC is sensitive to radiation, radiotherapy (RT) alone and concurrent chemo-radiotherapy are common treatment choices for early and advanced stages of the disease respectively. There is a high prevalence of cervical lymph node metastasis in NPC patients (60-90%) [2]. Therefore, RT of the neck to at least 60 Gy is a standard strategy for treating or preventing the nodal metastases in these patients [3]. In neck RT, however, ionizing radiation damages the carotid artery and may lead to the development of carotid atherosclerosis, which may result in cerebrovascular events, such as transient ischemic attack and stroke. Compared with non-irradiated controls, post-RT NPC patients tend to have thicker carotid artery wall, higher prevalence of carotid plaque and/or greater degree of carotid stenosis [4-7], which may lead to higher incidence of cerebrovascular events [7,8]. It has been reported that the risk of ischemic stroke is doubled in post-RT NPC patients than in non-irradiated controls [8].

Conventional cardiovascular risk factors, such as diabetes mellitus (DM), hypertension, hypercholesterolemia, coronary heart disease (CHD) and smoking, are also associated with the development of carotid atherosclerosis, which is commonly known as spontaneous carotid atherosclerosis. DM, mainly type 2 DM, is a metabolic disease and increasingly a health problem worldwide. Moreover, other conventional

cardiovascular risk factors are the common complications of DM patients and these risk factors have accumulative effects on carotid atherosclerosis [9,10]. Thus, DM patients with different number of cardiovascular risk factors are highly susceptible to carotid atherosclerosis.

Although previous studies have investigated the difference in carotid atherosclerosis between irradiated patients and non-irradiated controls, the independent effects of radiation on carotid atherosclerosis have not been fully determined with the conventional cardiovascular risk factors excluded. In addition, animal models have shown the difference in carotid atherosclerosis between irradiated cases and those with conventional pro-atherosclerotic burden (e.g. high cholesterol) [11-13]. In human study, however, the literature has scant information regarding the difference in carotid atherosclerosis between irradiated cancer patients and non-irradiated patients with conventional cardiovascular risk factors.

Ultrasonography is a safe, non-invasive and cost-effective imaging modality, and is useful in the assessment of carotid atherosclerosis. The present study aimed to use ultrasonography to investigate the difference in carotid atherosclerosis among post-RT NPC patients, type 2 diabetics and healthy controls. The findings of the present study will enhance the understanding of potential variations between radiation-induced and spontaneous carotid atherosclerosis, which may offer a justification for assessing carotid atherosclerosis in the routine follow-up for NPC.

Materials and Methods

Subjects

Post-RT NPC patients were recruited from the Department of Clinical Oncology of Queen Mary Hospital in Hong Kong. The inclusion criteria of post-RT NPC patients were Chinese NPC patients, older than 18 years, completed a single course of RT, and the post-RT duration was 4 years or more, whilst the exclusion criteria of post-RT NPC patients were more than one course of RT, history of carotid atherosclerosis prior to RT, previous carotid endarterectomy or stenting, current smoker, and history of DM, hypertension, hypercholesterolemia and/or CHD.

Patients with type 2 DM were recruited from a local Chinese non-profit-making organization for patients with diabetes (Angel of Diabetics, Hong Kong). The inclusion criteria of DM patients were Chinese patients with type 2 DM, older than 18 years and diagnosed with type 2 DM for at least 4 years, whereas the exclusion criteria of DM patients were previous RT, carotid endarterectomy and carotid stenting.

Healthy controls were recruited via posters in the Hong Kong Polytechnic University. The inclusion criterion of healthy controls was Chinese older than 18 years, whereas the exclusion criteria of healthy controls were previous RT, carotid endarterectomy, carotid stenting, current smoking, and history of DM, hypertension, hypercholesterolemia and/or CHD.

This study was approved by the Human Subject Ethics Subcommittee of the Hong Kong Polytechnic University and the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Written and informed consent was obtained from all participants before the commencement of the interview and ultrasound examination.

Clinical history

Archived clinical records of post-RT NPC patients and the most recent blood test reports of DM patients were reviewed, and individual face-to-face interviews were conducted for all participants to obtain their clinical history. The presence of cardiovascular risk factors was identified as follows: 1) DM, diagnosed with DM in the clinical record, taking medications to lower the glycemic level and/or fasting plasma (blood) glucose ≥ 7.0 (6.1) mmol/L [14]; 2) hypertension, diagnosed with hypertension in the clinical record, undergoing anti-hypertensive medications and/or the measured blood pressure ≥ 140/90 mmHg [15]; 3) hypercholesterolemia, diagnosed with hypercholesterolemia in the clinical record, undergoing medications to lower the cholesterol level and/or fasting total cholesterol ≥ 5.2 mmol/L [16]; 4) CHD, diagnosed with coronary vascular disease in the clinical record and/or had coronary stenting [17]; 5) smoking, current smoker consuming 10 cigarettes per day for at least six months [17].

Ultrasound examinations

All ultrasound examinations were performed by the same operator with the Esaote MyLab Twice ultrasound unit in conjunction with a 4-13 MHz linear transducer (Esaote, Genoa, Italy). The operator was blinded to whether subjects were diabetics or healthy controls, but knew who were post-RT NPC patients. A sphygmomanometer (Tensoval; Hartmann, Germany) was used for measuring the brachial blood pressure of the participants. The blood pressure measurements and ultrasound examinations were conducted in a 22^{o}C air-conditioned examination room. In order to ensure the measurements were performed with the subjects in a resting state, all subjects were allowed to sit and rest comfortably on a chair for at least 10 minutes before the blood pressure measurement. For each subject, the brachial blood pressure was measured with the sphygmomanometer at the left upper arm in sitting posture. The measured systolic and diastolic pressures were then inputted into the ultrasound unit for the evaluation of carotid arterial stiffness (CAS). Afterward, subjects were asked to lie supine on the examination couch with the neck slightly extended and the head turned away from the side under examination. Both the left and right carotid arteries were evaluated.

Carotid intima-media thickness (CIMT) and CAS were evaluated using the automated quantification programs of the ultrasound unit: Radiofrequency-Based Quality Intima-Media Thickness (RF-QIMT) and Radiofrequency-based Quality Arterial Stiffness (RF-QAS) respectively (Figure 1). CIMT was measured at the far wall of the common carotid artery (CCA) at a 1-cm segment 1 cm proximal to the inferior end of

carotid bulb. CAS was evaluated over the near and far walls of the CCA at the same segment. In the evaluation of CIMT and CAS, the mean and standard deviation (SD) of the measurements in six consecutive cardiac cycles were automatically and continuously recorded by the ultrasound system, and the mean measurement with an SD of < 20 μm for CIMT or < 30 μm for stroke change in vessel diameter for CAS were obtained for data analyses. Each carotid artery was scanned three times for measuring CIMT and CAS. The intra-operator reliability of RF-QIMT for measuring CIMT and RF-QAS for assessing CAS was excellent with intraclass correlation coefficient > 0.9.

In the evaluation of the CAS, five arterial stiffness parameters were investigated in the study: Distensibility Coefficient (DC), Compliance Coefficient (CC), the indices of α and β, and Pulse Wave Velocity (PWV). The lower DC and CC and the higher α, β and PWV indicate the stiffer the carotid artery. The equations of these parameters are:

$$DC = \frac{\Delta A/Ad}{\Delta P} \ (1/KPa)$$

$$CC = \frac{\Delta A}{\Delta P} \ (mm^2/KPa)$$

$$\alpha = \frac{Ad \cdot \ln(SBP/DBP)}{As - Ad}$$

$$\beta = \frac{Dd \cdot \ln(SBP/DBP)}{Ds - Dd}$$

$$PWV = \sqrt{\frac{\alpha \cdot DBP}{\rho}} \text{ (m/s)}$$

where Ds and Dd represent the systolic and diastolic diameters of the artery; As and Ad are the systolic and diastolic lumen areas, SBP and DBP indicate the systolic and diastolic blood pressures; ΔD, ΔA and ΔP are the stroke change in the vessel diameter, the lumen area and the blood pressure, respectively; and ρ is the blood density, which is a constant.

For each subject, the carotid plaque burden in the carotid artery was assessed. Carotid plaque was defined as focal thickening >50% of the adjacent intima-media layer [18]. When a carotid plaque was identified, transverse gray-scale images of the plaque were obtained and the degree of carotid stenosis was expressed as the percentage reduction of lumen diameter at the most stenotic site (Figure 2). Carotid plaque score was evaluated using an adjusted plaque scoring system [17]. In the scoring system, the carotid artery was divided into five segments: 1. proximal common carotid artery (≥2 cm proximal to carotid bifurcation); 2. distal common carotid artery (<2 cm proximal to carotid bifurcation); 3. carotid bulb and bifurcation; 4. internal carotid artery; and 5. external carotid artery. The degree of carotid stenosis in each segment was measured and the carotid plaque score was expressed as the summation of the degree of carotid stenosis of all segments in both carotid arteries (Figure 2).

Statistical analysis

CIMT, CAS and carotid plaque score were continuous variables, whilst the presence

of carotid plaque and the presence of ≥50% carotid stenosis were categorical data. Continuous data were expressed as means ± SD. The normality of distribution was checked using Shapiro-Wilk test. CIMT, CAS and carotid plaque score were transformed logarithmically in analyses because they were not normally distributed. The comparisons between study groups were performed using ANCOVA (continuous variables) or logistic regression (categorical variables) with the adjustment of age and gender. Multiple comparisons in the three study groups were corrected using Benjamini-Hochberg step-up false discovery rate controlling procedure [19]. All statistical analyses were performed using SPSS 20 (IBM, Armonk, New York, United States). Corrected *P* value (P_{cor}) < 0.05 was considered to be significant.

Results

A total of 76 healthy controls, 70 type 2 diabetics and 69 post-RT NPC patients were included in the present study. The neck of the NPC patients were treated with 2-D convertional RT with a mean radiation dose of 66.87 ± 3.45 Gy (range: 58.0 to 73.72 Gy). The mean age of the subjects in the three study groups was 42.8 ± 15.5 (range: 20 to 82), 59.5 ± 8.5 (range: 39 to 78) and 52.6 ± 8.4 (range: 36 to 69) years respectively. There were 39, 25 and 41 males, and 37, 45 and 28 females in the three study groups respectively. After adjustment with age and gender, there was no significant difference in systolic, diastolic and pulse blood pressure (ΔP) in study groups (P_{cor}>0.05) except that post-RT NPC patients had higher diastolic blood pressure than healthy controls (P_{cor}<0.05) (Supplement 1). The prevalence of stroke in

healthy controls, type 2 diabetics and post-RT NPC patients was 0% (n=0), 1.4% (n=1) and 2.9% (n=2) respectively

The present study found that CIMT, CAS and carotid plaque burden (carotid plaque score, the presence of carotid plaque and ≥50% carotid stenosis) were significantly higher in patients with type 2 DM than in healthy controls after adjustment with age and gender (Figure 3 and Supplement 1). Compared with healthy controls, DM patients had 33.1% higher CIMT (701.9 ± 126.0 μm *vs* 527.2 ± 159.1 μm, P_{cor}<0.05), 29.1-55.4% larger CAS (P_{cor}<0.05, 44.8% and 37.5% lower DC and CC, and 55.4%, 53.7% and 29.1% higher α, β and PWV, respectively), 7 times greater carotid plaque score (0.16 ± 0.21 *vs* 0.02 ± 0.06, P_{cor}<0.001) and 4.7 times higher prevalence of carotid plaque (48.6% *vs* 8.6%, P_{cor}<0.01).

In addition, post-RT NPC patients showed higher degree of carotid atherosclerosis than DM patients and healthy controls after the adjustment of age and gender (Figure 3 and Supplement 1). Post-RT NPC patients had 29.3% higher CIMT (681.7 ± 132.2 μm *vs* 527.2 ± 159.1 μm, P_{cor}<0.001), 48.3%-107.3% higher CAS parameters (P_{cor}<0.001, 48.3% and 43.1% lower DC and CC, and 107.3%, 103.4% and 48.8% higher α, β and PWV, respectively), 58.5 times greater carotid plaque score (1.19 ± 1.18 *vs* 0.02 ± 0.06, P_{cor}<0.001) and 9.1 times higher prevalence of carotid plaque (87.0% *vs* 8.6%, P_{cor}<0.001) when compared with healthy controls. Moreover, post-RT NPC patients had 6.3%-33.4% higher CAS parameters (P_{cor}<0.05, 6.3% and

8.9% lower DC and CC, and 33.4%, 32.4% and 15.3% higher α, β and PWV, respectively), 6.43 times greater carotid plaque score (1.19 ± 1.18 *vs* 0.16 ± 0.21, P_{cor}<0.001), 79.0% higher prevalence of carotid plaque (87.0% *vs* 48.6%, P_{cor}<0.001) and 4.48 times higher prevalence of ≥50% carotid stenosis (15.9% *vs* 2.9%, P_{cor}<0.05) when compared with DM patients.

Discussion

DM patients with different number of cardiovascular risk factors were included to represent subjects with spontaneous carotid atherosclerosis. Results of the present study showed that DM patients tended to have higher CIMT, CAS and carotid plaque burden than non-diabetic controls, and these findings were consistent with previous studies [20-22].

The independent effect of radiation on inducing carotid atherosclerosis was not fully investigated because previous studies did not exclude the effect of coexisting conventional cardiovascular risk factors of the patients, such as DM, hypertension, hypercholesterolemia, CHD and smoking. In order to eliminate the effect of these risk factors, the present study included post-RT NPC patients who did not have conventional cardiovascular risk factors. Chemotherapy was not included as a confounder of radiation-induced carotid atherosclerosis, because the extent of carotid atherosclerosis in patients with or without chemotherapy was not significantly different. Compared with healthy controls without both RT and conventional

cardiovascular risk factors, post-RT NPC patients had higher degree of carotid atherosclerosis. Thus, finding of the present study suggested that neck RT for NPC is an independent risk factor of carotid atherosclerosis.

No previous study has compared the extent of radiation-induced and spontaneous carotid atherosclerosis. The present study compared the degree of carotid atherosclerosis in post-RT NPC patients and DM patients. Results illustrated that radiation-induced carotid atherosclerosis in post-RT NPC patients was more severe than the spontaneous carotid atherosclerosis in DM patients in terms of stiffer carotid artery wall and higher carotid plaque burden.

More severe cell damage and excess oxidative stress may attribute to the higher severity of carotid atherosclerosis in post-RT NPC patients. Endothelial cells (ECs) in the intima layer and vava vasorum are the cell type most responsive to radiation in the carotid artery [23]. In neck RT, ionizing radiation damages ECs in the intima layer of the carotid artery and induces the expression of adhesion molecules [24-26]. These molecules recruit the circulating immune cells (i.e. leukocytes) on the endothelium and mediate the transmigration of the immune cells into sub-endothelium, initiating inflammatory responses and subsequent atherosclerotic processes [27]. ECs of vava vasorum are also damaged after irradiation. The radiation-induced depletion and swelling of the ECs may lead to the obliteration of vava vasorum and reduce the blood supply to the carotid artery wall, resulting in the formation of focal lesion and

angiogenesis [23]. In addition, ionizing radiation triggers the proliferation, migration and differentiation of smooth muscle cells (SMCs) in the media layer of the carotid artery [28]. Irradiated SMCs synthesize excess collagen and alter the extracellular matrix modelling, which lead to the thickening and stiffening of the carotid artery and the development of carotid plaque [28]. Moreover, ECs (the intima layer) and SMCs (the media layer) demonstrate crosstalk after irradiation. Irradiated ECs increase the proliferation and migration of SMCs, and induce the overproduction of collagen [28].

In addition to cell damage, radiation produces extreme reactive oxygen species (ROS) either directly by oxidation of H_2O or indirectly by cellular responses [29,30]. ROS (i.e. hydrogen peroxide) oxidizes low density lipoprotein (LDL). The oxidized LDL is then taken up by macrophages to form foam cells, and it also acts as a mediator to induce inflammatory responses [31]. Besides, ROS itself acts as the activator up-regulating numerous signalling pathways in ECs and SMCs in terms of growth, proliferation and inflammatory reaction, facilitating the development of atherosclerosis [31].

Because of these radiation-induced biological processes, higher extent of carotid atherosclerosis was observed in post-RT NPC patients than in DM patients and healthy controls in the present study. Since there is a high prevalence and severity of carotid atherosclerosis in post-RT NPC patients, the risk of stroke in these patients is doubled when compared with non-irradiated controls [32], which was consistent with

the result in the present study that the prevalence of stroke was higher in post-RT NPC patients than in type 2 diabetics and healthy controls. In addition, the benefit is still limited in treating radiation-induced high-grade stenosis using endarterectomy or stenting [32]. Thus, regular assessment of carotid atherosclerosis is essential for this group of patients so that diagnosis can be made and prompt treatment can be given to the patients in the early stage of carotid atherosclerosis.

Since carotid plaque is a causal risk of stroke, the assessment of carotid plaque may be useful for post-RT NPC patients. Prior to RT, a screening of the presence/absence of carotid plaque should be conducted for the NPC patients. If patients have carotid plaque, annual monitoring the progression of carotid plaque may be necessary after RT. In contrast, if patients do not have carotid plaque, the screening of carotid plaque may be performed in the fouth year after RT. This is because, firstly, it has been shown that carotid plaque is less likely to be found in the first two years after RT [4]; secondly, in the present study, carotid plaque was found in 60% of patients with post-RT duration of 4 years (3 out of 5 patients), which was consistent with the finding of a previous study in which 42.5 months was suggested as the cut-off value of post-RT duration for the presence of carotid plaque (around 3.5 years, a sensitivity of 68.4% and specificity of 50%) [33]. When carotid plaque is found, the degree of carotid stenosis and the stability of carotid plaque can be assessed, and appropriate and prompt medications can be given to these patients to alleviate the progression of carotid atherosclerosis, which may reduce the risk of stroke. Nevertheless, the

cost-effectiveness of the proposed examination strategy for preventing stroke in post-RT NPC patients remains to be investigated in future studies. In addition, this study demonstrated the difference in the CIMT, CAS and carotid plaque burden between post-RT NPC patients and DM patients. Further studies to investigate the difference in the echogenicity and surface irregularities of carotid plaques (stability) between these two groups of patients are suggested [34-36].

Conclusions

The results of the study demonstrated that neck RT for NPC is an independent risk factor of carotid atherosclerosis. In addition, the radiation-induced carotid atherosclerosis in post-RT NPC patients is more severe than the spontaneous carotid atherosclerosis in DM patients. Thus, assessment of carotid atherosclerosis is necessary for patients treated with RT for NPC. Given that ultrasonography is a non-invasive, reliable, cost-effective and readily available imaging modality and is useful for the assessment of carotid atherosclerosis, carotid ultrasound examination should be indicated in routine follow-up of NPC.

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