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RESEARCH ARTICLE

DOES CHEMORADIOTHERAPY INDUCE THROMBOTIC RISK IN ADVANCED NASOPHARYNGEAL CARCINOMA

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ABSTRACT

Background: Nasopharyngeal cancer (NPC), a highly prevalent malignant disease is a leading cause of death in several regions in Southern China including countries in Southeast Asia. In Indonesia, NPC is a major multi-ethnic problem and it remains one of the most confusing and commonly misdiagnosed disease until advanced stages. Chemotherapy improves treatment but also contribute towards increased risk of thrombosis in cancer.
Methods: A total of 12 subjects (males n=8; Stage II n=2; Stage III n=2, Stage IV n=8) were recruited into the study. Blood sampling was performed at pre- and post-chemotherapy cycles 1 to 3 followed by radiotherapy. PT (INR), aPTT, TT ratios were determined together with D-dimer, platelets and platelet aggregation.
Results: The mean age of NPC patients was 43.3 ±9.0 years. Pre-chemotherapy ratios of PT, aPTT and TT were <1.0 which indicated a hypercoagulable state. No significant variation in these parameters were seen post-chemoradiotherapy except that D-dimer were elevated postradiotherapy and platelet numbers significantly reduced from cycle 2. However, 4 patients (33.3%) with Stage IV disease had D-dimer levels above the normal level (<500 ng/mL) at post-radiotherapy were further analysed. This suggest a further enhanced hypercoagulable and hyperfibrinolysis state and thromboembolic risk following radiotherapy in this small cohort study.
Conclusion: Chemoradiotherapy enhance the risk of thromboembolism in 33.3% of advanced NPC patients in this small study group. These findings indicate that not all NPC patients show enhanced risk of thromboembolism following chemoradiotherapy.

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) represents a distinct entity regarding epidemiology, clinical presentation and prognosis. NPC is endemic in several regions in Southern China including countries in Southeast Asia. It is not easily diagnosed until advanced stages. Highest prevalence in the world was seen in the Guangdong province in South China (Huang et al., 1998) and Hong Kong. In Indonesia, NPC is prevalent among different people and represents a major socio-economic problem with an overall incidence estimated at 6.2/100,000 or about 12,000 new cases per year (Soeripto, 1998; Adham *et al*., 2012). It is the fourth most common malignancy in the head and neck after cervical, breast and skin cancers. The disease is related to Epstein-Barr (EBV) virus infection and most common in the undifferentiated type of NPC (WHO type III) (Lo et al., 2004). Histologically NPC is divided into three categories; keratinizing squamous cell carcinoma (WHO type I), non-keratinizing squamous cell carcinoma (WHO type II), and undifferentiated carcinoma (WHO type III) which is most prevalence in Southeast Asia and other high incidence regions and is most commonly associated with EBV infections (Lo et al., 2004). Chemotherapy can increase the risk for venous thromboembolism (VTE) in cancer patients (Lee and Levine, 1999). Thrombotic complications have been shown in association with specific chemotherapeutic agents; including L-asparaginase, mitomycin C, cisplatin (Falanga, 1998). However, it improves treatment of NPC and combining with radiotherapy dramatically improve the survival outcome (Chen et al., 1989).

Cancer can confer a prothrombotic or hypercoagulable state through activation of the coagulation and fibrinolytic pathways, the vascular endothelium, monocytes and platelets (Caine et al., 2002). Blood coagulation pathway emerged as the significantly altered pathway in NPC plasma in proteomic analysis have been reported (Peng *et al*., 2011). D-dimer, the lysis product of cross-linked fibrin indicates hyperfibrinolysis in response to clotting activation and fibrin formation (Falanga et al., 2013). D-dimer assays have been extensively studied and has been shown to have high sensitivity and high predictive value for deep vein thrombosis (DVT) and negative value for DVT exclusion (Prisco and Grifori 2009). It is a marker for hypercoagulability and links with venous as well as arterial thrombotic events and have been used to determine hypercoagulable state leading to thrombosis in myeloproliferative disease (Kleinegris *et al*., 2013; Gomez *et al*., 2011).

The pathogenesis of the coagulation system imbalance in cancer is complex and involves multiple factors both clinical and biological. The direct injury of endothelial cells by chemotherapeutic agents or tumour-derived products leading to a loss of antithrombotic properties is thought to play a role in increased venous thromboembolism (VTE) risk in cancer (Falanga et al., 2013). Our study group has set the criteria that hypercoagulable state can also be measured using the ratios of patient's prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) against normal healthy subjects where the normal arbitrary ratio is 1.0. When any of the two parameters slide to less than 1.0, then it is deemed as hypercoagulable due to increase procoagulant factors that shortened the clotting times. PT measurement indicates the status of the Extrinsic Coagulation Pathway and aPTT in the Intrinsic Coagulation Pathway whilst TT determines the fibrinogen status. The objective of the study was to determine whether thromboembolic risk can be induced by chemoradiotherapy in advanced nasopharyngeal carcinoma in our small cohort study group.

METHODS

The study received ethical approval from the Health Research Ethical Committee (No: 121/KOMET/FK/USU/2016), Faculty of Medicine, University of North Sumatera, Indonesia. It was conducted at the Department of Clinical Pathology and the Department of Ear, Nose Throat (ENT), Head and Neck, Haji Adam Malik Hospital in Medan, Indonesia between January to April 2016.

Subjects: The subjects with nasopharynx carcinoma were recruited by the ENT specialists into the study after they have given written Informed Consent to take part in the study. The inclusion criteria include known diagnosis of nasopharyngeal carcinoma, no commencement of chemotherapy or other treatments for the disease. Exclusion criteria if patients have undergone treatment like tranexamic acid, oral anticoagulant or aspirin. A total of 12 patients (males n=8, females n=4) with nasopharynx carcinoma WHO Type I (n=1); WHO Type II (n=9) and WHO Type III (n=1) were recruited into the study and undergo treatment for the disease. Chemoradiotherapy. Cisplatin 75mg/m^2 were infused in day 1 and 5-fluorouracil (1000mg/m^2) for 4 days and this cycle was repeated every 3 weeks for 3 cycles before the patients undergo radiotherapy protocol of 3600 Gy given per day and divided into 15 fractions.

Blood collection: A clean venipuncture was performed and about 12 mL blood was separately drawn into four 3mL tubes containing 3.2% sodium citrate (9:1) and mix.The citrated blood is used for platelet determination and the others spun at 2500g for 15 minutes and the plasma used for determination of prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT) and plasma D-dimer level. Blood sampling was performed at pre-chemotherapy and at post chemotherapy cycles 1, 2, 3 and radiotherapy within 24 hours after each drug infusion.

Laboratory analysis: Automatic coagulation analyzer (Coatron A4, Neufahm, Germany) was used for the determination of PT, aPTT and TT ratios. D-dimer was determined using automated analyzerDimex JR. D-dimer, latex agglutination method based on the principle of immunoturbidimetry (15, 16). Platelets were determined using the automatic Hematology Analyzer Sysmex XN 4000.

Statistical analysis: The Statistical Package for Social Sciences (SPSS 22 IBM Corp) was used to perform statistical analysis. One-way Analysis of Variance (ANOVA) was performed to test for variations in the parameters studied. The independent t-test and Mann-Whitney test for differences between groups was also performed. A *P*-value of <0,05 was considered to be statistically significant.

RESULTS

Characteristics of nasopharyngeal carcinoma patients at admission to the study and at post-chemoradiotherapy. The mean age of the twelve NPC patients (male n=8) was mean 43.3 ± 9.0 years and ranged between 31 years and 61 years. The disease stage was Stage II n=2; III n=2; IV n=8 and histology classification: WHO Type 1 keratinizing squamous cell cancer n=1 (Stage IV), WHO Type 2 nonkeratinizing squamous cell cancer n=9 (Stage II n=2, III n=1, IV n=6) and WHO Type 3 undifferentiated cancer n=2 (Stage III n=1, IV n=1). Only five patients were smokers and five patients had lumps in the neck with five reported having headaches. All patients reported episodes of epitaxis on admission to the study but stopped after chemotherapy cycle-1. Reduction in tumour size (n=11) was seen post-chemotherapy and post-radiation therapy with significant reduction in tumour size (n=11) was seen and all had skin burn marks (Table 1).

PT (INR), aPTT, TT ratios, D-dimer and platelets in advanced nasopharyngeal carcinoma at pre- and post-chemoradiotherapy
One-way Analysis of Variance (ANOVA) showed no statistical significant variation in PT, aPTT and TT ratios before and post chemoradiotherapy including D-dimer levels except for significant variation in platelets (P= <0.001). At pre-chemotherapy, mean ratio of <1.00 in PT, aPTT and TT was seen indicating a hypercoagulable state which did not show any significant variations even at post-radiotherapy. However, D-dimer levels showed a significant increase (P=0.006) at post-radiotherapy only (mean 392.8 ±177.3, range between 125 to 669 ng/mL) compared with pre-chemotherapy state. Platelets showed a significant reduction from cycle-1 and post-radiotherapy compared to pre-chemotherapy state (P= 0.002 and P=0.001) respectively (Table 2).PT (INR), aPTT, TT ratios, D-dimer levels and platelets of 4 patients (WHO Type 2 Stage IV) with D-dimer levels above 500 ng/mL at post-radiotherapy compared with pre-chemotherapy state.

Table 1. Characteristics of nasopharyngeal carcinoma patients at admission to the study and at pre- and post- chemoradiotherapy

N = 12
Age, mean (SD) years: 43.3 (9.0); Range :31 – 61 years
Sex: Male n=8; female n=4
Smokers n=5
Lump in neck n=5
Nose blocked n=4
Headaches n=1
Epitaxis: n=12
Histology:
WHO Type 1 keratinizing squamous cell cancer n=1 (Stage IV)
WHO Type 2 non-keratinizing squamous cell cancer n=9 (Stage II n=2; III n=1, IV n=6)
WHO Type 3 undifferentiated cancer n=2 (Stage III n=1, IV n=1)
Post-chemotherapy: (epitaxis stopped after chemo-1), reduction in tumour size n=11
Post-radiation therapy: skin burn and significant reduction in tumour size n=8

Table 2. PT (INR), aPTT, TT ratios, D-dimer and platelets in advanced nasopharyngeal carcinoma compared between pre- and post-chemoradiotherapy. Paired sample t-test and one-way Analysis of Variance (ANOVA)

	PT INR ratio	aPTT ratio	TT ratio	D-dimer ng/mL
N	12	12	12	12
Pre-chemotherapy				
Mean (SD)	0.93 (0.09)	0.94 (0.07)	0.80 (0.07)	266.2 (106.2)
Range	0.74 – 1.05	0.84 – 1.07	0.74 – 0.98	105 - 440
Chemotherapy-1				
Mean (SD)	1.00 (0.12)	0.90 (0.10)	0.91 (0.30)	294.5 (94.6)
Range	0.79 – 1.11	0.74 – 1.05	0.70 – 1.80	200 - 441
P	0.14	0.28	0.21	0.50
Chemotherapy-2				
Mean (SD)	1.00 (0.12)	0.87 (0.10)	0.83 (0.09)	315.1 (111.5)
Range	0.86 – 1.21	0.71 – 1.03	0.72 – 1.05	200 - 518
P	0.15	0.09	0.38	0.28
Chemptherapy-3				
Mean (SD)	1.00 (0.10)	0.90 (0.07)	0.82 (0.14)	291.0 (114.1)
Range	0.82 – 1.17	0.77 – 0.98	0.72 – 1.18	160 - 502
P	0.49	0.19	0.62	0.59
Radiotherapy				
Mean (SD)	0.99 (0.07)	0.92 (0.07)	0.89 (0.20)	392.8 (177.3)
Range	0.91 – 1.11	0.75 – 1.03	0.70 – 1.46	125 - 669
P	0.12	0.53	0.16	**<0.05**
ANOVA *P*	0.45	0.49	0.46	0.14

Table 3. PT (INR), aPTT, TT ratios, D-dimer and platelets in 4 NPC patients (WHO Type 2Stage IV) with elevated D-dimer levels (>500ng/mL) at post-radiotherapy compared with pre-chemotherapy state

	PT (INR)	aPTT ratioratio	TT ng/mL	D-dimer	Platelets x10^9/L
N = 4					
Pre-chemotherapy					
Mean (SD)	0.98 (0.10)	0.93 (0.7)	0.82 (0.11)	379.5 (53.3)	369.8 (47.3)
Range	0.89-1.09	0.93-1.01	0.74-0.98	300 - 414	308 - 420
Post-radiotherapy					
Mean (SD)	0.97 (0.10)	0.91 (0.10)	1.02 (0.30)	597.8 (55.0)	252.3 (57.7)
Range	0.91 - 1.00	0.82 -1.05	0.82 – 1.46	538 – 669	212 – 337
P	0.89	0.41	0.28	0.001	0.02
ANOVA	0.40	0.90	0.42	<0.01	0.09

No significant differences in parameters studied between chemotherapy cycles 1 to 3 and pre-chemotherapy in the above 4 patients.

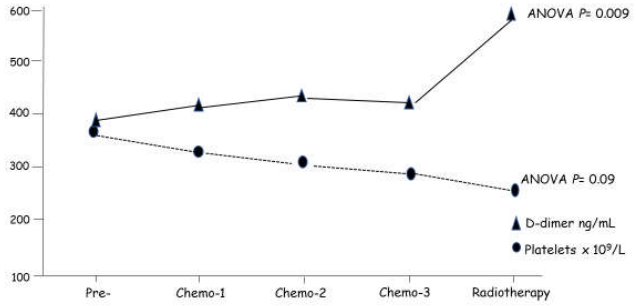

Figure 1. Mean platelets and D-dimer levels at pre- and post-chemotherapy in patients (n=4) with elevated D-dimer post-chemoradiotherapy.

Four patients (WHO Type 2 Stage IV) had significantly elevated D-dimer levels (P<0.01) of mean 597.8 ± 55.0 ng/mL (range between 538 and 669 ng/mL) and reduced platelets (P = 0.02) at post-radiotherapy. There was no significant variation in parameters studied except for D-dimer (P =<0.01). Their PT, aPTT and TT ratios did not show any statistical significant differences at post-chemoradiotherapy therapycycles compared with pre-chemotherapy state (Table 3). The platelets and D-dimer levels of these 4 patients at pre- and post-chemoradiotherapy are shown in Figure 1. This would suggest that a hypercoagulable state and enhanced fibrinolysis was seen in 33.3% (4/12) of patients with thrombotic risk after radiotherapy treatment.

DISCUSSION

Nasopharyngeal cancer is a highly prevalent malignant disease and a leading cause of death in several regions in Southern China including countries in Southeast Asia. The disease is not easily diagnosed until advanced stages. In Indonesia, NPC is prevalent among different native people and presents a major socioeconomic problem with an overall incidence estimated at 6.2/100,000 or about 12,000 new cases per year (Soeripto 1998; Adam et al 2012). In Indonesia, poor awareness of NPC early signs and symptoms among regional health workers was reported in an earlier study (Fles et al., 2010) and this contributed to miss diagnosis in early stage of the disease. Histologically, NPC is divided into three categories; keratinizing squamous cell carcinoma (WHO type 1), non-keratinizing squamous cell carcinoma (WHO type 2), and undifferentiated carcinoma (WHO type 3) which is most prevalent in Southeast Asia and other high incidence regions and is most commonly associated with EBV infections (Adham *et al*., 2012).

Our study in this small cohort group demonstrated that 75% of the patients were WHO type 2category, 8.3%% WHO Type 1 and 16.7% WHO Type 3. Chemotherapy can increase the risk of thromboembolism in cancer patients (Lee and Levine, 1999) associated with specific chemotherapeutic drugs (Falanga, 1998). The use of radiation plus chemotherapy drugs cisplatin and 5-fluoracil improves treatment results in a 3-year survival rate of 76% compared to the rate of 46% for patients receiving radiation therapy alone (Al-Sarraf *et al*., 1998). NPC patients in our study all had epitaxis before chemotherapy but this condition stopped after one cycle of chemotherapy and 91% had significant reduction in tumour size post-chemoradiotherapy. Cancer patients with solid tumours commonly present with abnormal laboratory coagulation tests characterized by varying degrees of clotting activation indicating a subclinical hypercoagulable state (Rickleset al., 1992). In our study, NPC patients present a hypercoagulable state as their PT, aPTT and TT ratios at prechemotherapy each were less than the normal ratio of 1.0. However, there were no significant variation during the course of post-chemoradiotherapy. The platelets numbers were significantly reduced after the first cycle of chemotherapy compared with pre-chemotherapy state suggesting the consumption of platelets during chemotherapy and radiation. D-dimer, a marker for hypercoagulability and links with venous as well as arterial thrombotic events (Kleinegris *et al*., 2013; Gomez et al., 2011) has high predictive value for deep vein thrombosis (DVT) and negative value for DVT exclusion (Prisco and Grifori 2009). D-dimer levels in our study showed significantly increased level post-radiotherapy but showed no significant variation (ANOVA) post-chemoradiotherapy. However, four of the patients had significantly elevated D-dimer levels (range between 538-669 ng/mL) post-radiotherapy together with significant reduction in platelets was seen. This suggests that a hypercoagulable and enhanced fibrinolysis state following radiotherapy treatment was evident in only a small group of patients. This represents that only 33.3% of advanced stage NPC disease are associatedwith enhanced risk of thrombosis post-chemoradiotherapy. An enlarged study is needed to confirm these findings. In conclusion, advanced NPC showed a hypercoagulable state and a more enhanced state of hypercoagulability and fibrinolysis was evident in 33.3% of advanced stage NPC patients following chemo radiotherapy. This small study indicated that not all advanced NPC patients show increased risk of thromboembolism following chemoradiotherapy

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Disclosure of Conflict of Interests

The authors stated they have no conflict of interests.

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