ORIGINAL ARTICLE

Bali Medical Journal (*Bali Med J*) 2018, Volume 7, Number 2: 330-334
P-ISSN.2089-1180, E-ISSN.2302-2914

Published by DiscoverSys

Association between high survivin expression and late clinical stage of nasopharyngeal non-keratinizing squamous cell carcinoma

Rosalina Susantio,* Mahendra-Dewi I. G. A. S., Susraini A. A. A. N.

ABSTRACT

Introduction: Nasopharyngeal carcinoma is one of the most common head and neck malignancy in Indonesia. Due to the evaluation of clinical stage is not enough to predict its prognosis, therefore other markers are needed to help to assess its progressivity. One of the markers is known as survivin, an apoptosis inhibitor, which usually shows increase expression in carcinoma. Roles of survivin in nasopharyngeal carcinoma carcinogenesis has not been studied well yet. The aim of this study was to analyze the association between high survivin expression and the late clinical stage of nasopharyngeal non-keratinizing squamous cell carcinoma.

Methods: This study was a cross-sectional observational analytic study, involved 30 histopathological samples of nasopharyngeal non-keratinizing squamous cell carcinoma, which have been examined at Pathology Anatomy Laboratory Sanglah Hospital by using stratified random sampling from January 2nd, 2014 to May 31st, 2017. The paraffin slides were stained with survivin immunohistochemical at Pathology Anatomy Laboratory Dr. Sardjito Hospital Yogyakarta. The cut-off point of survivin expression was analyzed by Receiver Operator Curve (ROC) analysis. The association between survivin expression and the clinical stage was analyzed with Fisher's Exact test.

Results: The cut-off point of survivin expression based on ROC analysis was 4.5. High survivin expression was significantly associated with late clinical stage cases ($p=0.026$; $p<0.05$).

Conclusion: High survivin expression has a significant association with the late clinical stage of nasopharyngeal non-keratinizing squamous cell carcinoma. Survivin can be used as a prognostic marker, which is useful for nasopharyngeal carcinoma therapy.

Keywords: survivin, clinical stage, nasopharyngeal non keratinizing squamous cell carcinoma

Cite This Article: Susantio, R., Mahendra-Dewi I.G.A.S., Susraini A.A.A.N. 2018. Association between high survivin expression and late clinical stage of nasopharyngeal non-keratinizing squamous cell carcinoma. *Bali Medical Journal* 7(2): 330-334. DOI:10.15562/bmj.v7i2.969

Department of Pathology Anatomy Faculty of Medicine Udayana University/ Sanglah General Hospital Denpasar-Bali

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is one of the malignant tumors of head and neck. This malignant tumor is commonly found in Asian, which is estimated to have an association with certain genetic factors. Another risk factor that plays a role in causing NPC is habits of consuming preserved food or food with high sodium content. In Indonesia, NPC is the most common head and neck malignancy and rank fourth as the most common carcinoma after cervical carcinoma, breast carcinoma, and skin carcinoma with recorded mean prevalence is 5.66/100.000, with 1.000 monthly new NPC cases.[1] Early stage of NPC commonly difficult to be clinically diagnosed because of its hidden location in nasopharynx and the early symptoms are similar to upper respiratory tract infection. This condition causes most of NPC cases are diagnosed at late stage thus the prognosis is poor. The early diagnosis is very important in improving in prognosis.

The carcinogenesis of NPC has not clearly understood. At this time, the prognosis of NPC is mainly based on clinical stage (primary tumor size, the involvement of regional lymph node, and metastasis), age, gender, histopathology type, and Epstein Barr Virus Deoxyribonucleic Acid (EBV-DNA) serum level. These factors are often not sufficient enough to predict the disease progressivity because the same clinical stage could show different outcomes.[2]

Survivin is an apoptosis inhibitor with double roles. First, it inhibits apoptosis via the intrinsic pathway (mitochondria signal) that is initiated by activating Bcl-2-Associated X protein/B-Cell Lymphoma 2 (Bax/Bcl-2).[3-5]

Second, survivin interferes the caspase 9 and extrinsic pathway by binding to death receptor at the cell surface, which then inhibits caspase 8.[3-5] The survivin is unique because it is weakly expressed in normal tissue compared to carcinoma cell activation. This characteristic makes survivin is suitable as diagnostic, prognostic, and anticancer treatment factors.[3] Based on the direct role in carcinogenesis, survivin also plays role in angiogenesis, metastasis, and chemoresistance.[6]

The role of survivin in carcinogenesis of NPC has not been studied much. The study about survivin

*Correspondence to:
Rosalina Susantio, Department of Pathology Anatomy
rosalina_susantio@yahoo.com

Received: 2017-11-27
Accepted: 2018-1-29
Published: 2018-5-1

and NPC in Indonesia is still infrequent. The aim of this study was to prove the association between high survivin expression and late clinical stage of nasopharyngeal non-keratinizing squamous cell carcinoma.

MATERIALS AND METHODS

Samples Collection

This cross-sectional observational analytic study involved 30 histopathological samples of nasopharyngeal non-keratinizing squamous cell carcinoma, consisting of 12 early clinical stage samples and 18 late clinical stage samples. These samples were gathered from Pathology Anatomy Laboratory of Sanglah Hospital, using stratified random sampling within the period of January 2nd, 2014 to May 31st, 2017.

The inclusion criteria were the preparation of nasopharyngeal non-keratinizing squamous cell carcinoma containing at least 100 carcinoma cells (at a magnification of 400x) and had enough tumor tissues for recutting, patients with complete medical records, and patients had not done radiotherapy nor chemotherapy. The exclusion criteria were preparation with bleeding and broken or molded paraffin blocks.

The clinical stage was available from patients medical record, supported with CT imaging and histopathological data according to Tumor Node Metastasis (TNM) classification system from Union for Internasional Carcinoma Control (UICC) and American Joint Committee on Carcinoma (AJCC). Early stage was defined as stage I and II of disease, while the late stage was defined as stage III and IV of disease.

Survivin Immunohistochemical Staining and Scoring

Paraffin-embedded tissue sections with 4 microns thick were mounted on positively charged slides. Paraffin sections were put in xylene to ensure deparaffinization and then rehydrated with alcohol. The sections were incubated with hydrogen peroxide and buffer solution followed by incubation with a primary antibody, survivin mouse monoclonal, clone 12C4 diluted 1:50 (DAKO Japan). 3,3'-diaminobenzidine (DAB) ~~(What is DAB)~~ substrate chromogen solution was applied for 5 minutes before the sections were counterstained with Mayer's hematoxylin. Prostatic tissue was chosen for positive control.

A semiquantitative immunohistochemical scoring system was applied. The IHC score of survivin was based on multiplying the percentage of survivin positive tumor cells in the nucleus and/or cytoplasm and the average intensity. The percentage of survivin positive tumor cells was categorized into 4 groups: 0: if ≤5%, 1: 6%-25%, 2: 26%-50%, and 3: >50%.[6,7,8,9] The intensity of survivin staining was scored as 0: negative, 1: weak, 2: moderate, and 3: strong. The IHC scores ranging from 0 to 9 and was analyzed using Receiver Operating Characteristic/ROC curve and was classified into high expression and low expression.[10]

Statistical Analysis

Data normality was tested using Shapiro Wilks test ~~(SPSS program?)~~. Characteristics of study samples were served in table and narration. The association between survivin expression and clinical stage was analyzed by using *Fisher's Exact* test with the level of confidence $p<0.05$.

RESULTS

The patient's age range in this study was varied from 31 to 73 years old, with the mean 49.30±10.3 years old. Male was predominant than female, with 2:1 ratio. Eighty-seven percent of samples were originated from Bali, and 40% of them worked as farmers. (Table 1)

Table 1 General characteristics of study samples

General characteristics	n=30
Sex	
Male	20
Female	10
Mean of age (years), (SD)	49.3 (10.3)
Place of origin	
Badung	1
Bangli	5
Buleleng	5
Denpasar	4
Gianyar	4
Jembrana	0
Karangasem	3
Klungkung	0
Tabanan	4
Outside Bali	4
Occupation	
Government employee	7
Entrepreneur	6
Farmer	12
Housewife	4
Others	1

SD: standard deviation

Table 2 Clinicopathological parameters of study samples

Clinicopathological parameters	n=30
Stage	
Early	12
Late	18
T stage	
T1	7
T2	13
T3	2
T4	8
N stage	
N0	3
N1	14
N2	7
N3	6
M stage	
M0	29
M1	1
~~Survivin percentage~~	
~~0 (…%)~~	~~0~~
~~1 (…%)~~	~~0~~
~~2 (…%)~~	~~0~~
~~3 (…%)~~	~~30~~
Survivin intensity	
0 (negative)	0
1 (weak)	6
2 (moderate)	9
3 (strong)	15
Location of spread	
Nucleus	1
Cytoplasm	17
Nucleus and cytoplasm	12

Table 3 Survivin expression score and clinical stage of the study samples

Survivin expression score	Clinical stage		Total	P
	Early	Late		
Low (<4.5)	5 (16.7%)	1 (3.3%)	6 (20.0%)	
High (≥4.5)	7 (23.3%)	17 (56.7%)	24 (80.0%)	0.026
Total (percentage)	12 (40%)	18 (60%)	30 (100%)	

The clinical stage characteristics in this study were classified into early stage (Stage I and II) and late stage (Stage III and IV). The ratio between early stage and the late stage was 2:3. Table 2 showed the clinicopathological parameters of the study.

The area under the curve (AUC) from ROC method for the survivin scoring system was 74.1% (CI 95%: 55.1%-93.1%), p=0.028. The cut-off point for survivin scoring system was 4.5, with sensitivity 94.4%, specificity 41.7%, positive predictive value (PPV) 70.83%, negative predictive value (NPV) 83.3%, positive likelihood ratio (LR+) 1.62, dan negative likelihood ratio (LR-) 0.13.

This study found that there were more cases with high expression of survivin (score ≥4.5) in late stage (17 of 24 cases; 70.8%) compare to early stage (7 of 24 cases; 29.2%). Table 3 showed sample distribution based on the survivin expression and clinical stages.

Analysis of the association between high survivin expression and late clinical stage using Fisher's exact test showed a statistically significant association (p=0.026).

Figure 1 showed the results of weak, moderate, and strong immunohistochemical staining of survivin.

DISCUSSION

The incidence of nasopharyngeal carcinoma (NPC) varies within regions and races, with the highest incidence in South China (>20/100.000). Most of the NPC cases in China occurs in the fifth and sixth decades, meanwhile in North Africa is bimodal, where it occurs in two age ranges (around 50 years old and between 10-25 years old). The epidemiology of NPC in North America and region of Mediterranean shows that the incidence of NPC occurs at the age of 10-20 years and 40-60 years, with the most common in range of 40-49 years (45.2%).[1,11,12] This result was similar to study by Umar and Ahmed in Pakistan that shows the mean age 42±19 years.[13] One study in Indonesia at Adam Malik Hospital Medan shows that the peak age of NPC patients was 40 to 49 years old with age range between 30 to 59 years.[14]

This study showed that NPC cases predominantly occurred two times in males than females. This result was consistent with the study by Zhang et al that showed the overall incidence ratio between males and females with NPC was 2.3:1.[15] The population study by Adham et al. (2012) in Indonesia showed that NPC is the most common head and neck carcinoma in Indonesia (28.4%) with male to female ratio 2.4:1 and endemic in Java region.[1] The predominance of male in NPC might be related to the smoking habit, proved by a report of IARC in 2012 that there was causal association between smoking and NPC based on 14 case-control and 6 cohort studies. This result was consistent to a meta-analysis of 28 case-control and 4 cohort studies that showed a higher risk of NPC in smokers compare to non-smoker with odds ratio (OR): 1.60, (CI 95%: 1.38–1.87).[16]

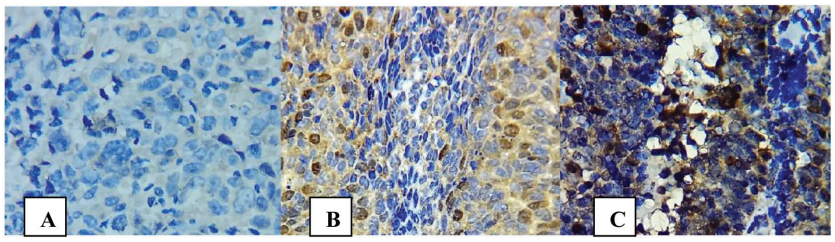

Figure 1 Immunohistochemical staining of survivin A. Weak B. Moderate C. Strong (400 x)

The distribution of subjects origin and occupation varied because Sanglah hospital is a referral center for Bali and Nusa Tenggara provinces. This result was different from the study in Adam Malik Hospital where most of the subjects were working as entrepreneurs (34.4%).[14] In this study, the author did not do further analysis about the association between occupation nor the geographic origin of the subjects' with the incidence or stage of NPC.

Survivin in this study was stained 100% of all samples with intensity varied from weak to strong. One study by Ma et al. (2011) showed that the functional polymorphism of -31C/G as a promotor of survivin gene might play a role in nasopharyngeal carcinoma events in Guangxi, Southern China. It was estimated that person with -31CG gene had a higher risk to suffer from nasopharyngeal carcinoma.[17] A study by Li et al (2008) found that 78.6% of NPC cases with positive survivin expression with 52.4% of them was considered as high survivin expression and 47.6% was low expression survivin.[6] The similar result was also found in another study done by Fu et al. (2008) that showed 70.37% of NPC cases with positive expression survivin.[18] Another study by Cai et al. (2015) showed that 79.5% of NPC cases with positive expression survivin with 68.2% of them were high expression.[8]

All of the above studies assessed the survivin expression by calculating the carcinoma cell percentage stained by survivin. This study is the first study using scoring method to assess survivin expression in NPC. The author had not found other studies using the similar method to assess the survivin expression in NPC cases. Some studies of survivin that use the scoring method were Youssef et al. (2008) in breast carcinoma, Aksoy et al. (2014) in endometrium carcinoma, Fu et al. (2014) in dogs with nasal carcinoma, Zhang et al. (2009) in prostate carcinoma, Farnebo et al. (2013) in head and neck squamous cell carcinoma, Zhou and Wang (2015) in cervix carcinoma, and Lee et al. (2009) in gastric carcinoma.[10,18-23]

There was a significant association between high survivin expression and clinical late stage of nasopharyngeal non-keratinizing squamous cell carcinoma. This result was similar to the study by Li et al. (2008) that found a significant association between survivin expression and clinical stage of non-keratinizing squamous cell nasopharyngeal carcinoma (p=0.018).[6] A similar result was also shown by Amer et al. (2013) in 49 cases of NPC (p<0.05).[24] Xiang et al. (2006) showed different result where there was no significant association between survivin expression and clinical stage of non-keratinizing squamous cell nasopharyngeal carcinoma (p=0.359).[25] High expression survivin in non-keratinizing squamous cell nasopharyngeal carcinoma is associated with the disease progressivity, advanced metastasis likelihood, resistant to therapy, and lower survival rate. This result indirectly shows the role of survivin as predictor and prognostic factor.[6,8,24]

This study has a clinical distribution of primary tumors (T), lymph node involvement (N), and metastasis (M) that varies in several stages. Several studies examined the association between survivin expression with each of these parameters such as in the study by Li et al. (2008) found that survivin expression was significantly associated with primary tumor (p = 0.004) and lymph node involvement (p = 0.028) and did not significantly correlate with the metastasis (p = 0.856).[6] Another study conducted by Xiang et al. (2006) found a significant relationship between survivin expression and metastasis (p = 0.040) and a non-significant association between survivin expression with primary tumor (p = 0.359) and lymph node involvement (p = 0.683).[25] This study did not analyze the relationship between expression survivin with each of these parameters.

Differences in the location of survivin staining are related to functional differences. Survivin in the nucleus may regulate cell proliferation while in the cytoplasm may be involved in cell survival but not in cell proliferation.[5] Survivin in the nucleus binds to microtubules in G2/M phase, whereas in the cytoplasm it inhibits apoptosis directly by inactivation of caspase-3, -7, and -9 or indirectly by inhibiting the Smac/DIABLO proapoptosis protein and preventing bonding with other IAPs such as XIAP.[18]

Some literature suggested that the difference site of survivin expression staining is related to prognosis, but the association of prognosis with the subcellular distribution of survivin in cancer remains unclear. Study of survivin expression in breast carcinoma by Youssef et al. (2008) found that survivin expression in the nucleus was a good prognostic factor.[10] Similarly, Zhang et al. (2009) suggested that survivin expression in the nucleus was associated with a better prognosis in prostate carcinoma.[25] Contrary to this study, Gonzalez et al. (2008) on carcinoma of the jaws and studies of Qi et al. (2010) in head and neck squamous

cell carcinoma found that survivin expression in the nucleus was unfavorable outcome and a poor prognostic factor.[26-7] These results were parallel to the studies performed by Fu et al. (2014) to obtain survivin expression in the nucleus was a factor of worse prognosis in nasal carcinoma in dogs.[18] This study did not look for an association between survivin sites with prognostic factors.

CONCLUSION

There was a significant association between high survivin expression and late clinical stage of nasopharyngeal non-keratinizing squamous cell carcinoma. Survivin could be one of the markers to predict the clinical course of NPC.

REFERENCES

1. Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, Tan IB, Middeldorp JM. Nasopharyngeal Carcinoma in Indonesia: Epidemiology, Incidence, Signs, and Symptoms at Presentation. *Chin J Cancer*. 2012; 31(4): 185-96.
2. Rosai, J.Respiratory tract: Nasal cavity, Paranasal Sinuses, and Nasopharynx, Larynx and Trachea, Lung and Pleura. In: *Rosai and Ackerman's Surgical Pathology.* 10th. Ed. London: Mosby Elsevier; 2011. p. 297-300.
3. Mita AC, Mita MM, Nawrocki ST, Giles FJ. Survivin: Key Regulator of Mitosis and Apoptosis and Novel Target for Cancer Therapeutics. *Clin Cancer Res*. 2008; 14(16): 5000-5.
4. Ryan BM, O'Donovan N, Duffy MJ. Survivin: A New Target for Anti-Cancer Therapy. *Cancer Treatment Reviews*. 2009; 35: 553-62.
5. Jaiswal PK, Goel A, Mittal RD. Survivin: A Molecular Biomarker in Cancer. *Indian J Med Res*. 2015; 141: 389-97.
6. Li YH, Hu CF, Shao Q, Huang MY, Hou JH, Xie D, Zeng YX, Shao JY. Elevated Expressions of Survivin and VEGF Protein are Strong Independent Predictors of Survival in Advanced Nasopharyngeal Carcinoma. *J of Trans Med*. 2008; 6: 1.
7. Chan ATC, Gregoire V, Lefebvre JL, Licitra L, Hui EP, Leung SF, Felip E. Nasopharyngeal Cancer: EHNS-ESMO-ESTRO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-up. *Ann Oncol*. 2012; 23(Suppl 7): vii83-5.
8. Khan Z, Khan N, Tiwari RP, Patro IK, Prasad GB, Bisen PS. Down-regulation of Survivin by Oxaliplatin Diminishes Radioresistance of Head and Neck Squamous Carcinoma Cells. *Radiother Oncol*. 2010; 96: 267-73.
9. Kleinberg L, Florenes VA, Silins I, Haug K, Trope CG, Nesland JM, Davidson B. Nuclear Expression of Survivin is Associated With Improved Survival in Metastatic Ovarian Carcinoma. *Cancer*. 2007; 109(2): 228-38.
10. Youssef NS, Hewedi IH, Raboh NMA. Immunohistochemical Expression of Survivin in Breast Carcinoma: Relationship with Clinicopathological Parameters, Proliferation and Molecular Classification. *J of The Egypt Nat Cancer Inst*. 2008; 20(4): 348-57.
11. Laantri N, Corbex M, Dardari R, Benider A, Gueddari BE, Khyatti M. Environmental, Genetic, and Viral Risk Factors of Nasopharyngeal Carcinoma in North Africa. *BMC Proceedings*. 2011; 5(Suppl 1):p30.
12. Liu W, Tang Y, Gao L, Huang X, Luo J, Zhang S, Wang K, Qu Y, Xiao J, Xu G, Yi J. Nasopharyngeal Carcinoma in Children and Adolescents-A Single Institution Experience of 158 Patients. *Rad Oncol*. 2014; 9: 274-80.
13. Umar B and Ahmed R. Nasopharyngeal Carcinoma, An Analysis of Histological Subtypes and Their Association with EBV, A Study of 100 Cases of Pakistani Population. *Asian of J Med Sci*. 2014; 5(4):17-20.
14. Melani W dan Sofyan F. Karakteristik Penderita Kanker Nasofaring di Rumah Sakit H. Adam Malik Medan Tahun 2011. 2013. Available from: https://jurnal.usu.ac.id/index.php/ejurnalfk. Accessed November, 23 2016.
15. Zhang LF, Li YH, Xie SH, Ling W, Chen SH, Liu Q, Huang QH, Cao SM. Incidence Trend of Nasopharyngeal Carcinoma from 1987 to 2011 in Sihui County, Guangdong Province, South China: An Age-Period-Cohort Analysis. *Chin J Cancer*. 2015; 34:15.
16. Lin JH, Jiang CQ, Ho SY, Zhang WS, Mai ZM, Xu L, Lo CM, Lam TH. Smoking and Nasopharyngeal Carcinoma Mortality: A Cohort Study of 101,823 Adults in Guangzhou, China. *BMC Cancer*. 2015; 15: 906.
17. Ma F, Zhang H, Zhai Y, Huang W, Zhao C, Ou S, Zhou H, Yuan W, Wang Z, Wang H, Yue W, Yu L, Li P, Xia X, Cai M, Zhang Y, Cui Y, He F, Ma Y, Zhou G. Functional Polymorphism-31C/G in the Promoter of BIRC5 Gene and Risk of Nasopharyngeal Carcinoma among Chinese. *PLoS ONE*. 2011; 6(2): e16748.
18. Fu DR, Kato D, Watabe A, Endo Y, Kadosawa T. Prognostic Utility of Apoptosis Index, Ki-67 and Survivin Expression in Dogs with Nasal Carcinoma Treated with Orthovoltage Radiation Therapy. *J Vet Med Sci*. 2014; 76(11): 1505-12.
19. Lee WS, Cho SB, Rew JS, Lee JH, Park CS, Joo YE. Expression of Survivin in Gastric Carcinoma and its Relation to Tumor Cell Proliferation and Apoptosis. *The Kor J of Path*. 2009; 43: 329-34.
20. Zhang M, Ho A, Hammond EH, Suzuki Y, Bermudez RS, Lee RJ, Pilepich M, Shipley WU, Sandler H, Khor LY, Pollack A, Chakravarti A. The Prognostic Value of Survivin in Locally Advanced Prostate Cancer: A Study Based on RTOG 8610. *Int J Radiat Oncol Biol Phys*. 2009; 73(4): 1033-42.
21. Farnebo L, Tiefenbock K, Ansell A, Thunell LK, Garvin S, Roberg K. Strong Expression of Survivin is Associated with Positive Response to Radiotherapy and Improved Overall Survival in Head and Neck Squamous Cell Carcinoma Patients. *Int J Cancer*. 2013; 133: 1994-2003.
22. Aksoy RT, Turan AT, Boran N, Tokmak A, Isikdogan BZ, Dogan M, Tulunay HG. Lack of relation of survivin gene expression with survival and surgical prognostic factors in endometrial carcinoma patients. *Asian Pac J Cancer Prev*. 2014; 16: 6905-10.
23. Zhou XL and Wang M. Expression Levels of Survivin, Bcl-2, and KAI1 Proteins in Cervical Cancer and Their Correlation with Metastasis. 2015. *GMR*; 14(4): 17059-67.
24. Amer, R.Z., El-Seaidey, A.Z., Elbadawy, A.E., Basuny, O.Y., Youssef, S.A. 2013. Prognostic Significance of Survivin and Nuclear Morphometry in Nasopharyngeal Carcinoma. *Med J Cairo Univ*; 81(2): 185-92.
25. Xiang, Y., Yao, H., Wang, S., Hong, M., He, J., Cao, S., Min, H., Song, E., Guo, X. 2006. Prognostic Value of Survivin and Livin in Nasopharyngeal Carcinoma. *Laryngoscope*; 116: 126-30.