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Expression of Caspase-9 as Diagnostic Value of Undifferentiated Nasopharyngeal Carcinoma (Type 3) Stage IV

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Abstract : Apoptosis is regulated by caspase-9 as an initiator, and caspase-3 as an executor, via an intrinsic pathway which can be used as diagnostic indicator. This study aimed to observe the expression of caspase-9 in undifferentiated nasopharyngeal carcinoma stage IV as a molecular marker. This was non-experimental, analytical, observational, quantitative study with cross-sectional approach. A biopsy was performed on patients of undifferentiated nasopharyngeal carcinoma. Patients were classified according to histopathology and clinical stage that refers to UICC. Expression of caspase-9 was measured with the immunohistochemical assay and further analyzed statistically. From 24 subjects, there were 12 subjects diagnosed as undifferentiated nasopharyngeal carcinoma stage IV. Receiving operating curve (ROC) of caspase-9 was between 50 % and 100 %. Value of AUC from ROC was 75.3 % (95 % IK 55.6 % - 95.1 %) p = 0.035. Expression of caspase-9 as an indicator of undifferentiated nasopharyngeal carcinoma stage IV was less than 2.294 with a sensitivity of 66.7 % and specificity of 66.7 %.
Keywords : Caspase-9, molecular diagnostic, sensitivity, specificity, undifferentiated nasopharyngeal carcinoma stage IV.

Introduction:

Nasopharyngeal carcinoma (NC) is head and neck malignancy with high incidence. In Moewardi Public Hospital, Surakarta, 81.48 % of NC WHO type 3 was administrated in otorhinolaryngology clinic in 2007, and increased in 2009 to 90.2 % of all cases, which was generally at critical stage III and IV[1]. In western, WHO Type 1 are 30-50 % cases, whilst WHO Type 2 and 3 are highly documented in Asia[2-4].

Epstein-Barr Virus (EBV) is often associated with lymphoid and epithelial malignancy including NC, in which express EBV latent gene, Epstein Barr – Virus Nuclear Antigen (EBNA 1, 2, 3A, 3B, 3C EBNA-LP), Latent Membrane Protein (LMP 1, LMP 2A and LMP 2B) and EBV-encoded small Ribo Nucleic Acid (RNA). LMP 1 is considered as key regulator in NC pathogenesis through activation of Nuclear Factor- Kappa β

Lymphocyte (NF-κβ) and Janus Kinase pathway (JAP) that causes excess proliferation, as well as induces encoding gene of A20 that inactivates p53, resulting in inhibition of apoptosis[5-7].

Middle drop[8], stated that EBV produces BCRF which is similar to human IL-10 that can suppress NK cells and CD8 T lymphocyte. Signal transduction of apoptosis relies upon caspase as responsible enzymes in programmed cell damage. Caspase-8 and 9 play its role as initiator, whereas caspase-3 as executor[9]. Caspase-3 is activated by caspase-9 in intrinsic pathway[10]. The active form of caspase-9 is a crucial marker in the early stage of apoptosis[11]. Thus, expression of caspase-9 is used as molecular diagnostic in NC WHO Type 3 stage IV.

Method:

Biopsy of NC was obtained from 24 subjects of undifferentiated NC (Type 3). The clinical degree of NC was measured in accordance with *Tumor Nodul Metastasis* (TNM) using UICC 1997. A biopsy was collected under supervision and management of Department of Otorhinolaryngology Clinic, Moewardi Public Hospital. Informed Consent was approved by Ethic Committee of Moewardi Public Hospital/Faculty of Medicine, Sebelas Maret University.

The Biopsy results were sent to Pathology Anatomy Laboratory, Moewardi Public Hospital for histopathology measurement. Histopathology of undifferentiated (Type 3) was further visualized with an immunohistochemical assay to observe the expression of caspase-9, in Biomolecular Pathology Anatomy Laboratory, Faculty of Medicine, Sebelas Maret University.

Positive cells were measured per mm^2 using a microscope. Data were analyzed statistically with Diagnostic Test using AUROC (Area Under Receiving Operating Curve).

Results and Discussion:

This study was conducted in NC patients administrated in Otorhinolaryngology clinic Moewardi Public Hospital between 2011-2014. According to the medical record and pathology measurement specimens, samples obtained were 434. Samples were classified based on demographic factors of NC which consisted of the population, sex, age, job, education, and histopathology type (Figure 1).

Characteristic of research subjects

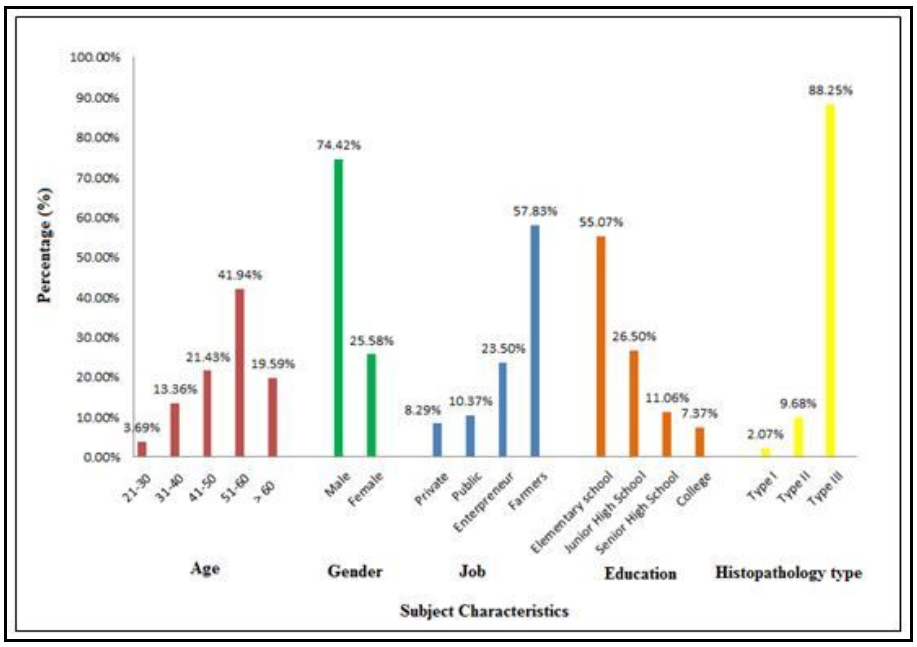

Figure 1 Characteristic of research subjects according to demographic factors

The result showed that most of the patients are old (51-60 years old) (41.94 %). The gender of the patient was dominated by the male patient (74.42 %). They are mostly works as the farmer (57.83 %) and they are not well-educated people because most of them are graduated from Elementary school only. We found that Type III is the most prevalence (88.25 %). This data give basic information about the NC patient in Moewardi Public Hospital in Surakarta, Indonesia.

In the present study, NC (Type 3) stage IV were 12 of 24 subjects, with the prevalence was 50 %. Receiving Operating Curve (ROC) showed caspase-9 expression possess good diagnostic value as between 50 % and 100 % line (Figure 2).

ROC of Caspase-9 expression

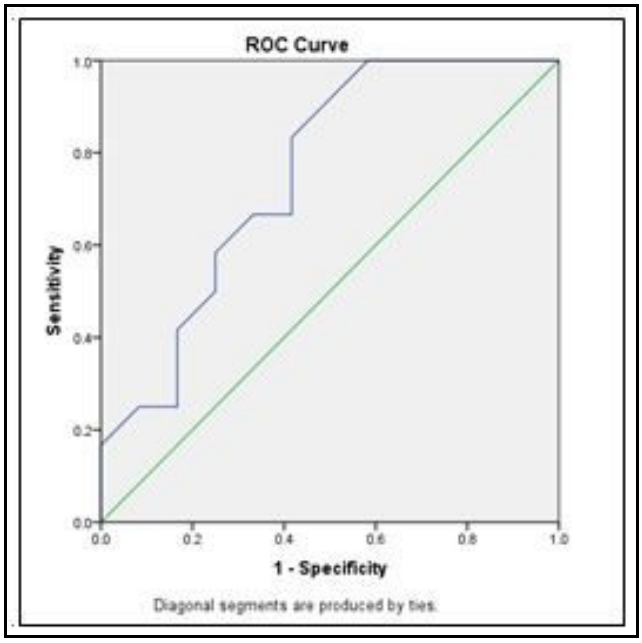

Figure 2 ROC of Caspase-9 expression as diagnostic value of NC WHO Type 3 Stage IV

AUC value obtained from ROC method was 75.3 % (95 % IK 55.6 % - 95.1 %), P= 0.035, statistically moderate (75.3 %). AUC value indicates caspase 9 expressions used as the diagnostic value of NC (Type 3) stage IV or not 100 % of patient, which means patients obtained were 75 patient.

Meeting point of sensitivity and specificity of Caspase 9

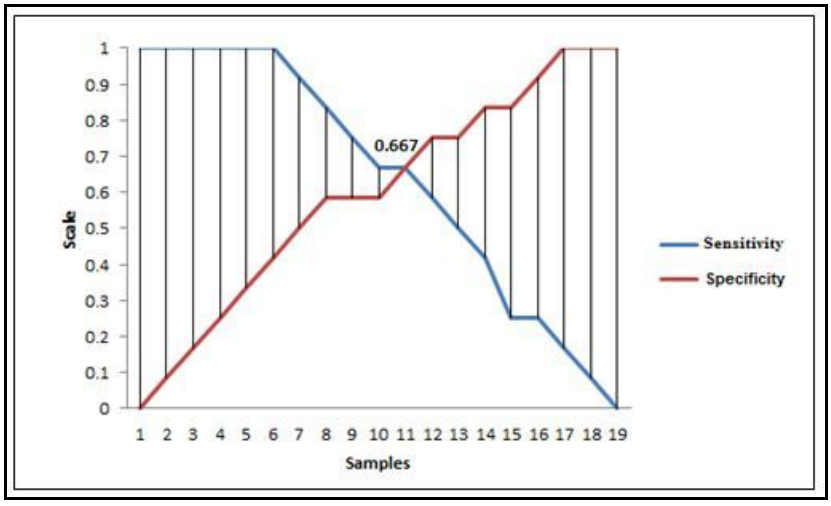

Figure 3 Meeting point of sensitivity and specificity of Caspase 9 as diagnostic value of NC WHO Type 3 Stage IV

AUC value of caspase 9 were 75.3 % (95% IK 55.6 % - 95.1 %), in which meeting point is supposed to be less than 2.94 with a sensitivity of 66.7 % and specificity of 66.7 %. According to the medical record and pathology specimen administrated in otorhinolaryngology clinic Dr. Moewardi General Hospital from 2011 to 2014, the highest case was NC WHO Type 3. In this study, NC WHO type 3 stage IV in otorhinolaryngology Clinic Dr.Moewardi General Hospital was 12 samples (50 %) of 24 advanced stage, which indicate patients in Dr. Moewardi hospital were predominantly in advanced stage (stage III and IV). This might be due to several factors, such as the social economy.

The result of the present study showed caspase 9 expressions possess good diagnostic value (between 50 % and 100 %, as shown in ROC). AUC value obtained indicates expression of caspase 9 can be used as a diagnostic value in NC WHO Type 3 stage IV. Diagnosis of PA histopathology refers to WHO criteria; Type 1, Type 2 and Type 3. Whereas stage is determined according to UICC.

This study showed that caspase 9 can be utilized as an alternative of molecular diagnostic of NC WHO Type 3 stage IV, due to its strategic pathway in apoptosis. Expression of caspase 9 as diagnostic value of NC WHO Type 3 stage IV, with a value of less than 2,94 with a sensitivity of 66.7 % and specificity of 66.7 %.

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