# Decrease of Epstein-Barr Virus Anti Early Antigen Imunoglobulin a Levels and Primary Tumor Size in Post-Cisplatin-Paclitaxel Chemotherapy in Nasopharyngeal Carcinoma Patients

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## Abstract

**Background:** Nasopharyngeal carcinoma (NPC) associated with Epstein-Barr virus (EBV) chronic infection is a common head and neck malignancy in South China and Indonesia. Although radiation and chemotheraphy is the main theraphy, it requires repeated and invasive biopsy for pathological evaluation. Therefore, a marker is required for screening including the level of anti early antigen imunoglobin a serology level.

**Method:** Pre-pots test, longitudinal cohort design. The PTV of 18 samples were examined using CT scan, while their serum EBV anti EA IgA level were examined using pre and post three series-cisplatin-paclitaxel chemotherapy ELISA.

**Results**: Although there was no significant changes in the level of anti EA IgA, however we found a decrease in the mean of pre-chemotherapy anti EA IgA level from 136.49 U/ml to 124.61 U/ml. There was significant changes in the VTP in pre and post-chemotherapy ($p<0.05$). The mean of VTP in pre-chemotherapy was 66.26 cm3 (SD-38.61 cm3), while in post-chemotherapy was 31.64 cm3 (SD-27.5 cm3). The delta mean of changes in anti EA IgA level was 11.8 U/ml and in VTP was 34.62 cm3. No correlation was found between the changes of anti EA IgA and the changes of VTP in post-chemotheraphy ($p>0.05$). However, decreases were found in the level of EBV EA IgA and PTV in pre and post NPC patients.

**Conclusion:** There were decreases of serum EBV EA IgA level and PTV in pre and post-chemotherapy NPC patients.

***Keywords:*** *Imunoglobulin A, primary tumor volume, cisplatin-paclitaxel chemotherapy, nasopharyngeal carcinoma*

## Introduction

Nasopharyngeal carcinoma (NPC) is a rare head and neck malignancy except in South China and Southeast Asia including Indonesia. NPC incidence in South China is between 20-40 per 100,000 population per year and in Indonesia is 6.2 per 100,000 population per year or 12,000 new cases per year [1] In Dr. Soetomo General Hospital and Dr. Cipto Mangun Kusumo General Hospital as the main hospital in Surabaya and Jakarta, Indonesia, respectively, nasopharyngeal carcinoma ranks fourth in malignancy after carcinoma of the cervix, breast and skin [2].

EBV infection will be followed by the formation of specific antibodies against EBV antigens including anti viral capsid antigen (VCA), early antigen (EA), and Epstein-Barr nuclear antigen (EBNA). Increased

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levels of Immunoglobulin A (IgA) anti EA and VCA are commonly found in patients with KNF [3]. Anti-EBV antibody levels, particularly IgA in NPC patients are higher than healthy individuals or patients with other kinds of malignant head and neck tumor, other organ tumors and even in other nasopharyngeal disorders [3].

Post-therapy NPC monitoring associated with EBV infection is performed with painful repeated biopsy and pathology examination. EBV serology examination can be used as screening for at-risk patients and occult primary tumors as well as to detect recurrence [4]. A research shows that there is a significant association between serum EBV DNA levels of KNF patients with clinical staging and tumor progression [5] It is expected that EBV serology may replace the role of the biopsy.

Several studies have shown that the levels of IgA anti VCA and EA EBV increase with the appearance of NPC symptoms. Immunoglobulin A anti-EA is a tumor marker for the diagnosis of NPC because it exhibits high specificity compared to other tumor markers and IgA anti-EA will increase 1 - 5 years before NPC [3]. IgA levels in pre-therapy have diagnostic and prognostic value, whereas NPC patients with higher levels of antibodies have a worse prognostic [6]. The remaining high levels of IgA anti VCA and anti-EA after therapy are associated with poor prognostics. The increased serum immunoglobulin A (IgA) EBV serologic levels with normal histopathologic results should still be warranted for greater recurrence or higher risk of recurrence

Serum IgA anti EA EBV serologic examination is required for post-therapy NPC evaluation. The purpose of this study was to determine the relationship between changes in IgA anti EA EBV levels in serum with primary tumor volume in post cisplatin-paclitaxel NPC patients in Dr. Soetomo General Hospital Surabaya. The results of this study are expected to be used as a basis for assessment of therapeutic response, early detection of KNF recurrence, and prognosis determination.

## Method

This is an observational study with longitudinal cohort approach using pre-post test. The study was conducted in Department of ENT Dr. Soetomo General Hospital Surabaya in the period of August 2016 until January 2017. 24 new NPC patients were collected as the samples from a total of 25 patients that met the study criteria. Six samples were dropped-out due to changes in paclitaxel regiment, one patient due to an allergy, one patient continued the chemotherapy procedure in outside Dr. Soetomo General Hospital Surabaya, one patient refused to continue chemotheraphy, two patients have undergone radiotheraphy before the chemotherapy finished and one patient passed away.

The examination of serum EBV EA IgA level was conducted using.The basic data collected in this study consisted of the patients data based on age, gender and ethnicity. The examination results in the form of pre and post cisplatin-paclitaxel chemotherapy serum EBV anti EA IgA in NPC patients was assessed by Clinical Pathology Consultant. Pre and post cisplatin-paclitaxel chemotherapy primary tumor volume was assessed by radiology consultant. The data were analyzed using Wilcoxon Signed Rank Test and rho Pearson correlation test. This Research to find out the changes of serum EBV anti EA IgA and primary tumor volume (PTV).

## Results

**Table 1. The results of anti-EA IgA in post 3-series cisplatin-paclitaxel chemotherapy**

| Cisplatin – paclitaxel Chemotherapy | Anti EA IgA level (U/ml) | Primary Tumor Volume (PTV) (cm3) |
|---|---|---|
| Pre | | |
| Mean | 136.49 | 66.26 |
| Median | 86.54 | 59.30 |
| Standard Devation | 140.38 | 38.61 |
| Post | | |
| Mean | 124.61 | 31.64 |
| Median | 93.07 | 20.15 |
| Standard Deviation | 127.80 | 27.55 |
| Δ(delta) | | |
| Mean | 11.88 | 34.62 |
| Median | 8.41 | 23.50 |
| Standard Deviation | 55.88 | 36.85 |

The most NPC patients were in the age group of 40-49 years old with seven patients (38.89%). The youngest age was 19 years old and the oldest was 62 years old. Distribution of NPC patients based on gender was described in patients were male (13 patients or 72.22%) and there were 5 female patients (27.78%). The comparison between male and female was 2.6:1. Distribution of NPC patients based on ethnicity were Javanese with 14 patients (77.78%) compared to Madura 4 people (22.22%).

The result of anti-EA IgA level in pre-chemotherapy was found to be 136.49 U/ml and standard intersection of 140.38 U/ml. The results of anti-EA IgA in post 3-series cisplatin-paclitaxel chemotherapy were found to average 124.61 U/ml and standard intersection of 127.80 U/ml (Table 1). Statistic test using Wilcoxon Sign Rank Test showed p value of 0.053. This indicated no significant changes in anti-EA IgA level in pre and post cisplatin-paclitaxel chemotherapy in patients with NPC ($p > 0.05$)

The results of PTV in pre-chemotherapy cisplatin-paclitaxel showed the mean of 66.26 cm3 and standard deviation of 38.61 cm3. The results of precisplatin-paclitaxel showed the mean of 66.26 cm3 and standard deviation of 38.61 cm3. Statistic test using Wilcoxon Sign Rank Test showed p value of 0.001. The data indicated a significant difference of PTV in pre and post cisplatin paclitaxel chemotherapy in NPC patients ($p < 0.05$).

Changes in pre and post cisplatin-paclitaxel anti EA IgA levels showed Δ mean of 11.88 and Δ standard deviation of 55.88. Changes of PTV in pre and post-cisplatin-paclitaxel chemotherapy had Δ mean of 34.62 and Δ standard deviation of 36.85 (Table 4). Statistical test using Pearson correlation showed correlation coefficient (r) of 0.260 and p value of 0.298, suggestinga insignificant correlation between the changes in anti-EA Ig A level and primary tumor volume in post cisplatin-paclitaxel chemotherapy in NPC patients

## Discussion

Distribution of NPC patients based on gender was the 13 male patients (72.22%) and 5 female patients (27.78%). The ratio between men and women was 2.5:1. Distribution of KNF patients based on gender, most patients were male (70%) with the ratio between men and women being almost the same throughout Indonesia, i.e. 2-3:1.24 The habits such as smoking increase the risk of KNF 2-6 times and so did the exposure to steam, dust and chemical gas at the workplace also increase the same risk. Formaldehide exposure at workplace also increase the risk of NPC to 2-4 times. Increased risk also occured in workers who inhaled firewood smoke, and the risk increased 2 times in workers exposed to industrial heat and combustion products. This led to high incidence in men due to differences in living habits and occupations that cause males to have more frequent contact with carcinogens that caused NPC.2 Testosteron hormone which was dominant in male was suspected of causing immune response and tumor surveillance decrease and thus male are more prone to EBV infection and cancer [7]

The results of statistical analysis of changes in the levels of serum EBV anti EA IgA in pre and post-chemotherapy cisplatin-paclitaxelpada patients with NOC was not significant. Nevertheless, there was a mean decrease by 136.49 U/ml in pre-chemotherapy to 124.61 U/ml in post-chemotherapy (Table 1). This was incompatible with the chemotherapy mechanisms that caused humoral and cellular immune suppression. Humoral immunity expressed by B cells and assessed on the level of immunoglobulin.27 The similar mechanism in radiotherapy was given to NPC patients which often caused immunologic cell damage which resulted in decrease of cellular humoral immune response[8]

Decreased serum EBV anti EA IgA levels after cisplatin-paclitaxel chemotherapy in this study might be due to abundant EBV not only in NPC tumor cells but also from activation of T cell infiltration, B lymphocytes, and epithelial cells capable of producing antigen associated with EBV.28,29 In addition, there was a difference of individual immune responses to various antigens which made antibodies as important markers were highly dependent on the host response to the viral antigen on the tumor.10 Changes in the latent cycle into lytic cycles in NPC tumor cells could occur spontaneously or induced by cisplatin chemotherapy, γ ray radiation, phorbol ester, sodium butyrate and bortezomid.30,31

Levels of EBV EA Ig A highly increased in 2 patients with NPC i.e. 19.84 U/ml in pre-therapy to 184.31 U/ml in post-therapy and 84.07 U/ml to 113.72 U/ml and 4 patients with NPC had slight increase, i.e. around ¬ + 2.00 U/ml. The levels of EBV EA Ig A in patients with NPC in this study decreased in 12 patients. This was consistent with the study by Gu, et al.32 (2009) obtaining fluctuation of EBV antibody reactivity during therapy and steady follow-up. Fifteen of the 35 NPC patients tested for antibodies decreased after therapy but

13 patients showed small changes. Increased levels of EBV antibody after therapy were obtained in 5 patients while 2 patients that initially decreased had increase. This might be due to the different kinetic diversity of serologic EBV for each KNF patient during therapy as well as illustration of differences in radiosensitivity and immunological reactions[9]

The results of the measurement of primary tumor volume (PTV) in post-chemotherapy cisplatin-paclitaxel showed a decrease of PTV mean in post-chemotherapy cisplatin-paclitaxel. The PTV change was statistically significant and therefore it was concluded that there was a significant PTV change in pre and post chemotherapy [10] This corresponded to the use of cisplatin-paclitaxel chemotherapy in the management of malignant tumors, i.e, to eradicate tumor cells or for locoregional control when used in conjunction with surgery or radiotherapy. Chemotherapy was used to treat macroscopic and microscopic metastases. Microscopic metastasis that was clinically invisible and deposited in the body would turn into macroscopic if not treated [11].

Pearson correlation test result on serum EBV anti EA IgA level changed with primary tumor volume change (PTV) in post- cisplatin-paclitaxel chemoterapy got the value r = 0,260 and p = 0,298. The results showed a non-significant relationship (p> 0.05). However, in general we found a decrease in EBV EA IgV levels and decreased primary tumor volume in patients with NPC who received pre and post cisplatin-paclitaxel chemotherapy. Interaction of cancer cells with lymphocytes or chemotherapy and radiotherapy that could have a significant effect on the immune system my explained it, and also the imbalance of humoral immunity and decreased cellular immunity triggered cancer progression and treatment failure.33 In this study changes in serum anti-EA IgA might be due to direct effects of chemotherapy or indirect effects of viral replication associated with growth of NPC tumor[12] The immediate effects of chemotherapy and cytostatic drugs were known to greatly damage lymphoid as an antibody producer but some studies have found that chemotherapy did not destroy all B memory cells and memory T cells even when examination did not get humoral immune response.34 Increased response of various antibodies to EBV protein at a higher stage of malignancy suggested viral replication associated with NPC tumor growth [9]

A study [13] showed no statistically significant association between gender, age, and distant metastases with anti-VCA IgA antibodies, anti-EA IgA, anti-RGA IgG, and anti-EBNA IgA. There was a tendency to increase the levels of anti-VCA IgA and anti-EA IgA with a classification of N, but there was no association with the classification of T. This might be related to lymphocyte infiltration in NPC which significantly contributed to increase antibody to EBV antigen lytic phase.

The results of this study were consistent with a study30 that various chemotherapeutic drugs such as cis-platinum, fluorouracil, and taxol trigger alteration of latent cycle into lytic EBV virus infection in tumor cells. This change occured through the protein signal kinase C δ, phosphatidylinositol 3'-kinase, and p38 stress mitogen-activated protein kinase but not caspase activation 3. This study was also conducted on mice containing KNF cells and found that combination of GCV therapy with 5 FU or a combination of GCV with cis-platinum being more effective in triggering KNF cell apoptosis than single therapy. Spontaneous induction of viral replication was more determined by the intracellular plasma environment than the factors that cause plasma cell differentiation. Lit replication of infected latent cell lines might be performed by induction including anti-immunoglobulin antibody (anti-IgG), activation of transforming growth factor β (TGF β), and activation of CD4 + T cells. The transition from the latent cycle to the EBV lytic cycle could be induced by DNA destruction agents such as chemotherapy (cisplatin), γ ray radicals, phorbol ester, sodium butyrate, and bortezomid.30,31

## Conclusion

There was a decrease in serum EBV EA IgA level and primary tumor volume n post-chemotherapy compared to in pre-chemotherapy and therefore could be used to monitor the success of post-chemotherapy medication.

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