Abstract

Background: Nasopharyngeal carcinoma is a malignancy in nasopharynx cavity originated from mucosal epithelial cell of nasopharynx. In Indonesia, nasopharyngeal carcinoma is the most considerable malignancy in head and neck area with the highest mortality rate.

Objective: To perceive that photodynamic therapy in nasopharyngeal carcinoma either residual or recurrence patients after receiving radiation or in chemo radiation therapy will yield a well-responded therapy, better life expectancy compared to those who didn’t receive PDT, and inducing systemic anti-tumor response which was marked by the increased level of immune-response cytokines.

Methods: This research took place in Department of Otorhinolaryngology Head and Neck Surgery Faculty of Medicine University of Gadjah Mada/Dr Sardjito General Hospital Yogyakarta, Indonesia using cohort retrospective design. The subjects of this research are patients with residual or recurrence nasopharyngeal carcinoma who had received photodynamic therapies between the years 2005-2011. Pre and posttest examination were used to test the hypothesis of the cytokines level difference before and after photodynamic therapies.

Results: As much as 58.1% patients were men, and 41.9% of patients were female. Most of the patients were above 40 years of age. As much as 21 out of 31 patients (67.7%) were previously diagnosed as nasopharyngeal carcinomas in late stages, in this case the given treatment was chemo radiation. The 5 years life expectancy of residual or recurrence nasopharyngeal carcinoma is 60.7%. This number was preferable compared to the life expectancy of nasopharyngeal carcinoma patients who received standard therapy of either radiation or chemo radiation. Residual and recurrence nasopharyngeal carcinoma patients diagnosed between year 2009-2013 without photodynamic therapy showed the 5 year overall survival rate as much as 22.9% with the median 23.6 months. There was a significant difference of life expectancy either clinically or statistically in patients with residual or recurrence nasopharyngeal cancer who received PDT compared to those who did not (p=0.004).

Conclusion: In long-term observation, it was reported that photodynamic therapy (PDT) increased the 5 years survival rate in patients with residual or recurrence nasopharyngeal carcinoma. Patients who received PDT had life expectancy 5 years longer than patients who did not receive PDT.

The result of prognostic factors analysis showed that gender and age <40 years old each played a role as factors which affect the life expectancy.

Keywords: Nasopharyngeal carcinoma; Photodynamic Therapy

Abbreviations: NPC: Nasopharyngeal Carcinoma; PDT: Photodynamic Therapy; TAA: Tumor Associated Antigen; IFNγ: Interferon Gamma; TNFa: Tumor Necrosing Factor Alpha

Introduction

Nasopharyngeal carcinoma (NPC) is a malignancy in nasopharynx cavity that originates from mucosal epithelial cell of nasopharynx. Nasopharyngeal carcinoma has high mortality rate. Most incident were found in South of Asia, including Malaysia and Indonesia with an incident rate around 5-15 patients per 100,000 people each year [1]. In Indonesia, nasopharyngeal carcinoma is the most common malignancy in head and neck area with the highest mortality rate. A survey held by Health Ministry of Indonesia in the year 1987 showed that the incidence rate of NPC is 4.7 per 100,000 each year, with the ratio of 3.5:1 between male and female [2]. Few researches were held in some hospitals showed that NPC is the most common malignancy compared to other malignancy in head and neck area with the prevalence of 40-80% [3].
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The issue encountered while managing NPC is the availability of radiotherapy device in Indonesian which is not equivalent to the number of NPC patients who need treatments, therefore there’s tardiness in administrating standardized therapy. Among 50 patients diagnosed with NPC in the year 2009-2010 in Department of Otorhinolaryngology Head and Neck Surgery Dr. Sardjito Hospital, Yogyakarta, Indonesia, 37 of these patients (74%) had to wait for more than 2 months to receive radiotherapy, 10 of these patients had to wait until up to 6 months even longer. The procrastination of the therapy given to NPC has consequence of wretched therapeutic outcome and may lead to considerable cases of residual and recurrence.

Photodynamic therapy (PDT) is a non-surgical therapy, which is minimally invasive, using source of light to activate photosensitizer drug. PDT has been used for additional therapy in many cancers including NPC. Generally, PDT can be given before or after chemotherapy, radiotherapy, or surgery without undermining the effectiveness of those modalities mentioned. In Yogyakarta, research about PDT for residual or recurrent NPC patients has been held since 2005, as much as 34 patients received PDT. Evaluation of the therapeutic response was done on twelve weeks after the administration using nasopharyngeal endoscopy, CT-scan, and nasopharyngeal biopsy. There were 25 patients who showed good results, 5 patients were still sustaining residue, 2 patients were unable to undergo the evaluation because they didn't show up for the examination and 2 other patients were still waiting for the evaluation schedule [4].

Previous researchers performed a study of PDT in 4 patients with recurrent NPC. It was reported that all patients were able to survive for up to 5 years [5]. Another research performed on PDT in 12 patients with recurrent NPC, a good therapeutic result was proved by CT-Scan or MRI 6 months after receiving PDT [6].

There was another clinical research on Foscan-mediated PDT of 20 patients with oral cavity malignancy. This research showed that PDT gave a pretty good result on therapy for squamous cell carcinoma of the oral cavity, also the ability to readminister the Foscan-mediated therapy to the same patient without any side effect [7]. In accordance to previous research done by Koberlik et al (2008) did research on PDT from mice implanted with fibrosarcoma tumor cells. This research proves the existence of acute phase response after PDT administration [8].

In addition, another researcher went over the systemic immune response in basal cell carcinoma patients. The research was applying the measurement of systemic immune response against Tumor Associated Antigen (TAA) from patients who received PDT compared to those who had surgery. The result of the research showed that PDT given locally to the patients was able to enhance the systemic immune response against tumor [9].

This research was held in Department of Otorhinolaryngology Head and Neck Surgery Dr. Sardjito Hospital, it was differ from other previous researches for it was arranged as aresearch to find out antitumor systemic immune response in NPC patients who received photodynamic therapy (PDT). In this research, there will be a few examination on some systemic immune response parameter in residual or recurrent NPC patients who received PDT.

Materials and Methods

This research is a clinical study on photodynamic therapy on NPC patients. The study was held in Department of Otorhinolaryngology Head and Neck Surgery Dr. Sardjito Hospital during the period of 2005-2011 who met the inclusion and exclusion criteria. The inclusion criteria of the patients were patients who had received photodynamic therapy (PDT) at the Department of Otorhinolaryngology Head and Neck Surgery Dr. Sardjito Hospital during the period of 2005-2011, informed consent forms were given to ensure their willingness to participate in this research. Patients who were willing to participate would undergo laboratory examination on the serum samples which had already taken before and after PDT administration, and during the follow up period, the serum samples then stored in the Laboratory of Biomolecular Faculty of Medicine Universitas Gadjah Mada.

There were 31 patients who met both inclusion and exclusion criteria which then registered as a research subjects to be analyzed in clinical parameter. The analysis of cytokines: Interleukin-12, Interleukin-18, Interleukin-2, and Interferon Gamma were applied to the serum samples of the patients where these serum samples were completed and stored in Laboratory of Biomolecular Faculty of Medicine Universitas Gadjah Mada. All clinical data of the patients were collected from medical records.

The clinical data collected were therapeutic response to PDT, recurrence (if existed), also an analysis for 5 years life expectancy was performed. Determination of the therapeutic response in NPC patients after PDT was performed in the same manner as NPC patients who received chemoradiation, which includes complete ear, nose, throat, head, and neck physical examination, nasopharynx endoscopy, nasopharynx CT-Scan, and nasopharynx biopsy guided by endoscopy. Distant metastasis was excluded by lung radiologic examination and abdominal USG. These data were recorded in patients’ medical records. Examination of Interleukin-12, Interleukin-18, Interleukin-2, and Interferon Gamma in qualified serum samples were performed by using ELISA method.

As comparisons are the NPC patients who had gone through radiation or chemoradiation and were undergoing residue or recurrence but did not receive PDT. The clinical data of these patients were also recorded and analyzed for life expectancy. Statistical analysis was executed using SPSS program. The characteristic of the subjects were presented in a characteristic table. Results reported include therapeutic response in the form of nasopharynx appearance in biopsy. Five year life expectancy as an
overall survival rate was analyzed, along with prognostic factors of PDT on NPC patients by comparing the life expectancy based on prognostic factors which were gender, age, biopsy results, and NPC stages.

Paired t-test was applied for comparative study for numeric non-parametris variables, Wilcoxon test was applied to analyze the mean difference between the cytokine levels; IL-12, IL-18, IL-2 and IFNγ before and after PDT, follow up 1 and follow up 2. This research had already gained permission from Ethical Committee Faculty of Medicine Universitas Gadjah Mada by firstly submitting a proposal and presentation in the presence of Ethical Committee evaluation team.

Results

The research was carried out on residual or recurrent nasopharyngeal carcinoma patients who had received PDT. As much as 31 patients fulfilled the inclusion and exclusion criteria; therefore were registered as subjects of this research.

Life sustainability of residual or recurrent NPC patients (Table 1) & (Figure 1)

Table 1: Description characteristics of research subjects.

| Variables                  | PDT (n=31) | Non PDT (n=53) | p   |
|----------------------------|------------|----------------|-----|
| Variables                  | Amount     | %             | Amount | %     |     |
| Gender                     |            |               |       |       |     |
| Male                       | 18         | 58,1          | 41     | 77,4  | 0.062|
| Female                     | 13         | 41,9          | 12     | 22,6  |     |
| Age                        |            |               |       |       | 0.125|
| <40 years old              | 9          | 29,0          | 8      | 15,1  |     |
| ≥40 years old              | 22         | 71,0          | 45     | 84,9  |     |
| Stages on First Diagnosis  |            |               |       |       | 0.399|
| Stadium Awal               | 10         | 32,3          | 22     | 41,5  |     |
| Stadium Lanjut             | 21         | 67,7          | 31     | 58,5  |     |
| Treatment Before Receiving PDT |       |               |       |       | <0.001|
| RT                        | 10         | 32,3          | 1      | 1,9   |     |
| CRT                        | 21         | 67,7          | 52     | 98,1  |     |
| Biopsy Result Post PDT     |            |               |       |       |     |
| Tumor (+)                  | 5          | 16,1          |       |       |     |
| Tumor (-)                  | 26         | 83,9          |       |       |     |

Five years life expectancy of residual or recurrent nasopharyngeal carcinoma patients who received PDT is 60,7%.

Nasopharyngeal carcinoma patients diagnosed between the year of 2009-2013 who had been undergoing residual or recurrent but did not receive PDT showed 5 years life expectancy or 5 years overall survival rate of 22,9% with median of 23,6 months. There’s a significant difference in life expectancy whether it’s clinically or statistically between residual or recurrent NPC patients with PDT compared to without PDT (p=0,004) (Figure 2).

The analysis of prognostic factors affecting residual or recurrent NPC patients with PDT was performed, the factors analyzed were gender, age, nasopharynx biopsy result post PDT, also the clinical stages when the diagnosis of NPC was first made. The evaluation of five years life expectancy was based on those prognostic factors. Based on gender, it was found that the five years life expectancy of male patients are higher (73, 5%) compared to females (42, 2%) where this difference is statistically mattered (p=0.045). Mean overall survival on males is 72%, whereas female patients is 45, 257%, it can be concluded that male patients have higher five years life expectancy than female patients (Figure 3).
In this research, the analysis of 5 years life expectancy based on age showed that patients ≤40 years old have higher life expectancy (76, 2%) compared to those who are >40 years old (53%), even though it is not statistically significant (p=0.417). Younger age NPC patients (≤40 years old) commonly have better health status, therefore 5 years life expectancy are higher compared to those who are over 40 years old. Mean overall survival on patients age ≤40 years old are 68,381, whereas patients >40 years old are 57,591. Although it is not statistically significant, it can be concluded that residual or recurrent NPC patients who were ≤40 years old have higher life expectancy (Figure 4).

The nasopharynx biopsy result post PDT showed 26 out of 31 (83.9%) patients are negative, there are no malignancy in nasopharynx cavity, while 5 (16.1%) patients still have positive result, some malignancy are still found in nasopharynx cavity. Local response rate 83.9% proves that photodynamic therapy is an effective therapy to control local tumors in residual or recurrent NPC patients (Figure 5).

In analysis of life expectancy based on nasopharynx biopsy result post PDT, it was found that some patients who still have tumors in nasopharynx have higher life expectancy (80%) compared to patients who have negative biopsy result (57.8%). This result is not statistically significant (p=0.638). These results possibly caused by the amount of positive result patients are only 5 compared to negative result patient, which are 26. Mean overall survival on negative result patients is 59,637 meanwhile patients with positive result are 50,400 (Figure 6).

In analysis of life expectancy based on stages on firstly diagnosed post PDT, it was found that patient with early stage have lower life expectancy (53, 3%) compared to late stages (62, 1%) even so it is not statistically significant (p=0.521) (Figure 7).
The analysis of factors affecting life ability of NPC patients who receive PDT, which are gender, age, biopsy result, and clinical stages, using cox-regression analysis with backward method. In this method, all prognostic factors were entered into multivariate analysis. Periodically, variable that had no effect will be cut off of the analysis. The process will stop if there’s no more variable being cut off of the analysis. Gender was found to be the factor that remained still in the analysis. Male gender as prognostic factor on life ability of the NPC patient post PDT even though it is not statistically significant with OR 0.280 (p=0.073) (Table 2).

Changes in cytokines’ level

In this research, after the sample serum collection was done which had been stored in Laboratory of Bimolecular Faculty of Medicine Gadjah Mada University, some samples were found to be unqualified for analysis. This was caused by the lack of volume of the stored samples; therefore the examination of cytokine level could not be done. Some other samples are found to have incomplete clinical data of patients and also be unqualified for analysis. The final amounts of qualified samples were 14 patients. Ten out of fourteen patients were males (71.43%) and the rest were females. Most patients are >40 years old (71.43%) with youngest of 18 years old, and the oldest of 63 years old. The average age for patients who received PDT was 43 years old. Most patients (85.71%) were on late stages when they were first diagnosed (Table 3).

Table 2: Cox Regression Analysis on PDT Prognostic Factors.

| Step    | B       | SE       | Wald | df | Sig. | Exp(B) 95.0% CI for Exp(B) |
|---------|---------|----------|------|----|------|-----------------------------|
| Step 1  |         |          |      |    |      |                             |
| Gender  | -1541   | 0.863    | 3.190| 1  | 0.074| 0.214 [0.039, 1.162]        |
| PA Results | 0.243  | 1.171    | 0.043| 1  | 0.835| 1.275 [0.129, 12.651]       |
| Age     | -0.836  | 0.829    | 1.017| 1  | 0.313| 0.434 [0.085, 2.201]        |
| Stages  | 0.328   | 0.763    | 0.185| 1  | 0.667| 1.389 [0.311, 6.200]        |
| Step 2  |         |          |      |    |      |                             |
| Gender  | -1.472  | 0.789    | 3.483| 1  | 0.062| 0.229 [0.049, 1.077]        |
| Age     | -0.811  | 0.820    | 0.979| 1  | 0.323| 0.445 [0.089, 2.216]        |
| Stages  | 0.296   | 0.750    | 0.155| 1  | 0.694| 1.344 [0.309, 5.851]        |
| Step 3  |         |          |      |    |      |                             |
| Gender  | -1.340  | 0.714    | 3.524| 1  | 0.060| 0.262 [0.065, 1.061]        |
| Age     | -0.754  | 0.806    | 0.874| 1  | 0.350| 0.471 [0.097, 2.285]        |
| Step 4  |         |          |      |    |      |                             |
| Gender  | -1.272  | 0.710    | 3.207| 1  | 0.073| 0.280 [0.070, 1.128]        |

In pathology anatomy examination of nasopharynx biopsy post PDT, most patients (85.71%) had no tumor (Figure 8).

Figure 7: Kaplan Meier graphic of 5 years life expectancy on NPC patients post PDT based on stages.

Figure 8: Pie Graphic of Pathology Anatomy results post PDT.

Pre PDT samples were taken 3 days before PDT administration; post PDT samples were taken 90 days after PDT. Follow up 1 sample were taken 181 days after PDT, and follow up 2 samples were taken 267 days after PDT. All of these samples were recorded and analyzed. The interleukin level was defined in pg/ml.

Interleukin-12 levels

In interleukin-12 examination, the mean value of interleukin-12 levels before PDT was 16,857 pg/ml. There was an elevation of IL-12 level post PDT and in follow up 1 and 2 (Table 4).

The graphic below describes changes in IL-12 levels in NPC patients who received PDT. The elevation can be observed in post PDT, follow up 1 and follow up 2 periods (Figure 9 & 10).

In pre post PDT analysis IL-12 levels post PDT compared to pre PDT, there was an elevation of 4,214 (24.9%) even though it’s clinically significant but statistically not significant (p=0.531) (Table 5) (Figure 11).

Pre and post analysis of Interleukin-12 level in follow up 1 period compared to pre PDT showed an elevation of 10,071 (59.7%), this elevation is not statistically significant (p=0.283). But, this nearly 60% elevation is clinically significant (Table 6).
Changes in Interleukin-12 levels in NPC patients post PDT was also found in follow-up 2 period. There was an elevation of 10,571 (62.7%) showed in follow-up 2 period compared to pre PDT. This result is clinically significant (Figure 12).

Pre and post analysis of Interleukin-12 level in follow up 2 period compared to pre PDT showed an elevation of 10,571 (62.7%), this elevation is not statistically significant (p=0.200) (Table 7).

Table 3: Characteristics of the Subjects (n=14).

| Variables               | Amounts | %     |
|-------------------------|---------|-------|
| Gender                  |         |       |
| Male                    | 10      | 71.43 |
| Female                  | 4       | 28.57 |
| Age (year)              |         |       |
| <40 years old           | 4       | 28.57 |
| ≥40 years old           | 10      | 71.43 |
| Minimum                 | 18      |       |
| Maximum                 | 63      |       |
| Average                 | 43      |       |
| Stages on First Diagnosis|         |       |
| Early Stage             | 2       | 14.29 |
| Late Stage              | 12      | 85.71 |
| PA Results Post PDT     |         |       |
| Tumor (+)               | 2       | 14.29 |
| Tumor (-)               | 12      | 85.71 |

Table 4: Average Level of Interleukin-12 pre and post PDT (pg/ml).

| IL-12 | Mean  | SD    |
|-------|-------|-------|
| Pre PDT | 16.857 | 24.156 |
| Post PDT | 21.071 | 32.226 |
| Follow-up 1 | 26.929 | 35.343 |
| Follow-up 2 | 27.429 | 30.744 |

Table 5: Paired T-test result on Interleukin-12 level.

| IL-12 Pre PDT | Mean | Difference (s.b) | CI95%        | P Value |
|---------------|------|------------------|--------------|---------|
| IL-12 Post PDT | 21.071 | 4,214 (24,502) | -9.933 - 18.361 | 0.531   |
Table 6: Paired T-test result of Interleukin-12 level (n=14).

|       | Average (s.d) | Difference (s.b) | CI95% | P Value |
|-------|---------------|------------------|-------|---------|
| IL-12 pre PDT | 16,857 (33,628) | 10,071 (33,628) | -9,345 |         |
| IL-12 Follow-up 1 | 26,929 (33,628) | -29,488 (33,628) | 0,283 |         |

Table 7: Paired T-test result of Interleukin-12 level (n=14).

|       | Average | Difference (s.b) | CI95% | P Value |
|-------|---------|------------------|-------|---------|
| IL-12 Pre PDT | 16,857 | 10,571 (29,330) | -6,363 -27,506 | 0,200 |
| IL-12 Follow-up 2 | 27,429 | -29,488 (29,330) | 0,200 |         |

Interleukin-2 level

Interleukin-2 level in NPC patients who receives PDT also higher in follow-up 1 period compared to pre PDT. The level difference found was 3,686 (1, 9%) (Figure 13).

Pre and post analysis of Interleukin-2 level in follow up 1 period compared to pre PDT showed an elevation of 3,686 (1, 9%), this elevation is not statistically significant (p=0, 380) (Table 8).

Table 8: Paired T-test result of Interleukin-2 level (n=14).

|       | Average | Difference (s.b) | CI95% | P Value |
|-------|---------|------------------|-------|---------|
| IL-2 pre PDT | 188,110 | 3,686 (15,164) | 5,069 -12,441 | 0,380 |
| IL-2 Follow-up 1 | 191,796 | -9,153 (18,049) | 1,268 -19,575 | 0,080 |

The Interleukin-2 level in follow-up 2 periods was found to be 9,153 lower than pre PDT (Figure 14).

Pre and post analysis of Interleukin-2 level in follow up 2 period compared to pre PDT showed a difference, even though statistically this difference is not significant (p=0, 080) (Table 9).

Table 9: Paired T-test result of Interleukin-2 level (n=14).

|       | Average | Difference (s.b) | CI95% | P Value |
|-------|---------|------------------|-------|---------|
| IL-18 | Mean | S.D          |       |         |
| Pre PDT | 258,500 | 57,173 |     |         |
| Post PDT | 277,071 | 43,120 |     |         |
| Follow-up 1 | 244,571 | 53,284 |     |         |
| Follow-up 2 | 226,714 | 54,934 |     |         |
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The graphic above showed changes in IL-18 level pre PDT, post PDT, follow-up 1 and follow-up 2 period. An elevation can be observed in post PDT compared to pre PDT. The IL-18 level then declined in follow-up 1 and follow-up 2 periods. Analysis on IL-18 level showed elevation of 18,571 (7.1%) in post PDT compared to pre PDT (Figure 16) (Table 11).

Pre and post analysis of Interleukin-18 level in post PDT compared to pre PDT showed an elevation even though it is not statistically significant (p=0.086) (Figure 17) (Table 11).

### Table 11: Paired T-test result of Interleukin-18 level (n=14).

|        | Average | Difference (s.b) | CI95%          | P value |
|--------|---------|------------------|----------------|---------|
| IL-18 Pre PDT | 258,500  | 18,571 (37,439)  | -3,045 - 40,188 | 0.086   |
| IL-18 Post PDT | 277,071  |                 |                |         |

Pre and post analysis of Interleukin-18 level in post PDT compared to pre PDT showed an elevation even though it is not statistically significant (p=0.086) (Figure 17).

IFNγ (Interferon Gamma) levels on NPC Patients who received PDT

The average level of IFNγ in NPC patients pre PDT are 45,643 pg/ml. There was a devaluation of IFNγ level in NPC patients post PDT also in follow-up 1 and follow-up 2 patients. Graphic below showed IFNγ level in NPC patient pre PDT, post PDT, follow-up 1 and follow-up 2 periods (Table 14).

### Table 14: Average Level of IFNγ pre and post PDT (pg/ml).

| IFNγ   | Mean       | S.D       |
|--------|------------|-----------|
| Pre PDT | 45,643     | 33,410    |
| Post PDT | 34,571     | 25,779    |
| Follow-up 1 | 21,643  | 22,697    |
| Follow-up 2 | 14,714   | 19,839    |

Pre and post analysis of Interleukin-18 level in follow-up 1 period compared to pre PDT showed a difference even though it is not statistically significant (p=0.140). The Interleukin-18 level in follow-up 2 period were found to be 31,786 (12, 3%) lower compared to pre PDT (Figure 18).

### Table 13: Paired T-test result of Interleukin-18 level (n=14).

|        | Average | Difference (s.b) | CI95%          | P Value |
|--------|---------|------------------|----------------|---------|
| IL-18 Pre PDT | 258,500  | -31,786 (63,899) | -68,680 - 5,109 | 0.085   |
| IL-18 Follow-up 2 | 226,714  |                 |                |         |

Pre and post analysis of Interleukin-18 level in follow-up 2 period compared to pre PDT showed a difference even though it is not statistically significant (p=0, 085) (Table 13).

### Table 14: Average Level of IFNγ pre and post PDT (pg/ml).

| IFNγ   | Mean       | S.D       |
|--------|------------|-----------|
| Pre PDT | 45,643     | 33,410    |
| Post PDT | 34,571     | 25,779    |
| Follow-up 1 | 21,643  | 22,697    |
| Follow-up 2 | 14,714   | 19,839    |

Graphic below showed the changes of Interferon Gamma level in NPC patient pre PDT, post PDT, follow-up 1 and follow-up 2 periods. There was a linear devaluation of interferon gamma level in residual and recurrent NPC patients after PDT (Figure 19).

The interferon gamma levels in NPC patient’s pre PDT were 45,643. There was a devaluation of level post PDT as much as 11,071 or 24, 3% compared to pre PDT. This devaluation is clinically significant (Figure 20).

Pre and post analysis of interferon gamma level in post PDT compared to pre PDT showed a devaluation of 11,071 or 24, 3%,
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Pre and post analysis of interferon gamma level in follow-up 2 period compared to pre PDT showed a devaluation of 30,929 or 67.76% which is statistically significant (p=0.001) (Table 17).

Table 15: Paired T-test result of IFNγ level (n=14).

|                | Average | Difference (s.b) | CI95%        | P Value |
|----------------|---------|------------------|--------------|---------|
| IFN Gamma Pre PDT | 45.643  | -11.071 (16.453) | 1.572 - 20.571 | 0.026   |
| IFN Gamma Post PDT | 34.571  |                 |              |         |

Table 16: Paired T-test result of IFNγ level (n=14).

|                | Average | Difference (s.b) | CI95%        | P Value |
|----------------|---------|------------------|--------------|---------|
| IFN gamma pre PDT | 45.643  | -24.00 (26.349)  | 8.786 - 39.314 | 0.005   |
| IFN gamma Follow-up 1 | 21.643  |                 |              |         |

Table 17: Paired T-test result of IFNγ level (n=14).

|                | Average | Difference (s.b) | CI95%        | P Value |
|----------------|---------|------------------|--------------|---------|
| IFN gamma pre PDT | 45.643  | -30.929 (25.527) | 16.189 - 45.667 | 0.001   |
| IFN gamma Follow-up 1 | 14.714  |                 |              |         |

Discussion

This research revealed that the residual or recurrent NPC patients who received PDT have higher life expectancy (60.7%) compared to those who did not received PDT (22.9%). It is important to note that the group of residual/recurrent NPC patients who received PDT had gone through a selection to fulfill the inclusion criteria for PDT, in which the tumor size in this group is less than one centimeter (<1cm) and if there is a lymph node metastasis. Therefore the clinical characteristics between PDT group and no PDT group might be slightly different. This difference could possibly affect the result of 5 years life expectancy analysis on both groups. Even so, this research is managed to
show that PDT could increase 5 years life expectancy in residual or recurrent NPC patients.

The analysis result of prognostic factors affecting PDT success outcome showed that males have higher life expectancy (73, 5%) compared to the females (42, 2%). Residual or recurrent NPC patients who received PDT who are 40 years old or younger (≤40 years old) has higher life expectancy (76, 2%) compared to those who are older than 40 years old (53, 0%). From multivariate analysis on prognostic factors, it was discovered that male gender is one of the factors that takes effect in longer life expectancy of residual and recurrent NPC patients who received PDT.

There were elevation in cytokines' levels; IL-12, IL-2, and IL-18, 90 days after PDT was administered in residual or recurrent NPC patients. Interleukin-12, which is a T-cell stimulating factor, was reported to be linearly raised after PDT administration until day 267 post administration. Interleukin-2 and interleukin-18 levels seemed to be declined after previously elevated on day 90th after PDT administration. IFNγ levels were found to come down in a linear pattern after administration of PDT until day 267th post PDT.

This research has a novelty aspect where it proves that PDT could enhance the life expectancy of residual or recurrent NPC patients, which is also attributed to the elevation of immune response proteins. This research also reports the pattern of immune response protein levels in period of 90 to 267 days after PDT administration. Proven that 5 years life expectancy of NPC patients who received PDT are higher than those who did not received PDT. A retrospective cohort method applied in this research provides an advantage in which the immune response proteins' level could be observed from time to time. The weakness of this research is the execution of immune response proteins' level measurement, which was done after the samples had been stored for quite a long time in laboratory. Also, the absence of complete clinical data collection for NPC patients who received PDT causing the inability to analyze the clinical impact of the elevation or devaluation of immune response protein levels to the clinical manifestation of the patients.

This research completes previous other research done by Mroz et al. [10] reported that PDT which brought up a great inflammatory response were able to activate steady state DC that tolerates into immunogenic activated DC. The activation was marked by the elevation of MHC and costimulator molecules, or cytokine products level such as IL-12, IL-18, and interferon [10].

Interleukin-12 is a cytokine produce by human dendrite cells, macrophages and lymphoblastoid T-cells. Interleukin-12 takes place in naïve T-cell differentiation into Th1 lymphocytes so then this cytokine is called as T-cell stimulating factor. Interleukin-12 also stimulates the production of IFNγ and Tumor Necrosing Factor Alpha (TNFα) by T-cells and NK cells. Interleukin-12 also plays an important role in T-cells and NK cells activity by increasing the cytotoxic activity, also has antiangiogenic activity through the elevation of IFNγ production.

Interleukin-18 also called Interferon-γ Inducing Factor. Interleukin-18 is a part of superfamily IL-1 and was produce by macrophages and some other cells. IL-18 works by bonding with IL-18 receptors and along with IL-12 induce cellular immunity (cell-mediated immunity) and activate T-cell proliferation. It is mentioned that IL-18 is capable of activating immune response without the help of IL-12, also induce NK cells proliferation, where specifically IL-12 will up regulates Perforin Mediated Cytotoxicity on NK cells.

Interferon Gamma (IFNγ) is a cytokine produced by lymphocyte, by some researchers it is classified as lymphokine. IFNγ classified in type II interferon or immune interferon. IFNγ is a cytokine that’s crucial for natural and adaptive immunity against viral infection, intracellular bacterial infection, and as a control against tumor. IFNγ works as macrophage activators, its importance in immune system mostly based on its ability to inhibit viral proliferation directly, also in immunostimulation and immunomodulation effects [11].

This research is using retrospective cohort method where samples used for the immune response marker cytokines level measurement had been stored in laboratory for a relatively long period of time. Although the sample storage was done properly, most serum stored could not be measured for the lack of volume and the scattered sampling collection moments. As a consequence to this matter, only 14 samples out of 31 samples could be measured. This major limitation in serum samples is also one of the weaknesses of this research. It is suggested to do this research using prospective cohort method to collect more samples with less scattered sampling collection moments. Going forward, it is expected to be able to do a deeper and more specific research on systemic immune response of NPC patients who received PDT, also an analysis on clinical impact of the immune response level measurement needs to be done in the future.

Conclusions

The result of PDT on residual or recurrent NPC patients after radiotherapy of chemo radiation that was evaluated locally based on nasopharynx biopsy showed gratified result. This result is better compared to re-radiotherapy or even brachytherapy. After long period observation, it was reported that residual or recurrent NPC patients who receive PDT have 5 years life expectancy greater than those who did not receive PDT.

The analysis of prognostic factors in PDT that include gender, age, nasopharynx biopsy, and clinical stages were performed and revealed that male and age 40 years old and younger each took effect in life expectancy result. Elevation of immune response cytokines of IL-12, IL-2, and IL-18 were found in residual and recurrent NPC patients who receive PDT 90 days after the administration. Interleukin-12 was reported to elevate linearly after PDT administration until 267 days later. IL-2 and IL-18 were seemed to devalue after previously rising on 90 days after PDT administration. Interferon-γ levels also devaluated after PDT administration until 267 days later.

References

1. Lee AW, Ko MW, Foo W, Choi P, Tung Y, et al. (1998) Nasopharyngeal carcinoma-time lapse before diagnosis and treatment. Hong Kong Med J 4(2): 132-136.
2. Syafril A (1989) Epidemiologi tumor ganas telinga, hidung, dan tenggorokan. Dalam: diagnosis and penatalaksanaan. Jakarta: Balai Penerbit FKUI: 1-9.

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Clinical Study on Photodynamic Therapy (PDT) in Patients with Nasopharyngeal Carcinoma in Yogyakarta Review of Life Expectancy and Cytokine Level

3. Mulyarjo (2002) Diagnosis dan penatalaksanaan karsinoma nasofaringg. Dalam: Mulyarjo et al. (Eds.), Naskah lengkap pendidikan kedokteran berkelanjutan III ilmu penyakit THT-KL, Perkembangan terkini diagnosis dan penatalaksanaan tumor ganas THT-KL. Fakultas Kedokteran Unair; Surabaya, Indonesia, pp. 38-48.

4. Indrasari SR, Timmermans AJ, Wildeman MA, Herdini C, et al. (2012) Remarkable response to photodynamic therapy in residual T4N0M0 nasopharyngeal carcinoma: a case report. Photodiagnosis Photodyn Ther 9(4): 319-320.

5. Lofgren LA, Hallgren S, Nilsson E, Westerborn A, Nilsson C, et al. (1995) Photodynamic therapy for recurrent nasopharyngeal cancer. Arch Otolaryngol Head Neck Surg 121(9): 997-1002.

6. Tong MC, van Hasselt CA, Woo JK (1996) Preliminary results of photodynamic therapy for recurrent nasopharyngeal carcinoma. Eur Arch Otorhinolaryngol 253(3): 189-192.

7. Kramer EB, Verwanger T, Schnitzhofer G (1997) Photodynamically induced cell cycle and gene expression changes: precursors of apoptosis introduction? In: Berg K et al. (Eds.), in Photochemotherapy: Photodynamic therapy and other modalities III, Proc SPIE 3191: 96-106.

8. Koberlik M (2006) PDT-Associated host response and its role in the therapy outcome. Lasers Surg Med 38(5): 500-508.

9. Kabingu E, Oseroff AR, Wilding GE, Gollnick SO (2009) Enhanced systemic immune reactivity to a basal cell carcinoma associated antigen following photodynamic therapy. Clin Cancer Res 15(13): 4460-4466.

10. Mroz P, Hashmi JT, Huang YY, Lange N, Hamblin MR, et al. (2011) Stimulation of anti-tumor immunity by photodynamic therapy. Expert Rev Clin Immunol 7(1): 75-91.

11. Subowo (2014) Sitokin dalam respon imun. Dalam: Subowo. Imunobiologi. Edisi 3. Sagung Seto, Jakarta, pp. 157-200.