Difference of protein 53 expression based on radiation therapy response in cervical cancer

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Abstract. Cervical cancer is one of most common gynecological cancer in women and the leading cause of death in developing countries. An analytic study with the case-control design was conducted to determine the difference of p53 expression based on radiation therapy response in cervical cancer. The study was performed in Obstetric and Gynecology Department and Pathology Department of Adam Malik General Hospital Medan from January to February 2017. 15 paraffin blocks of acervical cancer patient with incomplete response were obtained as study samples, and 15 paraffin blocks of acervical cancer patient with complete response were obtained as control samples, The samples were collected by consecutive sampling. Immunohistochemical assessment of p53 expression was done to assess apoptosis count and radiation response. Data were analyzed using Kruskal-Wallis with confidence interval 83.5% and p<0.05 was considered statistically significant. The study found that an increase of p53 expression in samples with abundant apoptosis (≥5 apoptosis cells/5 HPF), p=0.033, and in incomplete response group, p=0.046. It means that p53 expression before radiation therapy can be used as an early marker for radiation therapy response in cervical cancer.

1. Introduction
Cervical cancer is one of the most common gynecological cancer and the leading cause of death in developing countries. The high number of death in cervical cancer patients is usually caused by the delay in management, where 70% patients were diagnosed with advanced stage. Cervical cancer staging is significantly related to its management and prognosis. The gold standard for cervical cancer staging is histopathological examination which also correlates to its potential for invasion and metastasis.[1]

p53 is a polypeptide which expressed or coded by p53 gene, with an important role in maintaining cell or genome integrity by transcription or translation process. The other name for p53 gene is tumor suppressor gene. p53 plays a role in damaged DNA by stopping cell cycle (G1 arrest) to perform DNA repair or apoptosis process.[2]

p53 is associated with radiosensitivity in radiation therapy. If p53 functions were impaired, the carcinogenesis process would leave unchecked. Mutated p53 will also lose its function as tumor suppressor in apoptosis process, impairing radiation response and inducing radioresistance. This study aimed to determine the difference of p53 expression based on cervical cancer staging, histology type, cell differentiation type, and apoptosis count.[3]
2. Materials and Methods

2.1. Location, time and population
This study was done in Obstetrics and Gynecology Department and Pathology Departement, Faculty of Medicine Adam Malik General Hospital Medan from January to February 2017. The inclusion criteria are an advanced stage of cervical cancer tissue paraffin block (stage IIB or more) which haven’t undergone radiation therapy, and complete medical record as well.

2.2. Data collection
Thirty paraffin block samples of cervical cancer were collected, 15 of which were cervical cancer patients’ with incomplete response (study samples), and the other 15 were cervical cancer patients’ with complete response (control samples).

2.3. Ethics
Ethical Committee of Medical Faculty, Universitas Sumatera Utara has approved this study. Informed consents were obtained from all test participants.

2.4. Data analysis
This study was an analytic study with a case-control design, and immunohistochemistry was performed to paraffin block of complete and incomplete response cervical cancer tissue. Study instrument was done with IRS score, interpreted by 0-1 (negative), 4-8 (moderately positive), 9-12 (high positive). Kruskal-Wallis test was done to analyze the difference between variables.

3. Results
From table 1, it was found that the subjects in incomplete response group were mostly at stage IIB cervical cancer (10 samples; 66.7%). From the histologic type, there was no difference between keratinizing and nonkeratinizing squamous cell carcinoma.

The most common differentiation type in incomplete response group was well differentiation type (66.7%).

| Table 1. Distribution of cervical cancer frequency based on subject characteristics. |
|-----------------------------------------------|
| Characteristic | Complete Response | Incomplete Response |
|----------------|-------------------|---------------------|
| Stage          |                   |                     |
| II B           | 7 (46.7)          | 10 (66.7)           |
| III B          | 8 (53.3)          | 5 (33.3)            |
| SCJ histologic type |             |                     |
| Keratinizing   | 8 (53.3)          | 7 (46.7)            |
| Non keratinizing | 7 (46.7)        | 8 (53.3)            |
| Differentiation |                   |                     |
| Well           | 10 (66.7)         | 10 (66.7)           |
| Moderately     | 4 (26.7)          | 2 (13.3)            |
| Poorly         | 1 (6.7)           | 3 (20.0)            |
| Apoptosis Count |                  |                     |
| Low            | 3 (20.0)          | 0 (0)               |
| Moderate       | 11 (73.3)         | 3 (20.0)            |
| High           | 1 (6.7)           | 12 (80.0)           |
| p53 Expression |                   |                     |
| Negative       | 1 (6.7)           | 0 (0)               |
| Weak           | 6 (40.0)          | 3 (20.0)            |
| Moderate       | 8 (53.3)          | 6 (40.0)            |
Table 2 shows that there was no significant difference in p53 expression between the two staging groups (p=0.747).

Table 2. The difference of p53 expression based on cervical carcinoma staging.

| p53 expression | Stage | IIB n (%) | IIB n (%) | p* |
|----------------|-------|-----------|-----------|----|
| Negative       |       | 1 (5.9)   | 0 (0)     | 0.747 |
| Weak           |       | 5 (29.4)  | 4 (30.8)  |    |
| Moderate       |       | 7 (41.2)  | 7 (53.8)  |    |
| Strong         |       | 4 (23.5)  | 2 (15.4)  |    |

Kruskal-Wallis test

Table 3 shows that there was no significant difference in p53 expression in squamous cell carcinoma using statistic test, with p-value>0.05 (p=0.574).

Table 3. The difference of p53 expression based on SCJ histologic type of cervical cancer.

| p53 expression | SCJ Histologic Type | SCJ n (%) | NSCJ n (%) | p* |
|----------------|---------------------|-----------|------------|----|
| Negative       |                     | 0 (0)     | 1 (6.7)    |    |
| Weak           |                     | 5 (33.3)  | 4 (26.7)   | 0.547 |
| Moderate       |                     | 6 (40.0)  | 8 (53.3)   |    |
| Strong         |                     | 4 (26.7)  | 2 (13.3)   |    |

Kruskal-Wallis test

From table 4, we can conclude that there is no significant difference in ap53 expression based on cell differentiation type using statistic test, with p-value>0.05 (p=0.837).

Table 4. The difference of p53 expression based on cervical cancer differentiation type.

| p53 expression | Differentiation Type | Well n (%) | Moderately n (%) | Poorly n (%) | p* |
|----------------|----------------------|------------|------------------|--------------|----|
| Negative       |                      | 1 (5.0)    | 0 (0)            | 0 (0)        |    |
| Weak           |                      | 5 (25.0)   | 3 (50.0)         | 1 (25.0)     |    |
| Moderate       |                      | 10 (50.0)  | 2 (33.3)         | 2 (50.0)     |    |
| Strong         |                      | 4 (20.0)   | 1 (16.7)         | 1 (25.0)     | 0.837 |

Kruskal-Wallis test

From the table 5 shown below, it was concluded that there was a significant difference in apoptosis count based on radiation response using statistic test with p-value<0.05 (p=0.000).

Table 5. The difference in cervical cancer apoptosis count based on radiation therapy response.

| Apoptosis count | Radiation response | Complete response n (%) | Incomplete response n (%) | p* |
|-----------------|--------------------|-------------------------|---------------------------|----|
| Low             |                    | 3 (20.0)                | 0 (0)                     |    |
| Moderate        |                    | 11 (73.3)               | 3 (20.0)                  | 0.000 |
| High            |                    | 1 (6.7)                 | 12 (80.0)                 |    |
Kruskal-Wallis test

From table 6 we can conclude that there was a significant difference to a p53 expression based on apoptosis count, with p<0.05 (p=0.033).

| p53 expression | Low n (%) | Moderate n (%) | High n (%) | p*  |
|----------------|-----------|----------------|------------|-----|
| Negative       | 0 (0)     | 1 (7.1)        | 0 (0)      |     |
| Weak           | 1 (33.3)  | 5 (35.7)       | 3 (23.1)   | 0.033 |
| Moderate       | 2 (66.7)  | 8 (57.1)       | 4 (30.8)   |     |
| Strong         | 0 (0)     | 0 (0)          | 6 (46.2)   |     |

Kruskal-Wallis test

From table 7, it is found that there was a significant difference in a p53 expression based on radiation therapy response, with p<0.05 (p=0.046).

| p53 expression | Complete n (%) | Incomplete n (%) | p*  |
|----------------|----------------|-----------------|-----|
| Negative       | 1 (6.7)        | 0 (0)           |     |
| Weak           | 6 (40.0)       | 3 (20.0)        | 0.046 |
| Moderate       | 8 (53.3)       | 6 (40.0)        |     |
| Strong         | 0 (0)          | 6 (40.0)        |     |

Kruskal-Wallis test

4. Discussion

In this study, most samples with incomplete radiation response were from stage IIB group; this finding is relevant with the previous study by Duenas et al. which stated that 70% of IIB cervical cancer patients showed a complete response.[4] Based on table 2, this study result is similar to the previous study by Deepali et al., A. Singh et al., Lars-Christian Horn et al. which stated that no difference in p53 expression based on cervical cancer stadium.[5,6,7] This finding may be the result of mutant p53 existence which is not correlated with its progressivity and subjective clinical staging. This study limitation is in the unmatched number of samples from stage II and III patients.

From table 3, relevant with the previous study from A. Singh et al., Giarneriet al, which stated that there is no difference in p53 expression based on cervical cancer histology.[6,8] This study only collected one type of histology (squamous cell carcinoma). From table 4, a different result was found compared to the previous study by Wootipoom et al., most common cell differentiation type is moderate.[13] This finding may be caused by the limitation of samples amount, which in turn also limits the variety of differentiation type in this study. Table 5 results showed a similar result with Gasinska et al., which stated that apoptosis count reflects the hypoxic condition in the tumor which resulted in impaired response to radiation therapy.[9]

From table 6, relevant to study by A. Singh et al., we found that there is a significant difference in p53 expression based on apoptosis count.[6] In malignancy, a mutation in p53 will impair its functions in suppressing tumor and apoptosis process. Dysregulation of this cellular signaling pathway is caused by the cellular transformation of the cancer cell. Mutated p53 will reduce apoptosis sensitivity to genotoxic condition such as radiation.

Table 7 result, showed that mutant p53 are associated with high recurrence.[2,3,10] In invitro study, p53 gene plays an important role in apoptosis process and post radiation tumor cell cycle, which in turn underline its significance in radiosensitivity.
5. Conclusion

p53 expression before radiation therapy can be used as an early marker of radiation therapy response in cervical cancer.

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