International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 03, 2020
ISSN: 1475-7192

Comparison of 8-Hydroxy-Deoxyguanosine Levels in Cervical Cancer Advanced Stages Before and After Chemotherapy

[1]Sardina, [2]Syahrul Rauf, [3]Rina Previana Amiruddin, [4]Siti Nur Asni, [5]Maisuri Tadjuddin Chaliq, [6]Sharvianty Arifuddin

ABSTRACT **---** Cervical cancer is the most commonly diagnosed cancer and the third leading cause of death of women in poor and developing countries. 8-hydroxy-deoxyguanosine (8-OHdG) levels have been widely used as a biomarker of oxidative DNA damage including cervical cancer. This study aims to compare levels of 8-hydroxy-deoxyguanosine (8-OHdG) as a marker of oxidative stress in advanced cervical cancer (FIGO stages) before and after chemotherapy. This prospective study involved 18 patients stage IIB, 8 patients stage IIIA, 9 patients of stage IIIB and 2 patients stage IIIC of cervical cancer. 8-OHdG levels were measured with the ELISA method. The mean level of 8-OHdG before chemotherapy in stage II was 8.14±9.14 ng/ml and stage III 8.07±8.79 ng/ml whereas the mean level of 8-OHdG after chemotherapy in stage II was 24.24±12.46 ng/ml and at stage III 24.67±13.85 ng/ml. 8-OHdG levels increased significantly (p<.05) in stage IIA, IIIA and IIIB after chemotherapy. In contrast, 8-OHdG levels in stage IIIC was not significantly increased after chemotherapy. In addition, 8-OHdG levels were significantly different between SCC and adenocarcinoma. Likewise, the type of differentiation is good, moderate and non-classification. 8-OHdG levels increase significantly in advanced stages of cervical cancer after chemotherapy.

Keywords **---** 8-hydroxy-deoxyguanosine, cervical cancer, advanced stages

I. INTRODUCTION

Cervical cancer is the most commonly diagnosed cancer and the third leading cause of death in women in poor and developing countries (1). The incidence and mortality of cervical cancer have declined in countries with well-developed screening programs (2). Scientific evidence from virological, molecular, clinical and epidemiological studies has identified Human Papillomavirus (HPV) as the main etiological agent in cervical cancer (3,4).

Studies on carcinogenesis show oxidative stress adversely affects cells and reactive oxygen species (ROS) has implications for cancer pathogenesis (5). Oxidative stress is changes in the pro-oxidant/antioxidant balance as a result of increased oxidative metabolism. Increased oxidative stress at the cellular level as a results of

[1] *Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, dinaromy1802@gmail.com*
[2] *Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, syahrulrauf@yahoo.com*
[3] *Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, rinaroem@gmail.com*
[4] *Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, st_nurasni@yahoo.com*
[5] *Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, maisurichalid@gmail.com*
[6] *Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, sharvianty@yahoo.com*

Received: 03 Dec 2019 | Revised: 21 Jan 2020 | Accepted: 18 Feb 2020

several factors including exposure to alcohol, drugs, trauma, extrime temperatures, infections, poor diet, toxins, radiation, or heavy physical activity (6). Previous study show high rates of oxidative damage observed in cervical intraepithelial neoplasia (CIN) and cervical carcinoma (7). Another study also show an increase in lipid peroxidation as an indicator of oxidative stress in patients with cervical squamous cell carcinoma (8).

The 8-hydroxydeoxyguanosine (8-OHdG) has been widely used as a biomarker of oxidative DNA damage (9,10). 8-OHdG residues can be removed from DNA by an enzymatic repair system so that these residues circulated and subsequent excretion in urine (11). Therefore, the levels of 8-OHdG in the blood and/or urine can be measured as a marker of oxidative DNA damage. Studies reported 8-OHdG levels increase in various types of human cancer (12,13,14,15). Previous studies on 8-OHdG levels in cervical cancer were performed on patient urine samples before chemotherapy (7,16,17,18). Therefore, the effects of chemotherapy on 8-OHdG levels remain unknown. In our study, we compare levels of 8-hydroxy-deoxyguanosine (8-OHdG) as a marker of oxidative stress in advanced cervical cancer (FIGO stages) before and after chemotherapy.

II. METHODOLOGY

Subjects

This prospective study was performed on cervical cancer patients at advanced stages (stage II and III FIGO) from November 2018 until November 2019 at Wahidin Sudirohusodo General Hospital in Makassar. Patients age 20–55 years, no history hysterectomy, had completed the first cycle of chemotherapy, never had chemoradiation before this study and without a history of local or systemic infection were eligible. Written informed consent was obtained from all of the patients.

Laboratory testing --- 8-OhdG levels measurement

Levels of 8-OhdG from the patient's serum were measured using the Human 8-hydroxy-deoxyguanosine ELISA kit (Cat. No. E1436Hu, Bioassay Technology Laboratory, Shanghai, China) and ELISA reader (Multiskan FC type 357, Thermo Scientific) according to the manufacturer's instructions. Level s of 8-OhdG were measured before chemotherapy and after completing the first cycle of the treatment.

Statistical analysis

Levels of 8-OhdG presented as mean±SD ng/ml. To compare the difference of 8-OhdG level before and after chemotherapy, Wilcoxon's test was used. Data were analyzed using SPSS version 25.0. A *p*-value <.05 considered statistically significant.

III. RESULTS

This prospective study was conducted on 37 cervical cancer patients. The number of patients with stage II (n = 18; 48.6%) was comparable to stage III (n = 19; 51.4%). During the study period, no samples were obtained with stage IV. As shown in the characteristics of this study (table 1), the mean age of patients was 40.65±6.6 years and multiparity. Histopathological examination results show the proportion of squamous cell carcinoma

(SCC) was higher (67.6%) compared with adenocarcinoma (32.4%). Moderate differentiation cells have the highest proportion compared with other types of cervical cancer cell differerntiation.

The levels of 8-OHdG before and after 8-OHdG are shown in table 2. The mean levels of 8-OHdG before chemotherapy at stage II were 8.14±9.14 ng/ml and stage III 8.07±8.79 ng/ml whereas the mean levels of 8-OHdG after chemotherapy at stage II 24.24±12.46 ng/ml and stage III 24.67±13.85 ng/ml. 8-OhdG levels were significantly increased (p<.05) in stage IIA, IIIA and IIIB. The mean levels of 8-OHdG in stage IIB was comparable with stage IIIA and IIIB whereas the lower levels found in stage IIIC before chemotherapy. 8-OHdG levels increase for all stages after chemotherapy. There was a significant difference (p<.05) increase in 8-OHdG levels between before and after chemotherapy regarding stages, histopathological and differentiation type except for stage IIIC and poor differentiation type (p>.05).

Table 1. Patient's characteristics

Characteristics	n(%)
Age (mean ± SD years)	40.65±6.60
Parity	
≤4	16(43.2%)
≥5	21(56.8%)
FIGO stages	
IIB	18(48.6%)
IIIA	8(21.6%)
IIIB	9(24.3%)
IIIC	2(5.4%)
Histopathology	
Squamous cell carcinoma (SCC)	25(67.6%)
Adenocarcinoma	12(32.4%)
Differentiation	
Good	10(27%)
Moderate	19(51.4%)
Poor	2(5.4%)
Non-classification	6(16.2%)

Table 2. Levels of 8-OHdG before and after chemotherapy

Characteristics	8-OHdG levels (mean±SD ng/mL)		*p*
	Before chemotherapy	After chemotherapy	
Stages			
IIB (n=18)	8.14±9.14	24.24±12.46	0.003
IIIA (n=8)	8.40±9.10	22.20±10.36	0.012
IIIB (n=9)	8.16±8.90	25.10±13.80	0.008

Received: 03 Dec 2019 | Revised: 21 Jan 2020 | Accepted: 18 Feb 2020

IIIC (n=2)	4.45±1.08	21.71±0.47	0.180
Histopathology			
Squamous cell carcinoma (n=25)	8.19±8.88	23.55±12.25	0.000
Adenocarcinoma (n=12)	8.25±9.01	25.38±13.89	0.003
Differentiation			
Good (n=10)	8,25±9.01	25,38±13,89	0,007
Moderate (n=19)	8,19±8.88	23,55±12,25	0,002
Poor (n=2)	11,48±12.52	19,62±2,62	0,180
Non-classification (n=6)	8.51±9.22	22,59±10,26	0,028

IV. DISCUSSION

Our study show 8-OHdG levels significantly differed according to FIGO stages, histological type, and cell differentiation type before and after chemotherapy. 8-OHdG levels can be measured by immunohistochemical methods such as the ELISA test or high-pressure liquid chromatography, spectrometry, or mass electrochemical detection (HPLC-MS/MS; HPLC-EC) in serum or urine samples (19).

The previous studies show 8-OHdG levels to increase in various types of cancer including cervical cancer (12,13,14,15,18,20). The high levels of oxidative stress in cancer cells might be due to oncogene activation, high metabolic activity, and mitochondrial damage. Decreased or increased levels of oxidative stress on chemotherapy indicate the oxidative stress levels of cancer cells affect the treatment response (21). The results from previous studies are also consistent with our results that show increased levels of 8-OHdG in advanced stages of cervical cancer.

Our study found 8-OHdG levels significantly regarding the FIGO advanced stages, histological, and cell differentiation before and after chemotherapy. Similar results were also reported from Jelic et al study. This study show 8-OHdG levels tend to increase with increasing stage of cervical cancer (18). Another study also observed a significant progressive increase in levels of 8-OHdG from LSIL to HSIL in invasive carcinomas that indicate changes in 8-OHdG levels in the early stages or early stages of cancer development can be used as predictors of high-risk patients (17). Previously, Romano et al. study reported higher levels of 8-OHdG in cervical cancer cells compared with normal cells (16). In contrast, another study that measured 8-OHdG levels in urine using the same method with our study shows no significant difference in 8-OHdG levels between CIN and SCC in the cervix compared with controls (7).

In our study, 8-OHdG levels increased significantly after chemotherapy. This indicates that chemotherapy increasing the levels of 8-OHdG. Higher levels of 8-OHdG could also indicate higher levels of cancer cell damage at an advanced stage. The effect of chemotherapy on 8-OhdG as a result of clinically cancer cells responds to the treatment. However, further effects of high levels of 8-OHdG such as changes in tumor size or the possibility of patients who can be operated after chemotherapy are not assessed in our study. Therefore, further studies can be considered for the relationship between 8-OHdG levels and chemotherapy regarding the clinical character of cervical cancer after chemotherapy. Also, the provision of antioxidants that can reduce ROS

levels and improve the development of cells after chemotherapy can be taken into consideration for further studies.

Alteration in redox balance through increased ROS levels or decreased cellular antioxidant capacity to induce high ROS production are a therapeutic benefit in cancer cells (23,24,25). Most chemotherapy agents such as platinum and taxane kill cancer cells through the induction of ROS production which triggers cell damage in cancer cells (25). Several studies have shown that chemoresistance is caused by low levels of oxidative stress and high antioxidant (21,26). High levels of oxidative stress in cervical cancer patients indicate high rates of cancer cell death. Therefore, oxidative stress levels of ROS could be used as markers for responses to chemotherapy and to determine appropriate chemotherapy agents based on its response to chemotherapy.

The discrepancies of our study with other studies due to discrepancies in the type of sample (serum, urine, cervical smear), measurement methods, the presence of comorbidities or complications with other diseases such as Alzheimer's (27,28,29). In addition, toxicokinetic variations and/or the ability to repair DNA damage between individuals can also cause differences in individual oxidative stress levels (30). Decreased levels of 8-OHdG are caused by increased antioxidants, regular exercise, and inhibition of glycolysis (31).

Increased levels of ROS in patients with cervical cancer can occur because of ROS induces the death of cancer cells or as a side effect of chemotherapy that induces the death of cancer cells. Anthracycline (doxorubicin, daunorubicin, epirubicin) as chemotherapy agents that produce the highest cellular ROS (32). Platinum complexes, alkylating agents, arsenic and topoisomerase inhibitors also induce high ROS production (29,32,33) whereas taxan, vinca alkaloids, analog nucleotides and antimetabolites (antifolates and nucleosides) produce lower ROS levels compared with other chemotherapy agents (25). The formation of ROS as an effect of chemotherapy with cisplatin is influenced by the concentration and duration of exposure to cisplatin. During acute treatment, cisplatin binds to the cell structure over a short time, making the cell more time to recover from primary and secondary damage. Conversely, cells exposed to cisplatin continuously during chronic treatment require additional time to recover from damage (34). The anticancer effect of cisplatin is obtained through immune system defects. ROS is needed as the platinum toxin in vivo which mostly derived from inflammatory tumor cells (35).

Increased levels of ROS during chemotherapy can occur through the mechanism of mitochondrial ROS formation and inhibition of cellular antioxidant systems. Inhibition of the antioxidant system involves low molecular weight antioxidants such as GSH and ascorbic acid as enzymes that reduce antioxidants and ROS that interact with enzymes such as SOD, peroxidase, and catalase (36). Cellular response to chemotherapy that induces ROS formation is determined by the type of ROS, location, duration, and levels of ROS (37). Prolonged exposure to chemotherapy that induces ROS is reported to induce resistance to chemotherapy agents (38). Doxorubicin chemotherapy agents that induce mitochondrial ROS, particularly H_2O_2 leads to cell death through apoptosis and autophagy of the cancer cells (32) while ROS induced by arsenic causes cancer cell death through the mechanism of necrosis and ferroptosis (39,40). Chemotherapy can also increase the genetic instability of cancer cells due to mutations caused by ROS (41). Therefore, the effect of ROS on heterogeneity and changes in cancer cells still needs further studies.

The limitation of our study is we are not assessing the effect of chemotherapy agents on 8-OHdG levels before and after chemotherapy because of the small size samples. In addition, this study also did not assess the effects of 8-OHdG levels on the chemotherapy response.

V. CONCLUSION

In conclusion, 8-OHdG levels increase significantly in advanced stages of cervical cancer after chemotherapy.

REFERENCES

1. Torre LA, Bray F, Siegel L, et al. Global Cancer Statistics 2012. Ca Cancer J Clin 2015;65:87–108.
2. Forman D, de Martel C, Lacey CJ, et al. Global burden of human papillomavirus and related diseases. Vaccine 2012;30:F12-F23.
3. Woodman CBJ, Collins SI, Young LS: The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer 2007;7:11-22.
4. zur Hausen H. Papillomaviruses in the causation of human cancers – a brief historical account. Virology 2009;384:260-5.
5. Klaunig JE, Kamendulis LM. The role of oxidative stres in carcinogenesis. Annu Rev Pharmacol Toxocol 2004;44:29–67.
6. Kolanjiappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin Chim Acta. 2002;326(1-2):143-9.
7. Looi ML, Mohd Dali AZ, Md Ali SA, et al. Oxidative damage and antioxidant status in patients with cervical intraepithelial neoplasia and carcinoma of the cervix. Eur J Cancer Prev. 2008;17(6):555-560.
8. Beevi SS, Rasheed MH, Geetha A. Evidence of oxidative and nitrosative stress in patients with cervical squamous cell carcinoma. Clin Chim Acta. 2007;375(1-2):119-23.
9. Gao CM, Takezaki T, Wu JZ, et al. Polymorphisms in thymidylate synthase and methylenetetrahydrofolate reductase genes and the susceptibility to esophageal and stomach cancer with smoking. Asian Pac J Cancer Prev. 2004;5(2):133-8.
10. Hwang ES, Bowen PE. DNA damage, a biomarker of carcinogenesis: Its measurement and modulation by diet and environment. Crit Rev Food Sci Nutr 2007;47:27–50.
11. Ock CY, Kim EH, Choi DJ, et al. 8-Hydroxydeoxyguanosine: not mere biomarker for oxidative stres, but remedy for oxidative stres-implicated gastrointestinal diseases. World J Gastroenterol. 2012;18(4):302-308.
12. Miyake H, Hara I, Kamidono S, Eto H. Oxidative DNA damage in patients with prostate cancer and its response to treatment. J Urol. 2004;171:1533–36.
13. Weiss JM, Goode EL, Ladiges WC, Ulrich CM. Polymorphic variation in hOGG1 and risk of cancer: A review of the functional and epidemiologic literature. Mol Carcinog 2005;42:127–141.
14. Diakowska D, Lewandowski A, Kopec W, et al. Oxidative DNA damage and total antioxidant status in serum of patients with esophageal squamous cell carcinoma. Hepatogastroenterology 2007;54:1701–4.

Received: 03 Dec 2019 | Revised: 21 Jan 2020 | Accepted: 18 Feb 2020

15. Tanaka H, Fujita N, Sugimoto R, et al. Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. Br J Cancer 2008;98:580–586.
16. Romano R, A Sgambato A, Mancini R, et al., 8-hydroxy-2'-deoxyguanosine in Cervical Cells: Correlation With Grade of Dysplasia and Human Papillomavirus Infection. Carcinogenesis 2000;21(6):1143-1147.
17. Sgambato A, Zannoni GF, Faraglia B, et al. Decreased expression of the CDK in hibitor p27Kip1 and increased oxidative DNA damage in the multistep process of cervical carcinogenesis. Gynecol Oncol 2004;92(3):776–83.
18. Jelić M, Mandić A, Kladar N, et al. Lipid peroxidation, antioxidative defense and level of 8-hydroxy-2-deoxyguanosine in cervical cancer patients. J Med Biochem. 2018;37(3):336-345.
19. Cooke MS, Loft S, Olinski R. Measurement and meaning of oxidatively modified DNA lesions in urine. CEBP 2008;17:3–14.
20. Pylväs-Eerola M, Karihtala P, Puistola U. Preoperative serum 8-hydroxydeoxyguanosine is associated with chemoresistance and is a powerful prognostic factor in endometrioid-type epithelial ovarian cancer. BMC Cancer. 2015;15:493.
21. Filippova M, Filippov V, Williams VM, et al. Cellular levels of oxidative stress affect the response of cervical cancer cells to chemotherapeutic agents. Biomed Res Int. 2014;2014:574659.
22. Liu J, Wang Z. Increased oxidative stress as a selective anticancer therapy. Oxidative Med Cell Longev. 2015;2015:294303.
23. Leone A, Roca MS, Ciardiello C, et al. Oxidative stress gene expression profile correlates with cancer patient poor prognosis: identification of crucial pathways might select novel therapeutic approaches. Oxidative Med Cell Longev. 2017;2017:2597581.
24. Postovit L, Widmann C, Huang P, Gibson SB. Harnessing oxidative stress as an innovative target for cancer therapy. Oxidative Med Cell Longev. 2018;2018:6135739.
25. Conklin KA. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integr Cancer Ther 2004; 3: 294-300.
26. Wang J, Lin D, Peng H, et al. Cancer-derived immunoglobulin G promotes LPS-induced proinflammatory cytokine production via binding to TLR4 in cervical cancer cells. Oncotarget 2014;5(20):9727–43.
27. Lovell MA, Gabbita P, Markesbery WR. Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. J Neurochem 1999;72:771–776.
28. Hata I, Kaji M, Hirano S, et al. Urinary oxidative stres markers in young patients with type 1 diabetes. Pediatr Int 2006;48:58–61.
29. Wong RH, Kuo CY, Hsu ML, et al. Increased levels of 8-hydroxy-20-deoxyguanosine attributable to carcinogenic metal exposure among school children. Environ Health Perspect 2005;113:1386–1390.
30. Irie M, Tamae K, Iwamoto-Tanaka N, Kasai H. Occupational and lifestyle factors and urinary 8-hydroxydeoxyguanosine. Cancer Sci. 2005;96:600–606.
31. Rodic S, Vincent DM, Reactive oxygen species (ROS) are key determinant of cancer's metabolic phenotype. IJC. 2018 :440-448.
32. Mizutani H, Tada-Oikawa S, Hiraku Y, Kojima M, Kawanishi S. Mechanism of apoptosis induced by doxorubicin through the generation of hydrogen peroxide. Life Sci. 2005;76:1439–53.

33. Shi H, Shi X, Liu KJ. Oxidative mechanism of arsenic toxicity and carcinogenesis. Mol Cell Biochem. 2004;255:67–78.
34. Brozovic A, Ambriović-Ristov A, Osmak M. The relationship between cisplatin-induced reactive oxygen species, glutathione, and BCL-2 and resistance to cisplatin. Crit Rev Toxicol. 2010;40(4):347–359.
35. Raudenska M, Balvan J, Fojtu M, et al. Unexpected therapeutic effects of cisplatin. Metallomics. 2019;11(7):1182–1199.
36. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. 2004;55:373–99.
37. Yang H, Villani RM, Wang H, et al. The role of cellular reactive oxygen species in cancer chemotherapy. J Exp Clin Cancer Res. 2018;37(1):266.
38. Maiti AK. Gene network analysis of oxidative stress-mediated drug sensitivity in resistant ovarian carcinoma cells. Pharmacogenomics J. 2010;10:94–104.
39. Chou WC, Jie C, Kenedy AA, et al. Role of NADPH oxidase in arsenic-induced reactive oxygen species formation and cytotoxicity in myeloid leukemia cells. Proc Natl Acad Sci U S A. 2004;101:4578–83.
40. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an irondependent form of nonapoptotic cell death. Cell. 2012;149:1060–72.
41. Sallmyr A, Fan J, Datta K, et al. Internal tandem duplication of FLT3 (FLT3/ITD) induces increased ROS production, DNA damage, and misrepair: implications for poor prognosis in AML. Blood. 2008;111:3173–82.

Received: 03 Dec 2019 | Revised: 21 Jan 2020 | Accepted: 18 Feb 2020