Abstract. Ionizing radiation can damage deoxyribonucleic acid (DNA) by the production of reactive oxygen species. Through the DNA repair mechanism, one of the products from oxidative damage, 8-hydroxy-2'-deoxyguanosine (8-OHdG), is generally removed from DNA and excreted in urine. In this work, urinary 8-OHdG levels in 50 cancer patients were studied. A competitive immunoassay kit manufactured by Japan Institute for the Control of Aging was used to measure urinary 8-OHdG. Our data showed that levels of 8-OHdG in patients undergoing radiotherapy had elevated urinary 8-OHdG compared to those without radiotherapy. We also found no linear-correlation between urinary 8-OHdG and the accumulated radiation dose. In order to investigate how the radiation exposure is associated with the oxidative profile in the whole body, consecutive urine samples from a patient were measured. Reference range of urinary 8-OHdG for this patient was measured as 8.3 ± 0.5 ng/mg. Concentration of 8-OHdG averaged 10.3 ± 3.8 ng/mg creatinine in urines sampled during the radiotherapy process. The level of urinary 8-OHdG exhibited a declining trend through radio-therapeutic course and was back to the reference level even before the treatment was finished. Because the levels of urinary 8-OHdG varied from 0.6 to 2 times of the normal range during therapy, serial sampling through the whole therapeutic course was recommended to monitor the oxidative stress profile in patient.

1. Introduction
Ionizing radiation can directly induce ionizations in DNA molecule and cause DNA strand break. However, the majority of damage from low-LET radiation occurs in an indirect manner via the formation of free radicals, such as OH radicals, which lead to many DNA lesions, including oxidative DNA base production. Deoxyguanosine (dG) is one of the constituents of DNA and when it is oxidized, it may alter into 8-hydroxy-2'-deoxyguanosine (8-OHdG). Through the base-excision repair pathway, the oxidized nucleosides and bases are generally excreted into urine. Urinary excretion of 8-OH-dG has been described as a sensitive marker to evaluate oxidative DNA modification [1]. Cancer patients may be exposed to large amount of ionizing radiation for therapeutic purpose. Increases of 8-OHdG in the DNA of peripheral blood leukocytes of patients exposed to therapeutic doses of ionizing radiation were confirmed in several studies [2,3]. It was also reported that elevation of urinary 8-OHdG was observed after total cumulative doses of 10 Gy and the levels related to the response of treatment in non-small-cell carcinoma patients during the course of radiotherapy [4]. Either for assessing oxidative damage from radiation or to evaluate the response of treatment, urinary 8-OHdG should be a useful non-invasive marker to monitor the therapeutic process. Due to the radiation exposure as well as a number of diseases...
will affect the level of urinary 8-OHdG, it is necessary to clarify the relationship between exposure dose and urinary 8-OHdG. Random urine samples were collected from a variety of cancer patients and the concentrations of urinary 8-OHdG were related to the accumulate exposure doses. Variation of urinary 8-OHdG through the treatment course was also observed by measuring serial collected samples taken from one patient.

Analytical approaches, such as gas chromatography-mass spectrometry and high-performance liquid chromatography with electrochemical detection have been shown to be effective to quantify 8-OHdG [5,6]. After a monoclonal antibody specific for 8-OHdG was developed and an enzyme-linked immunosorbent assay (ELISA) kit was constructed [7], measurement of urinary 8-OHdG becomes much less tedious and no extraction step is required. A commercial ELISA system was utilized in this study for it is easier than HPLC-based method to carry out in a clinical laboratory.

2. Materials and methods

2.1. Urine sample collection

Fifty patients with different stages of cancer were included in this study. Thirteen of those patients were diagnosed as breast cancer, 14 of them were gastrointestinal cancer, and the others were hepatoma, prostate cancer, lung cancer, cervix cancer or lymphoma. Twenty-five of those patients were under radiotherapy when sampling. Prescribed dosage were delivered by high energy (6-10 MeV) x-ray from 180 to 300 cGy per day confined to the tumor volume and the calculated accumulation doses were varied from 400 to 8000 cGy when urine was sampled. The average age of those patients was 58 years with a standard deviation of 11 years.

Urine samples from one breast cancer patient during the whole radio-therapeutic treatment were collected once a day and referred as serial samples. Radiation was delivered to the chest part of this patient by x-ray at the dose of 180cGy per day, 5 days a week in the first 6 weeks. Dosage increased to 300cGy/day, but confined to a smaller area in the last week during the therapeutic process. Total accumulated dose was 7040 cGy for this patient.

Control samples were obtained from people who came to hospital for physical checkup. The average age of those people was 42±5 years.

After collection, urine samples were kept frozen at -20°C until analyzed.

2.2. Measurement of 8-hydroxy-deoxyguanosine (8-OHdG)

Urine samples were centrifuged at 2,000 rpm for 10 min and the supernatants were used for assay. Concentration of 8-OHdG in each urine sample was determined by using a competitive enzyme-linked immunosorbent assay kit (New 8-OHdG Check, Japan Institute for the Control of Aging, Fukuroi, Shizuoka). The monoclonal antibody in this assay kit recognizes 8-OHdG specifically according to the
manufacturer. Urine creatinine values were used to correct the daily excretion. Urinary creatinine was measured by the Jeffre reaction (reagents from Daiichi Chemicals, Japan). Concentration of urinary 8-OHdG was calculated as ng/mg of creatinine.

2.3. Statistics
One-tailed Student’s t-test was used to determine the significance of difference between two groups of data.

3. Results
3.1. Comparison of urinary 8-OHdG levels in cancer patients and in controls
The average urinary 8-OHdG level in 50 controls is 7.0±2.4 ng/mg creatinine. The 50 cancer patients had an average urinary 8-OHdG level of 11.8±7.5 ng/mg creatinine (FIG. 1.). Average urinary 8-OHdG in cancer patients is higher than that of controls and the difference in these two groups was statistically significant (P = 2 X 10\(^{-5}\)). Use a cutoff value of 11.8 ng/mg creatinine (average + 2 standard deviation of control group), there are 17 people of the cancer group had elevated urinary 8-OHdG.

FIG. 1. Concentrations of urinary 8-OHdG in 50 controls and 50 cancer patients. The average urinary 8-OHdG level was 7.0±2.4 ng/mg creatinine in controls, 11.8±7.5 ng/mg creatinine in cancer patients.
3.2. Urinary 8-OHdG in different ages
To figure out whether the elevation of 8-OH-dG was contributed by the age effect, urinary 8-OHdG concentrations in controls and in cancer patients were plotted according to their ages. The correlation between 8-OHdG and age were not indicated from our study groups as shown in FIG. 2. Age effect for elevation of 8-OHdG in cancer patients should not be significant in this study.

*FIG. 2. Age distribution of studied control and cancer patients. The age of control ranged from 32 to 64 and the average was 42 ± 5 years old. The age ranged from 31 to 78 years for cancer patients.*

3.3. Levels of urinary 8-OHdG in cancer patients under radiotherapy
As depicted in FIG. 3, the average concentration of urinary 8-OHdG in 25 cancer patients under radiotherapy was 15.2±9.0 ng/mg creatinine and is significantly higher than that of patients without radiotherapy (p = 0.003). To find out whether the level of urinary 8-OHdG was correlated to the accumulated exposure dose, the relationship of dosage and 8-OHdG was depicted in FIG 4. Apparently, linear correlation between these two parameters was not found from our results.
FIG 3. 8-OHdG levels in cancer patients during radiotherapy or not. The average concentration of 8-OHdG was 15.2 ± 9.0 ng/mg creatinine in radiotherapy patients, 8.7 ± 3.9 in patients without radiotherapy at sampling time.

FIG 4. Urinary 8-OHdG levels versus accumulated radiation exposure dose in cancer patients.

3.4. Variation of urinary 8-OHdG in serial samples from one patient during radiotherapy
In order to confirm the levels of oxidative damage is not correlated to the accumulated exposure dosage during radiotherapy, concentrations of urinary 8-OHdG of serial collected urine samples from a breast cancer patient were graphed in Figure 5. When patient was exposed to 180cGy per day, urinary 8-OHdG
levels were elevated in the first week but declined after 4 weeks. A few urinary 8-OHdG data elevated again when patient undergoing increased exposure dose, which is 300 cGy/day, but delivered to a smaller area on the chest. To fine out the normal level of urinary 8-OHdG of this patient, six urine samples were collected after this treatment course. The normal level was measured as 8.3±0.5 ng/mg creatinine.

4. Discussion

Excretion of the repair products in urine represents the average rate of damage in the total body. Although the metabolic and chemical stability of 8-OHdG exhibits favorable properties for biomarker purposes, the levels of urinary 8-OHdG show marked inter-individual variations. Urinary 8-OHdG in those 50 control people varied from 2.3 to 13.8 ng/mg creatinine in our study. Endogenous factors such as age, smoking, diabetes, and inflammations are associated with the oxidative profile in whole body. Data from cancer patients showed the urinary 8-OHdG were significant higher than that of control [8]. Age should not be the effective factors to interfere this comparison. Exposure to radiation was the main contribution to the elevation of urinary 8-OHdG as shown in our results. Although radiation was delivered to different part of the body for different type of tumor, it is confirmed that radiation can cause oxidative damage in the body and this damage can be detected by measuring urinary 8-OHdG.

The fact that levels of urinary 8-OHdG were not increase with the accumulated dose was obviously indicated in both random urine samples and serial collected samples, although some patients accumulated up to 80Gy of exposure. The radiation was delivered only to tumor area and in a discrete
dose per day. Repair of DNA may occur right after damage and production of 8-OHdG was clearly identified in human peripheral T cells in couple hours after exposure to 2 to 20Gy of radiation [9]. Neither the repair products will accumulate in the body nor the consecutive exposure will cause advance DNA damage was implied by urinary 8-OHdG.

We concluded that it is necessary to monitor the urinary 8-OHdG during whole therapeutic course and extend of some previous base damages should be identified before radiotherapy. Measuring urinary 8-OHdG by an ELISA method is a relatively simple technique to provide a useful assessment of variation of this DNA repair product during radiotherapy.

5. References