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# Determination of urinary 8-hydroxy-2′-deoxyguanosine by two approaches—capillary electrophoresis and GC/MS: An assay for in vivo oxidative DNA damage in cancer patients

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**Abstract**

8-Hydroxy-2′-deoxyguanosine (8OHdG) has been considered as an excellent marker of oxidative DNA damage associated with age-related diseases such as cancer. In this paper, two sensitive methods—capillary electrophoresis with electrochemical detection (CE-ECD) and gas chromatography/mass spectrometry (GC/MS) were developed for urinary 8OHdG analysis. The R.S.D. of the spiked recovery of the two methods for determining urinary 8OHdG was 4.03% and 8.25%, respectively, and the results from the two methods have a good consistency ($r = 0.999$, $P < 0.01$). The developed CE-ECD method was applied to investigate the urinary 8OHdG levels in different cancer patients and follow up the response of therapy. It was found that the excretion levels of urinary 8OHdG in cancer patients were significantly higher than those in healthy persons ($35.26 \pm 27.96$ nM versus $13.51 \pm 5.08$ nM, $P < 0.05$), and cancer patients receiving surgical therapy and chemotherapy showed a significant decrease in urinary 8OHdG.


*Keywords:* 8-Hydroxy-2′-deoxyguanosine; Cancer; Capillary electrophoresis; GC/MS

## 1. Introduction

DNA oxidative damage has been considered to be one of the most important contributors to aging, cancer and other age-related degenerative processes [1–4], a biomarker of DNA damage would be useful in the estimation of the cancer risk of various populations, aging and in monitoring the effects of cancer therapy. Among potential markers of DNA damage, 8-hydroxy-2′-deoxyguanosine (8OHdG), a typical form of oxidative DNA damage [5], has been extensively investigated because it induces mutation [6,7], and is found frequently in tumor-related genes [8,9]. Moreover, 8OHdG levels in target tissues are correlated with the incidence of some cancers in animal models [10,11] and higher mean values of 8OHdG have been found in DNA from cancer than non-cancer tissues in some studies [12–14]; thus, 8OHdG is a useful marker for the study of oxidative DNA damage in cancer patients.

8OHdG is believed to be excreted in urine without further metabolism, and the urinary levels of 8OHdG appear to be dependent on the rate of DNA damage in vivo and on the efficacy of the repair processes [15,16], thus determination of urinary 8OHdG has been proposed as a non-invasive assay of in vivo oxidative DNA damage. Tagesson et al. [17] reported that the levels of urinary 8OHdG were increased in a variety of cancer patients compared to healthy individuals, furthermore

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high levels of urinary 8OHdG were found in patients subjected to whole body irradiation and in patients receiving chemotherapy. Recently, Erhola et al. [18] used 8OHdG as a useful biomarker to evaluate the response to therapy of lung cancer patients. The urinary 8OHdG levels in patients with gynecologic cancer were also significantly higher than those in control subjects [19].

Since urinary 8OHdG has become increasingly popular as a sensitive, stable and integral marker of oxidative damage to DNA, several analytical methods, such as HPLC with electrochemical detection (HPLC-ECD) [17,20,21], gas chromatography (GC) [22,23], immunoassays [24], HPLC–MS/MS [25] and capillary electrophoresis (CE) [26,27] have been utilized for quantitative determination of urinary 8OHdG. Among these analytical methods, the highly sensitive and specific HPLC-ECD has been the most widely used method. However, these HPLC-ECD methods often employed complicated and time-consuming double or triple column switching [16,17] and two or three-step solid-phase extraction (SPE) [16,20]. They are too complicated to be clinically applied. Recently, we have applied CE with electrochemical detection (CE-ECD) and GC method for urinary 8OHdG analysis [28,29]. In this paper, these two methods for determining urinary 8OHdG levels are firstly compared. Furthermore, the applications of CE-ECD method are given in measuring the urinary 8OHdG levels between cancer patients and controls, monitoring and evaluating the response of urinary 8OHdG levels to surgical therapy and chemotherapy for cancer patients.

## 2. Experimental

### 2.1. Chemicals

8OHdG was from Sigma (St. Louis, MO, USA). Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$), borax (sodium tetraborate, $Na_2B_4O_7{\cdot}10H_2O$) and bis(trimethylsilyl)trifluroaetamide (BSTFA) were purchased from Merck. Acetonitrile ($CH_3CN$) and methanol were of analytical grade and obtained from Shanghai Reagent Corp. (Shanghai, PR China). Solid-phase extraction columns ($C_{18}$/OH 500 mg, 6 ml) were obtained from Chrom Expert Co. (Sacramento, USA).

### 2.2. Urine sample collection and pretreatment

#### 2.2.1. Urine sample collection

The morning urine samples were collected from nine healthy persons (four women, five men) between the ages of 22 and 55, and 28 cancer patients (10 women and 18 men) between the ages of 27 and 72 including lung cancer, colorectal cancer, gynecologic cancer, neck transfer tumor and peritoneal tumor. When urine samples were collected, four patients were being treated by chemotherapy about 2–6 days, and six patients were treated by chemotherapy 30 days ago. Eight patients had their carcinoma surgically removed several months ago and no recrudescent carcinoma was found at the time of collection.

#### 2.2.2. Solid-phase extraction procedure of urine samples

The pH of collected urine was adjusted to 4–5 with 1 M HCl. One step SPE method using $C_{18}$/OH cartridges to isolate the urinary 8OHdG was the same as described before [28]. After SPE, the eluate was evaporated to dryness under vacuum at 39–40 °C. For CE-ECD analysis, the residue was only dissolved in 0.1 ml 30 mM phosphate solution (pH 6.5) to give an extract concentrated by a factor of 10 compared with the original urine, and the concentrated sample solution could be injected into the separation capillary of CE-ECD method.

#### 2.2.3. Preparation of trimethylsilylated derivatives

For GC analysis, the residue of SPE procedure must be dissolved in 0.5 ml methanol and transferred into a derivatization glassware. After that, the organic phase was evaporated to dryness at 39–40 °C under a stream of nitrogen. At last, the residue was trimethylsilylated with a 50 μl mixture of BSTFA and $CH_3CN$ (2:1, v/v) by heating for 1 h at 100 °C.

### 2.3. Separation conditions

For CE-ECD analysis, all experiments were performed on an HPCE-01 capillary electrophoresis system (Shandong Institute of Chemical engineering, Shandong, China) in an uncoated fused-silica capillary (75 cm × 25 μm i.d.) from Yongnian Optical Fiber Factory (Hebei, China). The electrophoretic separations were performed with 30 mM borate buffer, pH 9.12 at 20 kV, and 20 kV × 10 s for electrokinetic injection. The electrochemical detection at a constant potential with CE was performed using the end-column amperometric approach with a JF-01 electrochemical detector (Shandong Institute of Chemical Engineering, Shandong, China). Electrochemical detection was carried out with a two-electrode system. A 400 μm long, 7 μm diameter carbon fiber (Goodfellow Co., Oxford, UK) microcolumn electrode was fabricated according to Huang's method [30] and used as the working electrode with an SCE as the reference electrode, and the detection potential was 0.8 V versus SCE. The carbon fiber working electrode was placed inside the separation capillary 30 μm from the outlet.

For GC analysis, a Shimadzu GC-17A gas chromatograph equipped with a Shimadzu QP-5000 mass selective detector was used, mass spectra were taken at 70 eV. Separations were carried out on a J&W DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The temperature of the injection port was 300 °C and that of the interface port was 260 °C. Upon injection, the column temperature was held at 210 °C for 5 min, raised at a rate of 10 °C $min^{-1}$ to 270 °C, and held for 5 min. Helium was used as the carrier gas at an average linear velocity of 52 cm $s^{-1}$, the split ratio was 10:1.

### 2.4. Statistical analysis

The urinary 8OHdG was identified by comparing the migration time, spiking and peak current ratios and was quantified by the external standard method. Data were expressed as mean ± standard deviation (S.D.). Student's *t*-test was used to test difference in the mean 8OHdG value between groups, and a value of $P < 0.05$ was considered statistically as significant.

## 3. Results and discussion

### 3.1. Analytical characteristic of the two methods developed

For CE-ECD method, the average interday and intraday coefficients of variation of this analytical method were 1.14% and 4.88%, respectively. The limit of detection of 20 nM (signal to noise ratio S/N = 3) was achieved, which is suitable to determine the extremely low concentrations of 8OHdG in urine and the sensitivity was higher than that of the CE-ECD method for determining 8OHdG reported by Weiss and Lunte [27], i.e. 50 nM. It can be observed from Table 1 that the recovery was constant in the concentration range from 10 to 100 nM.

In our previous report, GC/FID was used for quantitative analysis and the GC method was only applied to detect 8OHdG in spiked urine because of the lack of sensitivity to detect low concentration in urine [29]. In this paper, GC/MS-selected ion mode (SIM) was used to quantitatively measure urinary 8OHdG due to the higher selectivity and sensitivity of SIM. According to qualitative analysis, the most intense ion at *m*/*z* 383 corresponding to the B + 1 (base + 1) ion was a characteristic ion and was selected as the quantitative ion. The average interday and intraday coefficients of variation of this analytical method were 4.23% and 8.25%, respectively. The limit of detection of 0.5 nM (signal to noise ratio S/N = 3) could be achieved. This is excellent for the determination of very low concentrations of 8OHdG in urine. The recovery of spiked urine was 99.37%, and the R.S.D. was 8.25%.

### 3.2. Comparison of the CE-ECD and GC/MS-SIM method for the determination of urinary 8OHdG

Table 2 gives the results obtained by CE-ECD and GC/MS-SIM method for the determination of urinary 8OHdG, respectively. By regression analysis, it was found the correlation of the result of two methods was very good ($r = 0.999$, $P < 0.01$). For GC analysis, due to the weak volatility of 8OHdG, it must be trimethylsilylated before injection. At the same time, the concentration of urinary 8OHdG is very low, the trimethylsilylation reaction is not easy to control for trace amount sample, which could lower the precision of GC analysis. Compared with GC/MS-SIM method, the CE-ECD method doesn't need any additional derivatization procedure and is simpler, the precision of this method is better, and its instrument is cheaper, so the CE-ECD method is more suitable for clinical application. Although the sensitivity of this method is a little lower than GC/MS-SIM method, it is enough to measure low concentrations of 8OHdG in urine.

Table 1
The recovery of spiked 8OHdG in urine

| Concentration spiked (nM) | Recovery % | | Average recovery % | R.S.D. (%) |
|---|---|---|---|---|
| 10 | 104.14 | 99.50 | 99.36 | 4.03 |
| 20 | 98.01 | 103.15 | | |
| 50 | 97.34 | 101.50 | | |
| 100 | 100.57 | 90.67 | | |

Table 2
The results obtained by CE-ECD and GC/MS-SIM method for determination of urinary 8OHdG

| No. | CE-ECD (nM) | GC/MS-SIM (nM) |
|---|---|---|
| 1 | 66 | 64.03 |
| 2 | 13.66 | 14.05 |
| 3 | 13.65 | 13.32 |
| 4 | 72.15 | 76 |
| 5 | 135.12 | 140.45 |

### 3.3. Analysis of urinary 8OHdG from healthy persons and cancer patients

Fig. 1 gives a typical electropherogram of a urine extract from a healthy person by CE-ECD method. Using this method, the excretion levels of urinary 8OHdG in nine healthy persons and 28 cancer patients were measured. It was found that the excretion levels (from 13.83 nM to 130.12 nM) of urinary 8OHdG in cancer patients were significantly higher than those (from 6.34 nM to 21.33 nM) in healthy persons (35.26 ± 27.96 nM versus 13.51 ± 5.08 nM, $P < 0.05$).

The excretion levels of urinary 8OHdG from different groups of cancer patients were also investigated and are shown in Fig. 2. It was found that the excretion levels of urinary 8OHdG in patients with lung cancer were the highest and were significantly higher than those in healthy persons (39.12 ± 39.35 nM versus 13.51 ± 5.08 nM, $P < 0.05$). Significant differences were also found between patients with colorectal cancer (35.88 ± 24.73 nM) or gynecologic cancer (32.55 ± 23.78 nM) and healthy individuals ($P < 0.05$). The number of subjects from the patients with a neck transfer tumor and peritoneal tumor was too small ($n = 2$) to give typical excretion levels of urinary 8OHdG, statistical analysis to them has no meaning.

These results mentioned above demonstrated that patients with malignant tumor may have increased urinary excretion of 8OHdG, and therefore increased oxidative damage to DNA, which suggested the importance of oxidative damage

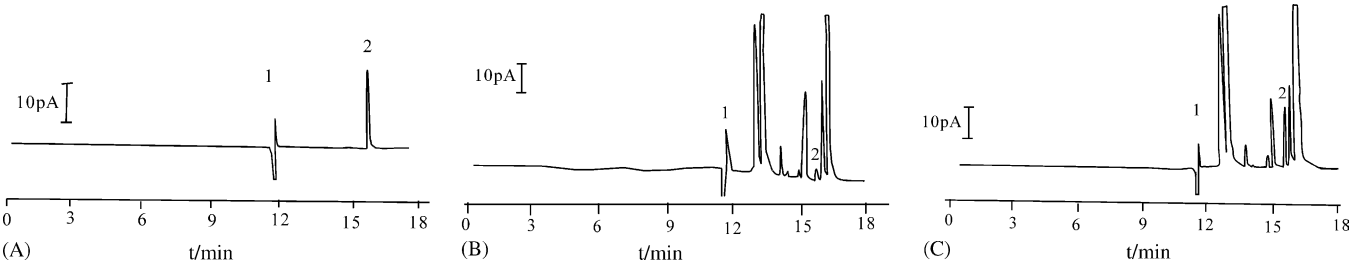


Fig. 1. Typical electrophoretogram of urinary 8OHdG analysis by CE-ECD method. (A) Aqueous standard of $5 \times 10^{-7}$ M 8OHdG; (B) an extract urine from a healthy person; (C) an extract urine adding $5 \times 10^{-8}$ M standard 8OHdG before solid-phase extraction procedure. Electrophoretic conditions: capillary: 25 μm × 75 cm; injection: 20 kV × 10 s; separation voltage: 20 kV; running buffer: pH 9.12, 30 mM borate buffer; sample matrix: pH 6.5, 30 mM phosphate buffer; detection potential: 0.8 V vs. SCE. Peak 1 and 2 represent EOF and 8OHdG, respectively.

to DNA in pathology research of aging and age-related degenerative disease such as cancer.

### *3.4. Association of the excretion levels of urinary 8OHdG with surgical therapy*

Surgical therapy was one of the most effective ways to treat cancer, and it was important to follow up the effectiveness of surgical therapy for the next treatment. In this study, the difference of the excretion levels of urinary 8OHdG between cancer patients before or after surgical therapy was investigated. Fig. 3 shows the difference of the excretion levels of urinary 8OHdG between some cancer patients with surgical therapy and the other cancer patients without surgical therapy. It was found that the excretion levels ($41.78 \pm 19.96$ nM) of urinary 8OHdG in cancer patients without surgical therapy were significantly higher than those ($13.51 \pm 5.08$ nM) of healthy persons ($P < 0.025$). By contrast, there was no significant difference of the mean value of 8OHdG between in those cancer patients with surgical therapy and healthy persons ($21.34 \pm 14.17$ nM versus $13.51 \pm 5.08$ nM). This result suggested that urinary 8OHdG might be used as a useful biomarker for monitoring the effect of surgical therapy.

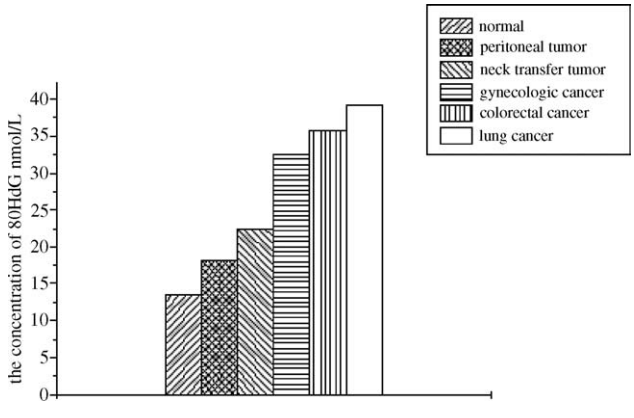


Fig. 2. The concentration of urinary 8OHdG from different groups of cancer patients. Number of samples analyzed was: normal, 9; lung cancer, 8; colorectal cancer, 4; gynecologic cancer, 12; neck transfer tumor, 2; and peritoneal tumor, 2.

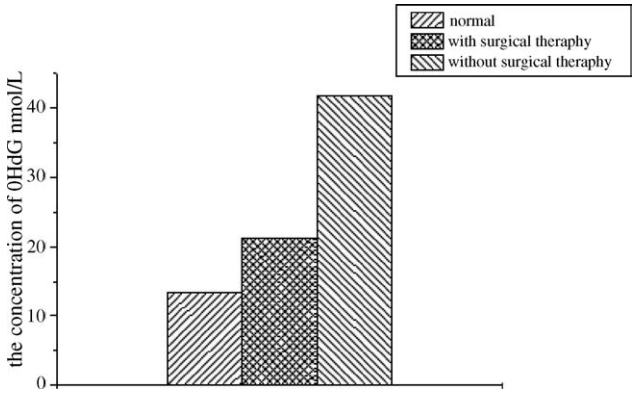


Fig. 3. The excretion levels of urinary 8OHdG from cancer patients with or without surgical therapy. Number of samples analyzed was: normal, 9; with surgical therapy, 8; and without surgical therapy, 10.

### *3.5. Association of the excretion levels of urinary 8OHdG with chemotherapy*

The urinary 8OHdG excretion in cancer patients with chemotherapy is illustrated in Fig. 4. There was a marked increase of the excretion levels of urinary 8OHdG from the patients just receiving chemotherapy, and decrease after a few days following chemotherapy. It was agreeable to other reports [17,18]. This indicated that increased urinary 8OHdG excretion after cytostatic drug treatment may reflect

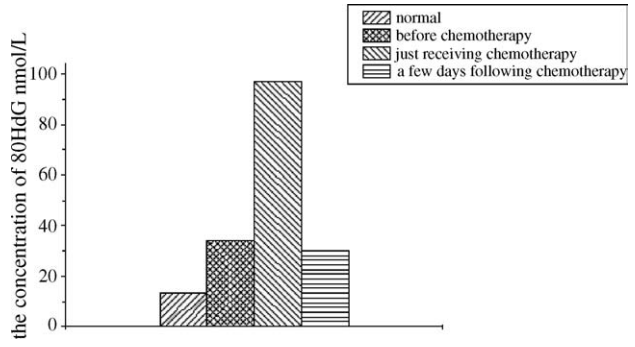


Fig. 4. Urinary excretion of 8OHdG in cancer patients receiving chemotherapy. Number of samples analyzed was: normal, 9; before chemotherapy, 10; just receiving therapy, 4; and a few days following chemotherapy, 6.

increased cell death and ensuing DNA turnover. Dead, disrupted cells were known to undergo liquid peroxidation faster than healthy cells and they would undergo oxidative DNA damage faster [31], thereby the excretion levels of urinary 8OHdG from patients following chemotherapy were significantly elevated.

## 4. Conclusions

The CE-ECD and GC/MS-SIM methods are firstly compared in this study and the results show that these two methods are both suitable to determine urinary 8OHdG with a sufficient sensitivity. The results obtained from two methods have a good consistency. CE-ECD method developed was applied to measure the urinary 8OHdG levels between cancer patients and the control, it is found that the excretion levels of urinary 8OHdG in cancer patients are significantly higher than those in healthy persons. In addition, the excretion levels of urinary 8OHdG in cancer patients receiving surgical therapy or chemotherapy were monitored. These results show that 8OHdG is a potential biomarker for oxidative DNA damage in cancer patients, moreover, a follow-up of urinary 8OHdG might be a useful tool to evaluate the response to therapy and help to know the effect of therapy for the next treatment. Of course, a large number of subjects should be investigated for further clinical prospective studies in the future.

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