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Measurement of 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine in Human Semen and Urine by Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry with On-Line Solid-Phase Extraction: Comparison with a Commercial Available Enzyme-Linked Immunosorbent Assay

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Abstract

This study aimed to assess the correlation between 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo) in semen and urine, and to compare the analytical methods of the isotope-diluted liquid chromatograph-tandem mass spectrometry (LC-MS/MS) coupled with an on-line Solid-Phase Extraction (SPE) and commercial Enzyme-Linked Immunosorbent Assay (ELISA) used for detecting 8-oxo-dGuo as an oxidative DNA damage marker. Semen and urine samples were simultaneously collected from 85 apparently healthy human subjects. An optimized DNA extraction method was employed to extract DNA from sperm while minimizing oxidation of DNA. All of the biological samples were analyzed by LC-MS/MS and ELISA. All of the biological samples were detected with 8-oxodGuo. ELISA consistently detected two to three times higher 8-oxodGuo levels in urine samples than LC-MS/MS. However, there was no significant correlation between measurements of 8-oxo-dGuo levels in urine and semen. In conclusion, the LC-MS/MS coupled with an SPE was a sensitive method to detect and quantify 8-oxo-dGuo in human sperm and urine. Urinary 8-oxo-dGuo may not be a reliable marker for detecting oxidatively damaged DNA in sperm.

Keywords: Oxidatively damaged DNA; Semen; Urine; LC-MS/MS; ELISA

Introduction

8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo) is one of the most abundant by-products of oxidatively damaged DNA, after being repaired by the nucleotide excision repair pathway [1]. Urinary 8-oxo-dGuo may originate from the hydrolysis of 8-oxo-7,9-dihydro-2'-deoxyguanosine 5'-triphosphate (8-oxo-dGTP) in the nucleotide pool [2,3]. 8-oxo-dGuo has mutation potential through the G to T transversion mutation upon replication of DNA [4]. Thus, the detection of the 8-oxo-dGuo has been considered important because of its abundance and mutagenic potential [5,6]. In the past decade, urinary 8-oxo-dGuo has been recommended as a reliable, useful biomarker of oxidative stress both in patients and healthy subjects [7]. Also, it has been widely studied as a noninvasive way to assess oxidative stress [8].

DNA damage in human sperm has been associated with a range of adverse clinical outcomes including infertility, disrupted preimplantation embryonic development, and disease in offspring [9,10]. Oxidative stress has been suggested as a molecular mechanism causing DNA base damage in human sperm. To date, reliable methods of detecting 8-oxo-dGuo as a biomarker to assess the extent of oxidative DNA damage in sperm have not been well established. Compared with the method used to detect somatic cells, the main difference in 8-oxo-dGuo analysis of human sperm is at the stage of DNA extraction. Sperm chromatin is very tightly compacted as a result of the unique association between DNA and nuclear proteins. DNA extraction and digestion procedures could introduce artificial DNA oxidation. To address this interference, the European Standard Committee on Oxidative DNA Damage recently recommended the DNA isolation method to minimize oxidation during DNA extraction [11-15].

Analytical methods, e.g. High-Performance Liquid Chromatography (HPLC) with Electrochemical Detection (ECD), using Gas Chromatography-Mass Spectrometry (GC-MS), have been established to detect urinary 8-oxo-dGuo. These chromatography-based methods require chemical derivatization, which can exhibit poor sensitivity or specificity on measuring the 8-oxo-dGuo in urine [16]. Recently developed liquid chromatograph-tandem Mass Spectrometry (LC-MS/MS) is a powerful technology with the sensitivity and selectivity to analyze DNA adducts [17,18]. Adding on-line sample extraction with a column-switching device to LC-MS/MS could reduce oxidation, since this method causes less ion suppression and has relatively short run times [17,18]. The mass spectrometric-based method has been applied to urine, blood, and saliva samples; but its application is still limited in quantifying 8-oxo-dGuo in sperm. Besides chromatographic-based methods, the immunological assay, Enzyme-Linked Immunosorbent Assay (ELISA), has been widely used to detect urinary 8-oxo-dGuo [12]. However, several reports have noted that the ELISA method often overestimates urinary 8-oxo-dGuo at 4-10 times higher levels than those quantified by chromatographic procedures

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including HPLC-ECD and GC-MS [12-15].

In the present study, we aimed to assess the correlation between 8-oxo-dGuo in semen and urine, and to compare the analytical methods of the isotope-diluted liquid chromatograph-tandem mass spectrometry (LC-MS/MS) coupled with an on-line Solid-Phase Extraction (SPE) and commercial Enzyme-Linked Immunosorbent Assay (ELISA) used for detecting 8-oxo-dGuo as an oxidatively damaged DNA marker. Sperm and urine samples were collected from 85 healthy human subjects. We optimized DNA extraction procedures to increase yields of DNA from sperm. In our prior study, the LC-MS/MS with an SPE had been successfully applied to analyze urinary 8-oxo-dGuo in urine [18]. We again applied this method to quantify 8-oxo-dGuo levels in sperm. The levels of 8-oxo-dGuo analyzed by the chromatographic method and immunological assay method were compared. Also, possible correlations between 8-oxo-dGuo in sperm and urine were examined.

Material and Methods

Chemicals

Solvents and salts were of analytical grade. Reagents were from the indicated sources: unlabeled 8-oxodGuo (Sigma-Aldrich, St. Louis,MO); [15 N5]-8-oxo-7,8-dihydro-2′-deoxyguanosine ([15 N5]-8-oxodGuo) (Cambridge Isotope Laboratories, Andover, MA).

Subjects and sample collection

This study was approved by the Institutional Review Boards of Old Dominion University, Norfolk, VA USA and Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan. Semen samples and corresponding spot urine samples were simultaneously collected from 85 apparently healthy individuals at the health examination center of the Kaohsiung Municipal Hsiao-Kang Hospital. A questionnaire was used to obtain data on age, Body Mass Index (BMI), smoking, and alcohol consumption. Semen samples were collected via masturbation. All samples were kept at 4°C during field sample collection. Once semen samples were delivered back to the laboratory, semen samples were centrifuged at 1000xg for 15 min to separate seminal plasma. Sperm pellets were washed two times with 1% HSD in PBS. Spot urine samples were collected right after the collection of the semen samples. No pretreatment was needed for the urine samples. Both sperm pellets and urine samples were then stored at -20°C before analysis.

Sperm DNA isolation

Sperm DNA isolation was performed according to the procedure recommended by the European Standard Committee on Oxidative DNA Damage (ESCODD) [11] with several modifications. Briefly, sperm samples (15-100 × 10^6 cells) were washed with 1% human serum albumin in PBS and centrifuged at 3000 g for 5 min. The resulting pellet was added to 600 µl of ice-cold extraction buffer (10 mM Tris/HCl, pH 8, 5 mM EDTA-Na_2, 0.15 mM deferoxamine), 70 µl of 10% (w/v) SDS and 30 µl of dithiothreitol (1M), followed by vortexing. After 30 µl of proteinase K was added, the samples were incubated at 55°C for 1 h. Then 30 µl of RNase A (10 mg/ml) and 8 µl of RNase T1 (1 U/µl), both in RNase buffer (10 mM Tris/HCl, pH 7.4, 1 mM EDTA-Na_2, and 2.5 mM deferoxamine), were added, and the mixture was vortexed and incubated at 37°C for 1 h. The mixture was cooled to 4°C for 5 min. Subsequently, 1.2 ml of NaI solution (7.6 M NaI, 40 mM Tris/HCl, pH 8.0, 20 mM EDTA-Na_2 and 0.3 mM deferoxamine) and 2 ml of 2-propanol were added. The sample was gently shaken and frozen at -20°C for 30 min for a better DNA precipitation. After centrifugation at 5000 g for 5 min, the DNA pellet was washed with 1 ml of ice-cold 40% (v/v) 2-propanol, centrifuged and further washed with 1 ml of ice-cold 70% (v/v) ethanol. Finally, the DNA pellet was collected by centrifugation (5000xg for 5 min) and dissolved in 200 µl of 0.1 mM deferoxamine overnight. DNA concentration was measured by absorbance at 260 nm. Protein contamination was checked using the absorbance ratio A260/A280; an absorbance ratio over 1.6 was acceptable.

Enzymatic hydrolysis of DNA

DNA hydrolysis was performed as described by Gedik et al. [11] with some modifications. Briefly, sperm DNA samples (10-20 µg) were spiked with 2.82 pmol of [$^{15}N_5$]-8-oxodGuo and 84.3 pmol of [$^{15}N_5$]-dG. Then 5 µl of 0.2 U/µl nuclease P1 (in 300 mM sodium acetate and 1 mM $ZnSO_4$, pH 5.3) was added to the DNA solutions and the DNA was incubated at 37°C for 2 h. Thereafter, 10 µl of 10× alkaline phosphatase buffer (500 mM Tris/HCl, pH 8, 1 mM EDTA) together with 4 µl of alkaline phosphatase was added and the incubation was continued at 37°C for 2 h. Subsequently, 10 µl of 0.1 M HCl was added to neutralize the solution and the neutralized DNA hydrolysates were ready for 8-oxo-dGuo analysis.

8-Oxo-dGuo analysis in sperm DNA and urine by online SPE LC-MS/MS

8-Oxo-dGuo concentrations in sperm DNA were measured using a validated method of LC-MS/MS with SPE as recently reported [19]. Briefly, after automatic sample cleanup, LC-MS/MS analysis was performed using a Agilent 1100 series HPLC system interfaced with a PE-SCIEX API 3000 triple quadrupole mass spectrometer with electrospray ion source. Detection was performed in the positive ion Multiple Reaction Monitoring (MRM) mode for simultaneous quantification of 8-oxo-dGuo and 2'-deoxyguanosine (dG), and the transitions of the precursors to the product ions were as follows: 8-oxo-dGuo (m/z 284→168), [$^{15}N_5$]-8-oxo-dGuo (m/z 289→173), dG (m/z 268→152), and [$^{15}N_5$]-dG (m/z 273→157). With the use of isotopic internal standards and on-line SPE, this method exhibited a low limit of detection (LOD) of 1.8 fmol for 8-oxo-dGuo, which corresponds to 0.13 adducts/10^6 dG when using 20 µg of DNA per analysis.

Urinary 8-oxo-dGuo concentrations in urine were also measured using a validated method of LC-MS/MS with on-line SPE as previously reported [20]. Twenty µl of urine was diluted 10-fold with 5% methanol containing 0.1% formic acid. After addition of 40 µl of [$^{15}N_5$]-8-oxo-dGuo solution (20 µg/l in 5% methanol/0.1% formic acid) as an internal standard, 100 µl of prepared urine sample was directly injected into the same on-line SPE LC-MS/MS as described above. The precision of the present method was determined by performing replicate determinations of 8-oxo-dGuo in three different urine samples. The intra- and inter-day CV were 2-3% and 4-5%, respectively [20]. The concentration of urinary 8-oxo-dGuo was adjusted to the urinary creatinine (ng 8-oxo-dG/mg creatinine) to control for variation in urinary output. Urinary creatinine was determined using the HPLC-UV method described by Yang [21].

Oxo-dGuo analysis in sperm DNA and urine by ELISA

Urine samples were thawed and centrifuged (300 g, 15 min). Urine sample supernatants in 0.1 mM deferoxamine were applied to the competitive ELISA plat (50 µl/well) according to the protocol supplied

by JaICA (Fukuroi, Japan). No other pretreatment of the samples was performed. Strict temperature controls were applied to the antibody incubations with two temperatures used for the primary incubation, 37°C for 1 h as recommended by JaICA and also 4°C overnight as the method of Evans et al. [22]. The determination range was 0.5-200 ng/ml. The monocloncal antibody, N45.1, with an established specificity [22], was used as the primary antibody. The external calibration curve was established with five standards of 8-oxo-dGuo (2.0, 10.2, 30.5, 70.6, and 282.5 nM). The values from each sample were calculated based on calibration sigmoid plots of absorbance at 492 nm of standard 8-oxo-dGuo at various concentrations, by fitting a logistic curve using computer aided analysis.

The levels of 8-oxo-dGuo in semen were also measured using the ELISA method (NOF, Tokyo, Japan). Five hundred microliters of semen fraction was deproteinized using an ultrafree-MC filter (NMWL=10,000; Millipore, Tokyo, Japan) with centrifugation at 5000xg at 4°C overnight. 100 µl of deproteinized semen was mixed with 100 µl of the 8-oxo-dGuo monoclonal antibody in the microtiter plate, which was precoated with 8-oxodGuo. After the plates were washed with PBS, a horseradish peroxidase-conjugated anti-mouse immunoglobulin antibody was added and the plates were further incubated at 37°C. The color reaction product was then detected with a spectrophotometer at 425 nm, and the concentration of 8-oxo-dGuo was calculated from a standard curve [23].

Statistical methods

The mean and SD were used to describe the distribution of 8-oxodGuo in sperm and urine as well as demographic data for study subjects. The data were analyzed using the SAS statistical package. Levels of 8-oxo-dGuo in urine and sperm measured by LC-MS/MS and ELISA were log-transformed to normalize their distributions before statistical analysis. The Student's t-test was used to compare levels of 8-oxo-dGuo in semen and urine analyzed by the two methods. Pearson correlation coefficients were used to study the correlations of 8-oxo-dGuo concentrations in sperm and urine after adjusting for other variables, i.e. age, BMI, smoking, and alcohol consumption

Results

A total of 85 healthy male subjects were recruited to this study. The demographic data for the study subjects are summarized in Table 1. The mean age and BMI were 38.4 ± 9.2 y and 24.6 ± 3.7 kg/m^2, respectively. 42 % and 29.7% of participants smoked and consumed alcohol regularly. Percentages of motility, viability, and normal morphology of sperm were 61.7, 77.3, and 23.1, respectively.

LC-MS/MS chromatograms for 8-oxo-dGuo and 15N5-labeled 8-oxo-dGuo in the urine and sperm are shown in Figures 1 and 2. The MS/MS transitions selected for 8-oxo-dGuo were m/z 284.13168.0 for quantification and m/z 284.13140.0 for qualification; the corresponding transitions for 15N5-8-oxo-dGuo were m/z 289.13173.0 for quantification and m/z 289.13145.0 for qualification. Limits of detection, defined as the lowest concentration that gave a signal-to-noise ratio of at least 3, was 5.7 ng/L (2.0 fmol) on column.

Table 2 summarizes mean levels of 8-oxod-dGuo in both semen and urine samples from smokers and nonsmokers. The levels of 8-oxo-dGuo in urine and semen were quantified using LC-MS/MS and ELISA. The levels of 8-oxo-dGuo in both urine and semen from all of the human subjects were detectable. The mean levels of urinary 8-oxo-dGuo were 3.26 ng/mg creatinine and 5.32 ng/mg creatinine measured by LC-MS/MS and ELISA, respectively. The mean level of 8-oxo-dGuo measured by ELISA was 1.8 times higher than that measured by LC-MS/MS. The mean levels of 8-oxo-dGuo in sperm were 21.3/10^6 dG and 5.82 (ng/ml) analyzed by LC-MS/MS and ELISA, respectively. Smokers had higher levels of 8-oxo-dGuo in urine and semen analyzed by LC-MS/MS and ELISA (3.92 ng/mg creatinine and 6.26 ng/mg creatinine for urine, respectively; 22.4 /10^6 dG and 6.98 ng/ml, respectively) than nonsmokers (2.64 ng/mg creatinine and 4.35 ng/mg creatinine for urine, respectively; 18.2 /10^6 dG and 4.66 ng/ml, respectively. However, the 8-oxo-dGuo levels were not significant different between smokers and nonsmokers (p=0.17 and p=0.23).

Figure 3a and 3b shows the comparison of 8-oxo-dGuo in urine and sperm measured using LC-MS/MS. There was no significant correlation between the levels of 8-oxo-dGuo in sperm and urine, either with creatinine adjustment or without.

Discussion

This study employed both direct (LC-MS/MS) and indirect (ELISA) approaches to quantify 8-oxo-dGuo in both urine and sperm. To our knowledge, this is the first study to employ both methods for detecting 8-oxo-dGuo levels in urine and sperm, and assess the relationship between 8-oxo-dGuo levels in urine and sperm. For the direct approach, DNA extraction and hydrolysis steps are important to obtain reliable results. The major limitation of DNA extraction for the direct approach is the possibility of generating potential artifactual DNA oxidation during its extraction [11]. In this study, we modified the DNA extraction method recommended by the ESCODD in order to minimize oxidation and show a high yield of sperm DNA. We observed incubation with DTT, and proteinase K was essential to decondense and extract sperm DNA efficiently. Our established method yielded more than 10 times the DNA as compared to a traditional DNA extraction kit (data not shown). Our method consistently recovered sperm DNA amounts ranging from 40-60 µg of DNA from sperm concentrations in the range of 15-20 × 10^6/ml. The high extraction yield of sperm DNA achieved in this study can minimize artifactual DNA oxidation. When lower amounts are extracted, side oxidation becomes more important [24].

Variable	Human subject N=84
Age (y)	38 ± 9
BMI (kg/m^2)	24.6 ± 3.7
Smoking (%)	42.0
Alcohol consumption (%)	30.0
Urinary creatinine (µg/ml)	1751.1 ± 367
Semen quality	
pH	8.1 ± 0.3
Concentration (10^6/ml)	116.8 ± 92.3
Motility (%)#	61.7 ± 19.2
Viability (%)	77.3 ± 16.5
Morphology(%)*	23.1 ± 6.1

Mean ± SD.

#Progressive, non-linear, and non-progressive motility.

*Percentage of sperm with normal morphology.

Table 1: Demographic characteristic and mean urinary creatinine level of study subjects.

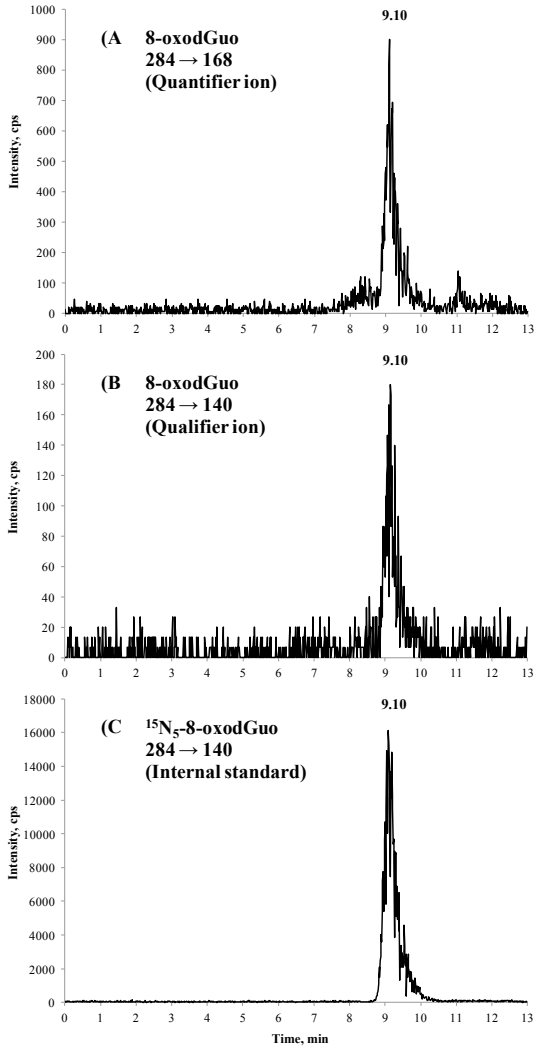

Figure 1: Chromatograms of 8-oxo-dGuo in human sperm DNA, as measured by LC-MS/MS coupled with online SPE. 8-oxo-dGuo was monitored at (A) m/z 284→168 and (B) m/z 284→140, and the internal standard [$^{15}N_5$]-8-oxo-dGuo was monitored at (C) m/z 289→173. cps, counts per second.

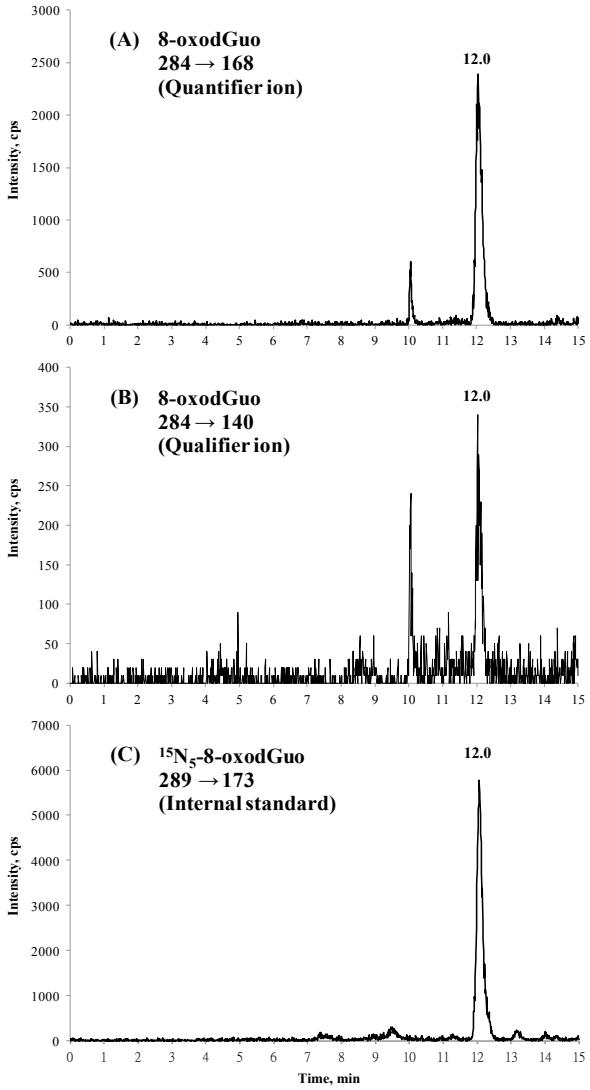

Figure 2: Chromatograms of 8-oxodGuo in human urine, as measured by LC-MS/MS coupled with online SPE. 8-oxodGuo was monitored at (A) *m/z* 284→168 and (B) *m/z* 284→140, and the internal standard [$^{15}N_5$]-8-oxodGuo was monitored at (C) *m/z* 289→173. cps, counts per second.

Using the LC-MS/MS technique only required 25 μg of DNA, which was much less than the amount (50 μg) of DNA required by HPLC-EC analysis. The requirements of a relatively small amount of DNA may provide the means for wide application in clinical samples, particularly semen samples that may contain limited sperm concentrations. HPLC-ECD was established following a protein precipitation and immune-affinity column purification to quantify 8-oxo-dGuo, which resulted in a low of detection of 50 fmol [25,26]. Nevertheless, due to a low specificity of ECD, that method required a large amount of specimen (e.g., 5-10 ml of plasma or 3-6 ml of saliva) in the combination with a tedious manual sample cleanup [25,26]. Our liquid chromatographic method with the use of a highly sensitive and specific mass spectrometry detector following only a one step manual SPE was relatively simple and also had a low LOD of 2.0 fmol. A weakness of the LC-MS/MS with the SPE method is that serious interference and ion suppression were observed in other cell types, e.g. plasma and saliva samples, due to the pre-treatment process of protein precipitation using methanol or acetonitrile [20]. For urine and sperm samples, however, such a pre-treatment process is not required. Thus, our liquid chromatographic method could be a reliable and accurate analytical method for detecting 8-oxo-dGuo, particularly in both urine and sperm.

Using the healthy individuals without any reproductive diseases, the mean of urinary 8-oxo-dGuo analyzed by LC-MS/MS was 3.26 ng/mg of creatinine, which was lower than other studies' findings measured by LC/MS/MS, HPLC, and GC/MS [27-38], but fell within the 95% interval range. After grouping the studied population by smoking status, we observed that smokers have higher urinary 8-oxo-dGuo levels than nonsmokers, but no statistical significance existed between

	LC-MS/MS			P	ELISA			P
	All N=84	Smokers N=36	Nonsmokers N=48		All N=84	Smokers N=36	Nonsmokers N=48	
8-oxo-dGuo in semen#	20.3 ± 20.9	22.4 ± 22.1	18.2 ± 19.2	0.09	5.82 ± 5.32	6.98 ± 6.03	4.66 ± 4.53	0.14
8-oxo-dGuo in urine (ng/ml)	3.63 ± 2.67	4.36 ± 4.94	2.88 ± 2.73	0.16	5.92 ± 4.98	6.33 ± 6.12	5.51 ± 4.45	0.20
8-oxo-dGuo in urine (ng/mg creatinine)	3.26 ± 2.12	3.92 ± 2.79	2.64 ± 1.94	0.17	5.32 ± 4.48	6.29 ± 5.98	4.35 ± 4.01	0.23

Mean ± standard deviation.

#Unit for 8-oxo-dGuo in seminal plasma analyzed by ELISA is ng/ml; Unit for 8-oxodGuo in sperm analyzed by LC-MS/MS is per10^6 dG.

Table 2: Means of 8-oxo-dGuo levels in semen and urine from study subjects with smoking and nonsmoking using LC-MS/MS and ELISA.

them. This observation was contrary to Hu's study and others [36,37]. The difference may be caused by the number of cigarettes consumed by the human subjects. Both Hu and the study at hand used human subjects in Taiwan. In Hu's case, the average number of cigarettes smoked per day was 17.8 [37] as compared with 6.5 cigarettes per day in this study. Other studies did not specifically indicate the number of cigarettes smoked. It may be a worthwhile effort to determine a dose-response between the number of cigarettes and the level of 8-oxo-dGuo in future studies.

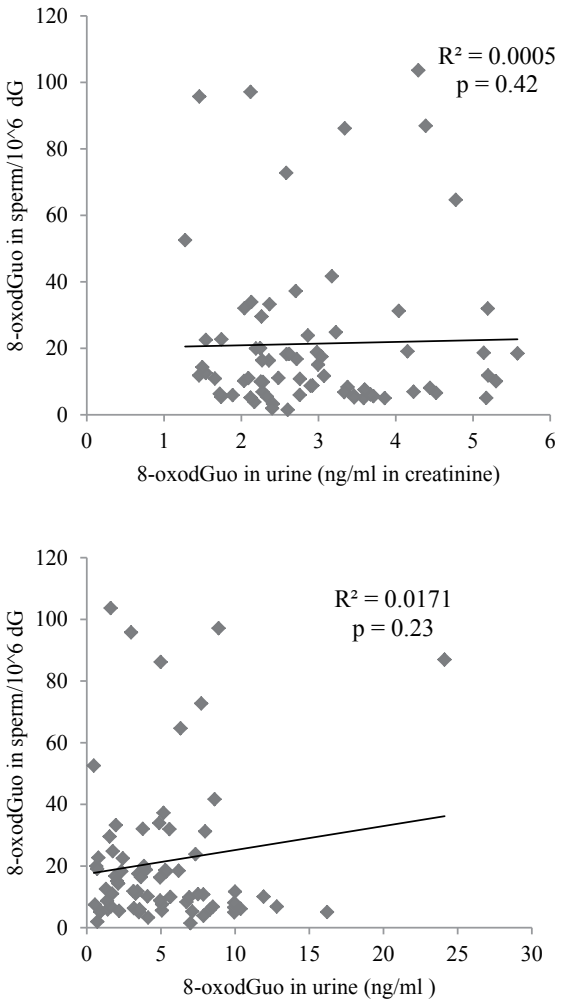

Figure 3: Correlation between 8-oxo-dGuo levels in sperm and urine as estimated by Spearman correlation (a) with creatinine adjustment on 8-oxo-dGuo in urine; (b) without creatinine adjustment on 8-oxo-dGuo in urine.

To date, limited studies have applied chromatography-based methods to detect 8-oxo-dGuo in sperm. Most have used HPLC-ECD for that purpose, while ours is the first known study to use LC-MS/MS with the SPE method. Comparing the results from studies using healthy individuals, the measurements in this study using LC-MS/MS were lower 8-oxo-dGuo concentrations than those measured by HPLC-ECD [34]. The advantage of LC-MS/MS with the SPE method is the reduction of oxidation by producing less ion suppression and having relatively short run times. The 8-oxo-dGuo level observed by LC-MS/MS likely comes from oxidative damage caused by factors of interest rather than false positives of 8-oxodGuo from analytical methods, e.g. HPLC and GS/MS.

Our data showed discrepancies between ELISA and LC-MS/MS measurements of individuals for 8-oxo-dGuo in both urine and sperm. Also, there was not a strong correlation between levels determined by the two methods. In both urine and sperm, the ELISA method yielded higher levels of 8-oxo-dGuo than LC-MS/MS. In line with the findings of other studies, the ELISA method may overestimate 8-oxo-dGuo levels in urine [27,28]. The ELISA method was found to generate significantly higher urinary 8-oxo-dGuo levels (4-10 times) than those measured by chromatographic methods [28,29]. In our study using the ELISA method, the results bear out similarly when analyzing semen samples. The ELISA method is designed to detect 8-oxo-dGuo in seminal plasma rather than directly detect 8-oxodGuo in DNA of sperm as measured by LC-MS/MS. Thus, we estimated 8-oxo-dGuo levels in seminal plasma analyzed by ELISA using the number of sperm per ml of seminal plasma and observed that the estimated 8-oxo-dGuo levels yielded by ELISA at 9 times higher than the 8-oxo-dGuo levels measured by LC-MS/MS.

The results suggested that the specificity of antibodies using this method is not sufficient to allow an accurate determination of the level of the lesions, since the antibodies may cross react with the antibodies of the parent unmodified guanine bas, which is structurally similar to 8-oxo-dGuo [24]. Future work should focus on improving the accuracy of the ELISA method by increasing the specificity of antibodies.

We observed that no significant correlation exists between the 8-oxo-dGuo in urine and sperm. The insignificant correlation may be due to the origination of the lesions from different pathways and different repair mechanisms involved. The 8-oxo-dGuo concentrations in urine reflect the overall oxidatively damaged DNA in biological systems. The repaired products of oxidatively damaged DNA were initially released into the bloodstream and then excreted into the urine [30]. Urinary 8-oxo-dGuo could possibly originate from enzymatic hydrolysis (e.g.

via the nudix hydrolases) of oxidized guanine nucleoside 5'-phosphates in the nucleotide pool as well as from the nucleotide incision repair or mismatch repair pathways [31]. Urinary 8-oxo-dGuo reflected the results of a cellular DNA excision repair process including nucleotide incision repair (ENR) in the whole body, rather than the result of changes within specific tissues or cell types [20]. On the other hand, although DNA adducts in most germ cell stages should be repairable, DNA repair does not occur in condensed spermatids and sperm in epididymis where protamine has replaced somatic histones, rendering the DNA transcriptionally inert [32]. In addition, NER is limited to testicular cells, spermatocytes, and round spermatids [33]. 8-oxo-dGuo in urine could simply reflect overall DNA repair and its clearance from the whole body, while 8-oxo-dGuo in sperm could reflect specific repair mechanisms in germ cells throughout spermatogenesis.

In conclusion, the study demonstrated an extraction method to minimize oxidation of DNA from sperm and quantified the baseline concentrations of 8-oxo-dGuo in human sperm and urine using a highly sensitive and selective on-line LC-MS/MS method. The mean baseline concentrations of 8-oxo-dGuo in sperm and urine were 20.2 per 10^6 dG and 2.76 ng/ml creatinine, respectively. Our data observed that the ELISA method had low specificity on its application to detect 8-oxo-dGuo in sperm and urine analysis [24,28]. The ELISA method tended to yield higher concentrations of 8-oxodGuo in urine and sperm than the LC-MS/MS method. Although urine samples are less invasive and easy to obtain, urinary 8-oxo-dGuo levels may not be suitable to assess oxidative DNA damage in sperm.

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