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The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma

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Abstract

To explore the associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma, 56 non-smoking subjects were asked to collect semen by masturbation into a sterile wide-mouth metal-free plastic container after 3 days of abstinence. The conventional semen parameters were analysed. The concentrations of Cd, Pb and Se in seminal plasma were detected using atomic absorption spectrophotometer. 8-OHdG levels in sperm DNA were measured using HPLC–EC. The results showed that the geometric mean concentrations of Cd, Pb and Se were 0.78, 7.8 and 51.4 μg/l, respectively. The geometric mean of 8-OHdG/10^6 dG was 51.4 (95% CI: 21.5–123.0). A significant inverse correlation exists between Cd and sperm density ($r = -0.28$, $P < 0.05$), and between Cd and sperm number per ejaculum ($r = -0.27$, $P < 0.05$). In contrast, there was a significantly positive correlation between Se and sperm density ($r = 0.50$, $P < 0.01$), between Se and sperm number ($r = 0.49$, $P < 0.01$), between Se and sperm motility ($r = 0.40$, $P < 0.01$), and between Se and sperm viability ($r = 0.38$, $P < 0.01$). No statistically significant correlation was observed between Pb and semen quality. A significant inverse correlation was observed between 8-OHdG and sperm density ($r = -0.34$, $P < 0.01$), between 8-OHdG and sperm number per ejaculum ($r = -0.30$, $P < 0.01$), and 8-OHdG and sperm viability ($r = -0.24$, $P < 0.05$). 8-OHdG was significantly correlated with Cd in seminal plasma ($r = 0.55$, $P < 0.01$). A significant but weak positive correlation was found between 8-OHdG and Pb concentration in seminal plasma ($r = 0.28$, $P < 0.05$). In contract, a significant inverse correlation was observed between 8-OHdG and Se concentration in seminal plasma ($r = -0.40$, $P < 0.01$). The results indicate that Cd in seminal plasma could affect semen quality and oxidative DNA damage in human spermatozoa. Se could protect against oxidative DNA damage in human sperm cells. Pb did not appear to have any association with the semen quality when concentration of Pb in seminal plasma was below 10 μg/l.

Keywords: Cadmium; Lead; Selenium; Spermatozoa; 8-Hydroxy-2′-deoxyguanosine; DNA damage

1. Introduction

There has been increasing concern about the decrease in semen quality in the general population. According to Carlsen et al.'s report, sperm density had

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fallen by 40% over the past 50 years [1]. Auger et al. found that there had been a decline in sperm density, motility and the percentage of morphologically normal sperm in fertile men in Paris over a 20-year period [2]. These findings led to much speculation about the cause and the mechanism of the decline of semen quality.

Cadmium (Cd) and lead (Pb) are two of the well-known reproductive toxicants. Cd has been found to accumulate in male reproductive organs and induce male reproductive toxicity in several animal species [3–6]. However, epidemiological studies have been equivocal about effects of Cd on hormone concentrations, male fertility and sperm parameters. Pb is a male reproductive toxicant in laboratory animal experiments. Occupational exposure to Pb has also been associated with impaired semen quality, decreases in fertility rates and increases in frequencies of spontaneous abortion and male infertility [4,7,8]. In contract, Se is an essential element for normal testicular development, spermatogenesis, spermatozoa motility and functions [9–12]. Some studies showed that Se supplementation in subfertile men with low Se status could improve sperm motility and increase the chance of successful conception [13]. Se has also been demonstrated to have protective effects against the toxicity of metals in the male reproductive system of experimental animals [14].

On the other hand, one of the significant developments in the last 10 years in the study of human infertility has been the discovery that oxidative sperm DNA damage plays a critical role in the aetiology of poor semen quality and male infertility. Fraga et al. [15] first demonstrated an inverse correlation between 8-OHdG in human sperm DNA and concentration of ascorbic acid in seminal plasma. Ni et al. [16] found a significant inverse correlation between 8-OHdG level and sperm density and sperm number. The most convincing evidence suggesting the involvement of oxidative sperm DNA damage in male infertility is the finding that infertile patients contained higher levels of 8-OHdG in sperm than control subjects. Kodama et al. [17] found that the levels of 8-OHdG in sperm DNA in 19 infertile patients were significantly higher than in the control. Shen et al. [18] found that the levels of 8-OHdG in sperm DNA in 60 infertile patients were significantly higher by nearly 110% than in the control.

In the present study, we detected the concentrations of Cd, Pb and Se in seminal plasma among 56 non-smokers by atomic absorption spectrophotometer and then examined the relationship between semen quality and concentrations of Cd, Pb and Se in seminal plasma.

We also investigated 8-OHdG levels in DNA isolated from the same sperm samples to explore possible association of DNA damage in spermatozoa with concentrations of Cd, Pb and Se.

2. Materials and methods

2.1. Materials

Dithiothreitol (DTT), RNase A, proteinase K, DNase I, nuclease P1 and alkaline phosphatase were purchased from Sigma. Proteinase K was dissolved in 10 mM Tris–HCl at a concentration of 2.5 U/μl. Alkaline phosphatase (from *Escherichia coli*) was dissolved in 0.1 M Tris–HCl (pH 7.4) at a concentration of 0.5 U/μl. Nuclease P1 (from *Penicillium citrinum*) was dissolved in 20 mM sodium acetate (pH 5.1). RNase A was dissolved in 10 mM Tris–HCl/1 mM EDTA (pH 7.0) buffer. All the above enzymes were kept at −20 °C after being dissolved. Standard of 8-OHdG was kindly given by Dr. G.N. Wogan (MIT, MA, USA). Standard of deoxyguanosine (dG) was purchased from Sigma. Solutions of Cd, Pb and Se (each a chloride salt) with concentrations of 1 g/l were purchased from BDH Laboratory Supplies (Poole BH151TD, UK) and used as standards.

2.2. Seminal analysis

Individuals with medical history or signs of defective androgenisation or abnormal testicular examinations were excluded from this study. As cigarette smoking is known to cause higher concentration of Cd in seminal plasma [19], poor semen quality and higher level of 8-OHdG in sperm DNA [20,21], smokers were excluded from this study. As alcohol drinking has been associated with poor semen quality, alcohol drinkers were also excluded from this study. Fifty-six subjects whose mean age was 34.5 ± 4.4 years with a range of 26–45 years were asked to collect their semen at home in the morning by masturbation into a

sterile wide-mouth metal-free plastic container after 3 days of abstinence. The semen was brought into the hospital immediately. Each sample was incubated at 37 °C. The conventional semen parameters for semen volume, density, sperm number per ejaculum, viability, motility, morphology were analysed within 1 h according to WHO guidelines for the examination of human sperm [22]. All remaining semen specimens was kept in −70 °C and used for extraction of sperm DNA, measurement of 8-OHdG and determination of Cd, Pb and Se in seminal plasma.

2.3. Extraction of sperm DNA

Sperm DNA was extracted following the protocol established in this laboratory [21]. Briefly, semen was thawed and centrifuged at 1500 × *g* for 5 min at 4 °C. Seminal plasma was transferred into an Eppendorf tube for the determinations of Pb, Cd and Se. Sperm sample was first washed three times with sperm wash buffer (SWB, 10 mM Tris–HCl, 10 mM EDTA, 1 M NaCl, pH 7.4), then incubated with 0.04 M DTT, 0.5 mg/ml proteinase K and 0.9% SDS for 1 h. Subsequently, sperm DNA was extracted with chloroform/isoamyl alcohol (12:1 (v/v)) and digested with ribonuclease. Finally, DNA was dissolved in 10 mM Tris–HCl for subsequent enzymatic DNA digestion.

2.4. Enzymatic DNA digestion and 8-OHdG analyses

DNA samples were denatured at 97 °C for 3 min and kept in ice immediately. DNase I (75 U) was added into each sample and incubated at 37 °C for 1 h. Nuclease P1 (5 U) was then added into each sample and the mixture was incubated at 37 °C for 1 h. Alkaline phosphatase (1 U) was finally added into each tube and the mixture was incubated for another hour. The digested DNA was dissolved in distilled, deionised water. 8-OHdG and dG were simultaneously detected by HPLC–EC and HPLC–UV systems. The HPLC–EC analysis system consisted of a Gilson pump, a Whatman partisphere 5 C 18 column, an electrochemical detector (Ag/AgCl reference electrode, glassy carbon working electrode, potential 0.7 V, range 50 nA), an ultraviolet detector (254 nm), an autosampler and an integrator. The mobile phase consisted of 20 mM $NH_4H_2PO_4$, 1 mM EDTA and 4% methanol (pH 4.7). The flow rate was 1 ml/min. The calibration curves for dG and 8-OHdG were established using standard dG and 8-OHdG from Sigma. The retention times for 8-OHdG and dG were 13 and 9 min, respectively. The results were expressed in 8-OHdG/10^6 dG.

2.5. Analyses of Cd, Pb and Se in seminal plasma

Cd, Pb and Se were detected by atomic absorption spectrophotometer (Varian Spectraa-220G double beam) according to protocols established by this laboratory [23]. Coated pyrolytic graphite tubes were used throughout the study. There was strict quality control. Precision of the method was measured by coefficients of variation. Mean CV for measurement of Cd in seminal plasma was 2.3% for within-day determinations and 2.9% for day-to-day determinations. Mean CV for measurement of Pb in seminal plasma was 2.1% for within-day determinations and 2.8% for day-to-day determinations. Mean within-day variation and day-to-day variation for measurement of Se were 2.0 and 2.7%, respectively.

2.6. Statistical analysis

The association among semen quality, oxidative DNA damage in human spermatozoa and concentrations of Cd, Pb and Se in seminal plasma was analysed using the SPSS for Windows (Version 10.0).

3. Results

First, conventional semen analyses for semen volume, density, sperm number per ejaculum, viability, motility, morphology were performed according to WHO guidelines for the examination of human sperm. The concentrations of Cd, Pb and Se in seminal plasma were detected by atomic absorption spectrophotometer. The results are showed in Table 1. The association between sperm quality and concentrations of Cd, Pb and Se in seminal plasma was then analysed. The results showed that a significant inverse correlation exists between Cd and sperm density ($r = -0.28$, $P < 0.05$), and between Cd and sperm number per ejaculum ($r = -0.27$, $P < 0.05$). In contrast, there was a significant positive correlation between Se and sperm density ($r = 0.50$, $P < 0.01$), between Se and sperm number ($r = 0.49$, $P < 0.01$), between Se

Table 1
Geometric mean and 95% confidence interval of various sperm parameters and concentrations of cadmium, lead and selenium in seminal plasma

Parameters	*n*	Geometric mean	95% Confidence interval
Semen volume (ml)	56	3.4	1.7–6.9
Sperm density (million/ml)	56	41.2	6.8–250.5
Sperm number (million)	56	134.8	20.3–895.6
Sperm motility (%)	56	55.8	36.1–86.2
Sperm viability (%)	56	69.4	44.0–109.4
Morphological defects (%)	49	80.4	61.6–104.8
Cd (μg/l)	56	0.77	0.48–1.22
Pb (μg/l)	56	7.8	4.6–13.1
Se (μg/l)	56	51.3	26.1–100.7

and sperm motility ($r = 0.40$, $P < 0.01$), and between Se and sperm viability ($r = 0.38$, $P < 0.01$). Partial correlation analysis was performed considering the influence of age as covariate. The results showed that a significant inverse correlation is present between Cd and sperm density ($r = -0.28$, $P < 0.05$), and between Cd and sperm number per ejaculum ($r = -0.28$, $P < 0.05$). A significant positive correlation was also found between Se and sperm density ($r = 0.49$, $P < 0.01$), between Se and sperm number ($r = 0.50$, $P < 0.01$), between Se and sperm motility ($r = 0.40$, $P < 0.01$), and between Se and sperm viability ($r = 0.38$, $P < 0.01$). No significant correlation was observed between Pb in seminal plasma and semen quality (Table 2).

Next, 8-OHdG was measured in DNA isolated from the same sperm samples. The geometric mean of 8-OHdG/10^6 dG was 51.4 (95% CI: 21.5–123.0). Linear correlations between 8-OHdG levels and various seminal parameters were first analysed. A significant inverse correlation was observed between 8-OHdG and sperm density ($r = -0.34$, $P < 0.01$), between 8-OHdG and sperm number per ejaculum ($r = -0.30$, $P < 0.01$), and between 8-OHdG and viability ($r = -0.24$, $P < 0.05$). Partial correlation analysis was performed considering the influence of age as covariate. A significant inverse correlation remained between 8-OHdG and sperm density ($r = -0.30$, $P < 0.01$), between 8-OHdG and sperm number per ejaculum ($r = -0.28$, $P < 0.05$).

Last, correlations between concentrations of the three metals in seminal plasma and 8-OHdG levels in sperm DNA were analysed. The results are presented in Figs. 1–3. 8-OHdG in sperm DNA was significantly

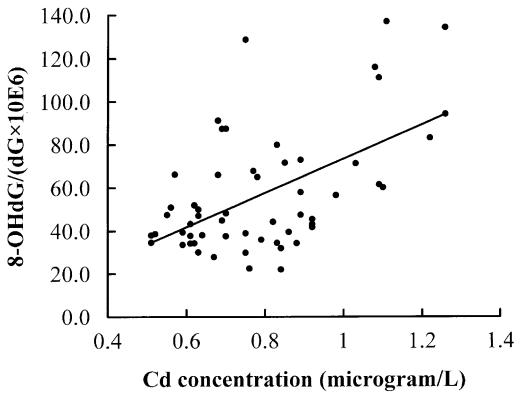

Fig. 1. Scatter diagram of 8-OHdG levels in sperm DNA and Cd concentration. Results showed that a significant positive correlation was observed between 8-OHdG and Cd in seminal plasma. $r = 0.55$, $P < 0.01$.

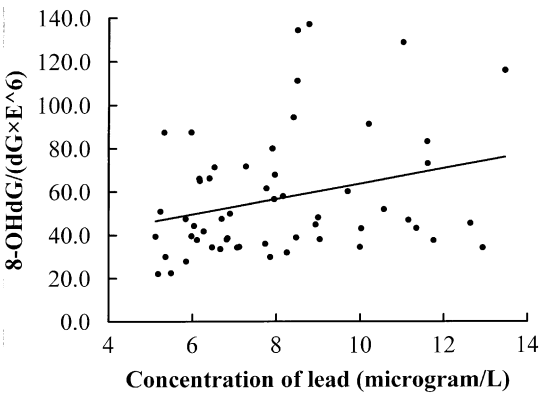

Fig. 2. Scatter diagram of 8-OHdG levels in sperm DNA and Pb concentration. Results showed that a significant but weak positive correlation was observed between 8-OHdG and Pb in seminal plasma. $r = 0.28$, $P < 0.05$.

Table 2
Correlations among 8-OHdG, sperm parameters and Cd, Pb and Se in seminal plasma

Elements	Linear correlation coefficient							Partial correlation coefficient						
	8-OHdG	VOL	DEN	SPN	MOT	VIA	SMD	8-OHdG	VOL	DEN	SPN	MOT	VIA	SMD
8-OhdG		−0.003	−0.34*	−0.30*	−0.18	−0.24**	0.17		−0.02	−0.30**	−0.28**	−0.15	−0.21	0.18
Cadmium	0.55*	−0.08	−0.28**	−0.27**	0.03	−0.07	0.06	0.56*	−0.08	−0.20	−0.22	0.03	−0.08	0.07
Lead	0.28**	0.17	−0.21	−0.08	−0.01	−0.07	0.14	0.27**	0.16	−0.20	−0.08	0.00	−0.05	0.15
Selenium	−0.40*	0.06	0.50*	0.49*	0.40*	0.38*	−0.19	−0.40*	0.07	0.49*	0.50*	0.40*	0.38*	−0.20

VOL, semen volume; DEN, sperm density; SPN, sperm number; MOT, sperm motility; VIA, sperm viability; SMD, sperm morphological defects.

* $P < 0.01$.

** $P < 0.05$.

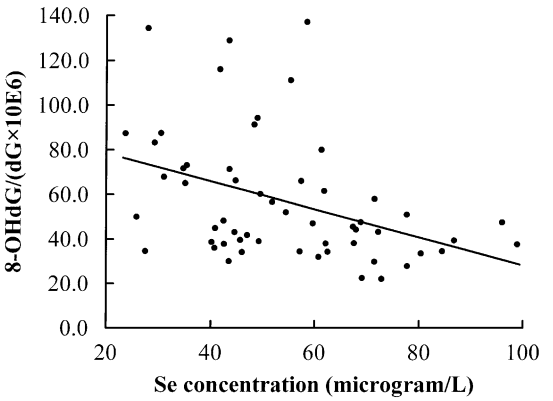

Fig. 3. Scatter diagram of 8-OHdG levels in sperm DNA and Se concentration. Results showed that a significant inverse correlation was observed between 8-OHdG and Se in seminal plasma. $r = -0.40$, $P < 0.01$.

correlated with Cd in seminal plasma (Fig. 1, $r = 0.55$, $P < 0.01$). A significant but weak positive correlation was also found between 8-OHdG and Pb in seminal plasma (Fig. 2, $r = 0.28$, $P < 0.05$). In contract, a significant inverse correlation was observed between 8-OHdG and Se in seminal plasma (Fig. 3, $r = -0.40$, $P < 0.01$).

4. Discussion

Cd is one of the well-known reproductive toxicants in experimental animals. However, the exact effects of Cd on male fertility and semen quality in human are still controversial. Keck et al.'s study showed that no significant correlation was observed between Cd in seminal plasma and conventional semen parameters [24]. Other studies indicated that Cd was associated with poor semen quality. According to Chia et al.'s and Xu et al.'s reports, there was a significant inverse relationship between Cd in blood and semen volume, density and normal morphology [25,26]. A significant inverse correlation was also found between semen volume and Cd concentration in seminal plasma [26]. Different conclusions may be attributed to Cd concentrations. The concentrations of Cd reported by Xu et al. and Keck et al. show significant differences. Mean Cd concentration in seminal plasma was 0.38 ± 0.64 μg/l in Keck et al.'s report, significantly lower than 0.61 ± 0.21 μg/l reported by Xu et al. Our study showed that geometric mean concentration of Cd in seminal plasma was 0.77 μg/l in non-occupational population, close to the earlier data reported by Xu et al. A significant inverse correlation was observed between Cd in seminal plasma and sperm density and sperm number per ejaculum. Based on concentrations of Cd in seminal plasma, the subjects were classified into four groups (<0.6, 0.6, 0.8, >1.0 μg/l). The associations between semen quality and the concentration of Cd in seminal plasma were further analysed. The results indicated that sperm density and sperm number per ejaculum decreased as the concentration of Cd in seminal plasma increased (Table 3).

It is not known whether Cd may have a direct effect on spermatogenesis. Cd is a mutagen in somatic cells. Cd was able to induce the formation of 8-OHdG and DNA strand breaks in human lymphoblastoid cells [27]. In this study, correlation between concentration of Cd in seminal plasma and 8-OHdG levels in sperm DNA was analysed. The results indicated a significant correlation between 8-OHdG levels in human sperm DNA and concentration of Cd in seminal plasma, suggesting that oxidative DNA damage in human sperm is related to Cd in seminal plasma. Until now, there were no literature on Cd and its oxidative DNA

Table 3
Relationships between semen parameters and Cd in seminal plasma

Concentration (μg/l)	*n*	Density (million/ml)	Sperm number (million)	Volume (ml)	Motility (%)	Viability (%)	Morphological defects (%)
<0.6	56	73.8	288.8	3.9	59.5	71.6	76.0
0.6	56	49.9*	169.3*	3.4	56.7	68.6	84.6
0.8	56	31.6*,**	103.9*,**	3.2	57.5	69.6	80.2
>1.0	56	30.5*,**	97.8*,**	3.3	55.0	66.9	82.1

* $P < 0.01$, compared with <0.6 group.
** $P < 0.05$, compared with 0.6 group.

damage in human sperm. The mechanism of Cd-induced oxidative DNA damage in germ cells remains unclear. Animal studies have demonstrated that Cd induced DNA damage in the rat testis. Cd-induced DNA damage in the rat testis may be triggered by reactive oxygen species (ROS), such as H_2O_2 [28]. Furthermore, animal experiments have shown that Cd increased glutathione peroxidase activity, increasing GSSG in rat testis, and decreased glutathione reductase and catalase activities in rat testis, reducing the level of GSH [28]. The deficiencies in metallothionines may leave mammalian testicular cells particularly susceptible to DNA damage and carcinogenesis of Cd [29].

Pb has been recognised as a male reproductive hazard in occupationally exposed workers. Occupational exposure to Pb has been associated with male infertility and poor semen quality [4,8,30–32]. Even long term blood Pb concentration below the currently accepted occupational health level (60 μg/dl) could adversely affect the sperm density and total sperm number [7]. The effect of Pb on spermatogenesis has also been demonstrated in rat testis, in which nuclei and acrosomes in round spermatids were observed swelled and nuclei in Sertoli framentated. Spermatid and Sertoli cell damage may lead to disruption of spermatogenesis in Pb-intoxicated rats [33]. Nevertheless, Pb concentration in blood and in seminal plasma have no association with the sperm parameters in non-occupational population whose mean concentrations were 77.2±31.3 μg/l in blood and 12.7±2.9 μg/l in seminal plasma [26]. In the present study, the concentration of Pb in seminal plasma was determined in 56 non-smokers. Geometric mean concentration of Pb in seminal plasma was 7.8 μg/l, lower than 12.7 μg/l reported by Xu et al. No significant correlation was observed between Pb concentration in seminal plasma and semen quality (including semen volume, sperm density, sperm number per ejaculum, motility, viability and morphological defects). However, our study shows that when Pb concentration in seminal plasma was above 10 μg/l there was a significant reduction in sperm density and sperm number per ejaculum.

Until now, there was no literature concerning the Pb-induced oxidative DNA damage in human sperm. In this study, relationship between oxidative DNA damage in human sperm and Pb concentrations in seminal plasma was analysed. The results showed a significant but weak positive correlation between 8-OHdG levels in sperm DNA and Pb concentration in seminal plasma. Although the present data do not conclusively indicate that Pb in semen induces oxidative DNA damage in human sperm, this finding would provide an important clue in studying mechanism of Pb on male reproductive toxicity. Because the subjects of present study came from a non-occupational exposure population, geometric mean concentration of Pb in seminal plasma was 7.8 μg/l, which was much lower than 411 μg/l in seminal plasma among occupational workers [8]. It would be interesting to explore whether exposure to a higher concentration of Pb in occupational population can induce oxidative DNA damage in human sperm.

Se is an essential trace element for human and its role in human reproduction has been studied extensively. However, reports on the relationships between semen quality and concentration of Se in seminal plasma were inconsistent. Bleau et al. [9] found that the Se level in seminal plasma had a significant positive correlation with sperm density. In contract, Saaranen et al. [34] and Roy et al. [35] were unable to show such a relationship. A few years later, Xu et al. [24] demonstrated there was a statistically significant relationship between sperm density and concentration of Se in seminal plasma in normospermic men but not in oligospermic men. This may explain the discrepancy of earlier studies, as some of the earlier investigations were on infertile men and thus unable to show such a relationship. In the present study, the subjects came from healthy non-smokers. There was a significantly positive correlation between concentration of Se in seminal plasma and sperm density, sperm number, motility and viability. Based on concentrations of Se in seminal plasma, the subjects were classified into three groups (<40, 40–60, >60 μg/l). The associations between semen quality and the concentrations of Se in seminal plasma were further analysed. Results demonstrated that sperm density, sperm number per ejaculum, motility and viability increased in a concentration-dependent manner (Table 4).

The effect of Se on spermatogenesis has been demonstrated in animal experiments. In rat testis and seminal vesicles, Se was converted to phospholipid hydroperoxide glutathione peroxidase (PHGPx) [36,37]. PHGPx exists as a soluble peroxidase in spermatids but persists in mature spermatozoa as

Table 4
Relationships between semen parameters and Se in seminal plasma

Concentration (μg/l)	*n*	Density (million/ml)	Sperm number (million)	Volume (ml)	Motility (%)	Viability (%)	Morphological defects (%)
<40	56	21.6	76.9	3.6	46.1	58.7	82.7
40–60	56	39.1*	123.1**	3.2	55.7*	69.2*	80.2
>60	56	72.2**,†	216.8**,†	3.0	75.3**,‡	90.5**,†	85.3

* $P < 0.05$, compared with <40 group.
** $P < 0.01$, compared with <40 group.
† $P < 0.01$, compared with 40–60 group.
‡ $P < 0.05$, compared with 40–60 group.

an enzymatically inactive, oxidatively cross-linked, insoluble protein. In the midpiece of mature spermatozoa, PHGPx protein represents at least 50% of the capsule material that embeds the helix of mitochondria [12]. On the other hand, Se is also an antioxidant, which may protect against oxidative DNA damage in somatic cells [38–40]. Our data indicated that there was a significant inverse correlation between 8-OHdG levels in sperm DNA and concentration of Se in seminal plasma, suggesting that Se did also protect against oxidative DNA damage in human sperm cells.

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