ORIGINAL ARTICLE

Seminal oxidation–reduction potential and sperm DNA fragmentation index increase among infertile men with varicocele

Takashi Tanaka, Yoshitomo Kobori, Kazutaka Terai, Yasuyuki Inoue, Akiyoshi Osaka, Naoki Yoshikawa, Yukihito Shimomura, Keisuke Suzuki, Tetsuji Minami, Toshiyuki Iwahata, Shin Onota, Atsushi Yamamoto, Kouhei Sugimoto, and Hiroshi Okada

Department of Reproduction Center, Dokkyo Medical University Saitama Medical Center, Koshigaya, Japan

CONTACT: Yoshitomo Kobori, Department of Reproduction Center, Dokkyo Medical University Saitama Medical Center, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan. Tel: +81-48-965-1111; Fax: +81-48-965-8743; Email: ykobori@dokkyomed.ac.jp

ABSTRACT

Varicocele is a common cause of male infertility. It is reported that low sperm concentration, motility and morphology are indicative of increased sperm DNA fragmentation index (DFI) in men with varicocele. Although research has been conducted into the relationship between varicocele and DFI, little is known about seminal oxidation-reduction potential (ORP) in varicocele patients. We assessed the relationship of varicocele with seminal ORP and sperm DFI in both fertile and infertile men. This prospective case-control study compared the findings of infertile men with varicocele to those of men with normal spermatogenesis without varicocele. Semen samples were collected and assessed using the WHO (2010) guidelines. ORP was measured (mV) and normalized to sperm concentration (mV/ 10^6 sperm/mL). DFI was measured using the SCSA method. For group comparisons, only samples with a concentration $>1x10^6$ sperm/mL were included. Infertile men with varicocele had significantly lower mean sperm concentration, motility, and total sperm count. Conversely, infertile men with varicocele had a significantly higher mean serum FSH level, and higher ORP and DFI values than fertile controls. ORP was higher in patients with varicocele and positively correlated with DFI ($p < 0.01$). ORP and DFI showed significant negative correlations with semen parameters (sperm concentration, motility, and total sperm count) in infertile patients with varicocele.

KEYWORDS: Varicocele; semen analysis; DNA fragmentation index; oxidation-reduction potential

Introduction

Male infertility refers to a male's inability to achieve pregnancy in a fertile female. Male factor infertility is regarded as an alteration in sperm concentration and/or motility and/or morphology (World Health Organization, 2010). In humans, it accounts for 40%–50% of infertility cases (Brugh & Lipshultz, 2004; Hirsh, 2003; Lotti & Maggi, 2014) and affects approximately 7% of all men (Pasqualotto et al., 2008).

It has been reported that 30.2% of male infertility cases in Japan are caused by varicocele (Yumura et al., 2018). Varicocele is considered the leading cause of male factor infertility and can be detected in about 40% of male partners in all infertile couples. Varicoceles are found in up to 15% of the adult male population, presenting in approximately 35% of men with primary infertility and up to 80% of men with secondary infertility. Varicocele is caused in part by high temperature in the testis, increased oxidative stress, and intratesticular anoxia. Oxidative stress leads to lipid peroxidation of sperm membranes as well as intracellular lipids and proteins, increases apoptosis, and results in DNA damage (Pastuszak & Wang, 2015).

Oxidative stress is the most common factor leading to infertility in men with varicocele (Majzoub et al., 2018). An accurate measure of oxidative stress is the oxidation-reduction potential (ORP). The ORP provides an overview of the redox system through assessment of the net balance between oxidants and reductants in any given medium. Recently, the ORP in semen has been easily and comprehensively measured using the MiOXSYS system in a multi-centre clinical study that included nine countries (Agarwal et al., 2019).

Higher ORP and DNA fragmentation index (DFI) are associated with low sperm quality and provide reliable information that synergises the predictive value of semen analysis during male fertility evaluation (Agarwal, Majzoub, et al., 2016). While ORP and DFI have been previously correlated with different semen parameters, their relationship with varicocele has not been investigated. In this study, we compared conventional semen parameters (semen volume, sperm concentration, motility, progressive motility, total sperm count, average path velocity, straight-line velocity, and curvilinear velocity) as measured by computer assisted semen analysis and advanced sperm function tests (ORP and DFI) between infertile men with varicocele and fertile controls. In addition, we explored the correlation between ORP and DFI with and without varicocele.

Materials and methods

Study design

This prospective study was conducted at the Reproduction Center of Dokkyo Medical University Saitama Medical Center, Koshigaya, Japan. Over a period of two years (2017–2019), 138 infertile men with varicocele (Grade 1, 24 cases; Grade 2, 47 cases; Grade 3, 67 cases) and 102 men with normozoospermia without varicocele were evaluated. Serum LH, FSH, testosterone level, and bilateral testicular size were evaluated in each patient. The study was approved by the Institutional Review Board at Dokkyo Medical University (Reference number:

1929XXXXXX), and written informed consent was obtained from all participants before enrolment.

Semen analysis parameters

Each participant provided a semen sample after 1–10 days of sexual abstinence. Standard semen analysis was performed in conjunction with computer assisted semen analysis (CELOS II; Hamilton Thorne, Beverly, MA). Sperm motility was assessed, and straight-line velocity, curvilinear velocity, and average path velocity were calculated by CELOS II.

ORP and DFI

ORP was measured in unprocessed post-liquefied semen to assess test reproducibility using the MiOXSYS. As the analyzer applies a low-voltage steady current measured in millivolts (mV), oxidative stress reflects the relationship between sperm (producers of free radicals) and seminal plasma (an antioxidant reservoir). Thus, raw ORP values (mV) were normalized to sperm concentration, a value that reflects both semen volume and sperm number. Data for ORP are presented as $mV/10^6$ sperm/mL throughout (Agarwal, Sharma, Roychoudhury, Du Plessis, & Sabanegh, 2016).

DFI was evaluated using the sperm chromatin structure assay (SCSA) method (CytoFLEX; Beckman Coulter, Inc., Atlanta, GA). SCSA, a high-precision flow-cytometric test, is probably the most widely used flow-cytometer and cell analyzer for research. It is the

only sperm DNA fragmentation test that simultaneously measures both DNA strand breaks and chromatin structure (Jerre, Bungum, Evenson, & Giwercman, 2019).

Statistical analysis

Summaries of quantitative variables are reported as means ± standard error (SE) for infertile patients and fertile controls. The Mann–Whitney U test was used to compare means of age, count, motility, DFI, and ORP between infertile men with varicocele and normozoospermia men as fertile controls. Z-test was used to determine normal distribution and Spearman's rank test was used to test the correlations of DFI and ORP for each sample specimen. All statistical analyses were performed with the Statcel 3 program (OMS Publishing, Tokyo, Japan), and differences were considered significant at $p < 0.05$.

Results

The baseline characteristics and semen analysis characteristics of the infertile patients with varicocele and normozoospermia controls without varicocele are shown in Table 1.

Infertile men with varicocele had a significantly higher level of serum FSH and lower left testicular volume. They also had significantly lower values at semen analysis and following CASA. However, ORP and DFI values were significantly higher in the infertile patients with varicocele than in the normozoospermia controls without varicocele ($p < 0.01$; Figure 1). The mean ORP ($mV/10^6$ sperm/mL) in the semen of the infertile men with a varicocele was three

times higher than that of the normozoospermia controls ($p < 0.01$). There was a significant correlation between ORP and DFI among all participants (Figure 2).

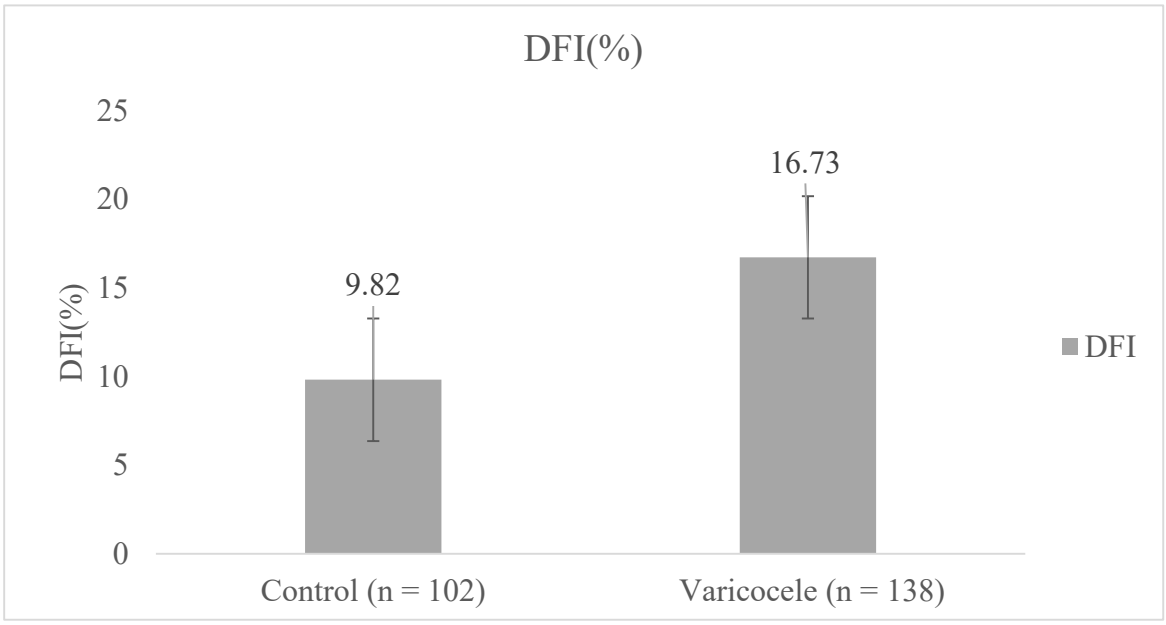
Table 1

Baseline characteristics and semen analysis characteristics of the sample.

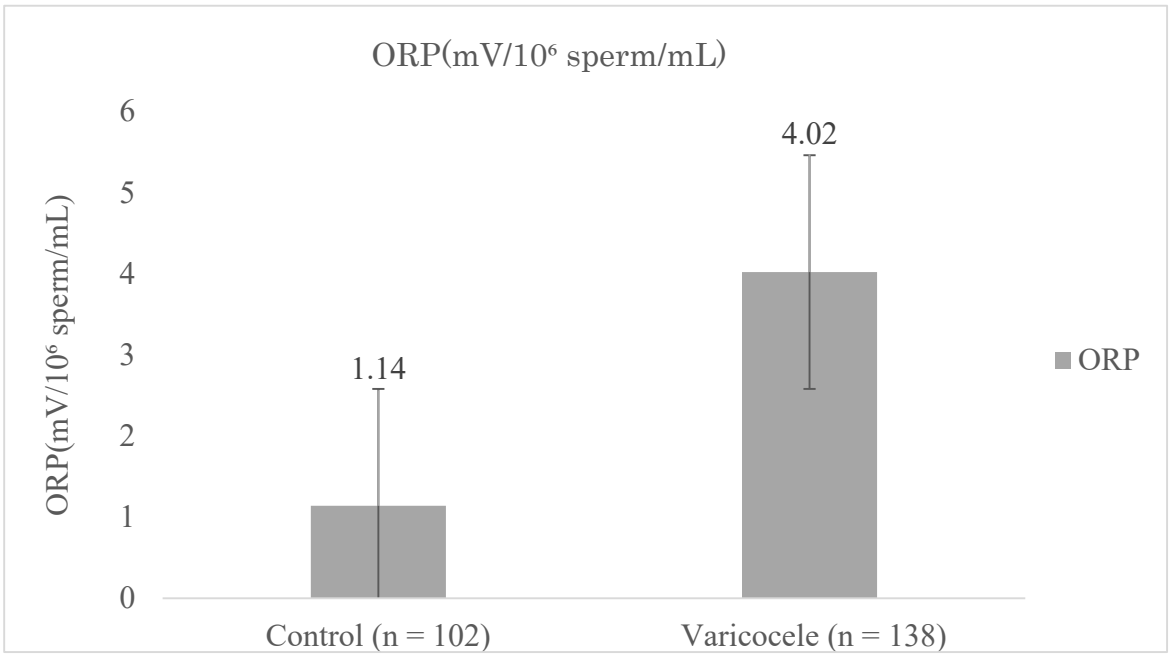
Variable, mean (SE)	Normospermic men (n = 102)	Varicocele patients (n = 138)	P Value
Demography			
Age (years)	37.38 ± 5.75	36.82 ± 5.89	0.46
Abstinence (days)	3.82 ± 4.71	4.05 ± 5.68	0.74
Prolactin (ng/mL)	11.63 ± 5.47	11.45 ± 9.75	0.87
Luteinzing hormone (mIU/mL)	4.80 ± 2.12	4.70 ± 2.24	0.73
Follicle stimulating hormone (mIU/mL)	4.70 ± 1.96	5.72 ± 3.07	$P < 0.01$
Testosteron (ng/mL)	4.66 ± 1.49	4.84 ± 1.96	0.43
Rt. testis (mL)	20.52 ± 4.11	19.67 ± 4.01	0.12
Lt. testis (mL)	20.16 ± 4.33	18.86 ± 4.06	$P < 0.05$
Body height (cm)	172.70 ± 6.16	172.35 ± 6.07	0.66
Body weight (kg)	70.83 ± 11.13	69.75 ± 9.38	0.42
Body mass index	23.75 ± 3.48	23.33 ± 3.43	0.36
Semen analysis			
Volume (mL)	2.73 ± 1.36	2.85 ± 1.45	0.51
Concentration ($\times 10^6$/mL)	68.23 ± 58.89	34.39 ± 40.86	$P < 0.01$
Total sperm count ($\times 10^6$)	179.91 ± 216.50	97.15 ± 141.72	$P < 0.01$
Motility (%)	59.29 ± 22.15	47.42 ± 23.06	$P < 0.01$
Progressive motility (%)	23.46 ± 58.89	18.25 ± 12.20	$P < 0.01$
Average path velocity (μm/s)	43.81 ± 9.75	39.39 ± 10.54	$P < 0.01$
Straight-line velocity (μm/s)	32.57 ± 6.86	29.35 ± 7.79	$P < 0.01$
Curvilinear velocity (μm/s)	63.30 ± 16.08	57.94 ± 16.04	$P < 0.01$
DFI (%)	9.82 ± 10.31	16.73 ± 12.13	$P < 0.01$
ORP (mV/10^6 sperm/mL)	1.14 ± 1.78	4.02 ± 7.56	$P < 0.01$

Figure 1

DFI and ORP results of infertile men with varicocele and normospermia controls.



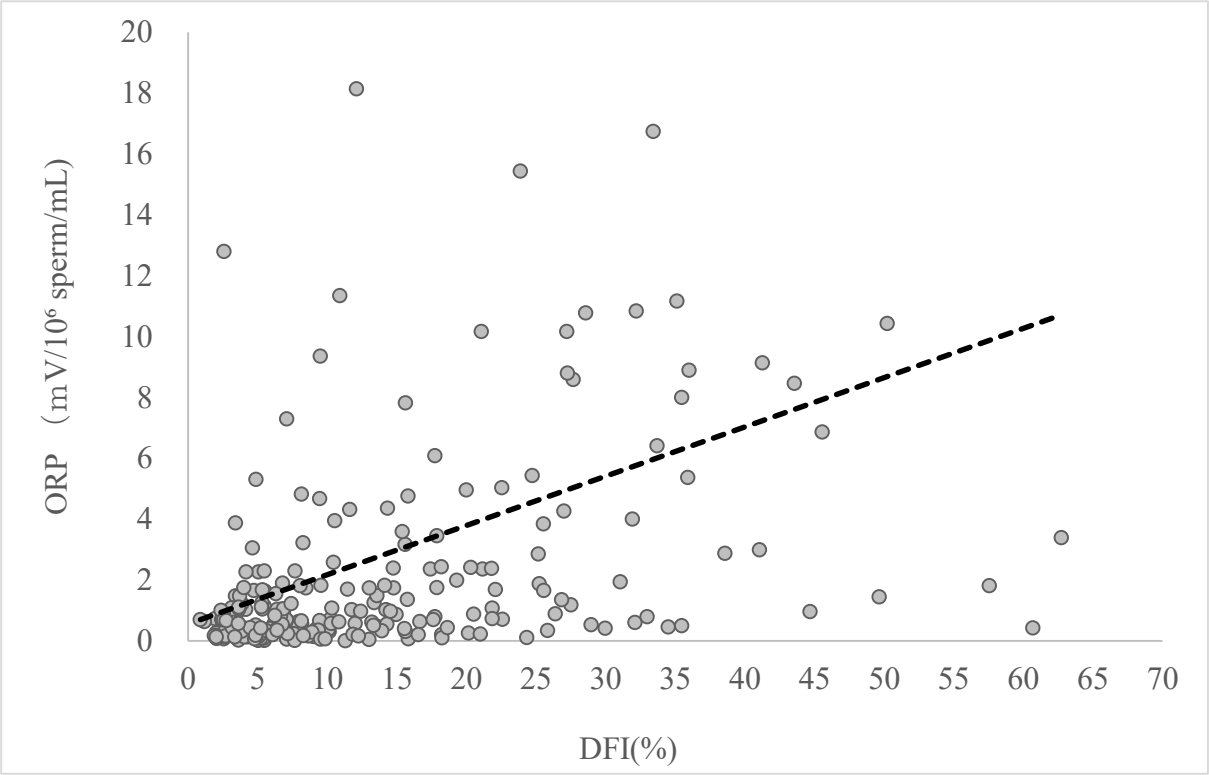
$P < 0.01$



$P < 0.01$

Figure 2

Correlation between DFI and ORP.



n = 240 r = 0.320075 $p < 0.001$

Discussion

The relationship between sperm DNA fragmentation and reactive oxygen species (ROS) levels in infertile men with a varicocele was investigated, this study is the first in the literature to examine the ORP and DFI of infertile men with a varicocele (Abdelbaki, Sabry, Al-Adl, & Sabry, 2017). We found that ORP, DFI, and semen analysis values were significantly higher in men with a varicocele comparsed to controls. Infertile men with a varicocele had significantly lower semen analysis parameters (sperm count, motility, and CASA parameters) and higher ORP and DFI levels than normozoospermia controls.

Oxidative stress is a factor contributing to the pathophysiology of many diseases, including male subfertility due to varicocele (Agarwal, Roychoudhury, et al., 2016). The total oxidant capacity and oxidative stress index in patients with varicocele has been shown to be significantly higher in the internal spermatic vein than in the peripheral circulation (Ko, Sabanegh, & Agarwal, 2014). It is also reported that there is a significant increase in (ROS) levels in the internal spermatic vein compared to the peripheral venous circulation in infertile patients with varicocele (Altintas et al., 2012). The total antioxidant capacity was shown to be significantly lower in patients with varicocele compared to the control group, and significantly lower in the peripheral spermatic veins than in the peripheral circulation (Liang, Wen, Dong, Zhao, & Shi, 2015). Many studies in the literature suggest that oxidative stress is the main factor that triggers the disorder in the etiopathogenesis of varicocele-related infertility.

Oxidative stress plays a central role in the pathogenesis of male infertility attributed to various etiologies, including varicocele (Barbieri, Hidalgo, Venegas, Smith, & Lissi, 1999). It is a result of an imbalance between ROS present in the ejaculate and the ability of the available antioxidants to quench these oxidants. Thus, excessive amounts of ROS have negative effects on sperm proteins and lipids and lead to DNA fragmentation (Dutta, Majzoub, & Agarwal, 2019).

ORP, also known as redox potential, is a measure of the potential for electrons to move from one chemical species to another. To quench the damaging effects of oxidants, antioxidants donate electrons to oxidants, thereby reducing the chance of oxidants acquiring electrons from other nearby structures and causing damage. ORP is a measure of this relationship between oxidants and antioxidants and provides a comprehensive measure of oxidative stress. Higher ORP levels indicate an imbalance in the activity of oxidants relative to antioxidants and can thus be used to differentiate the degree of oxidative stress-induced male factor infertility (Agarwal et al., 2019; Mostafa et al., 2006). ORP was significantly higher in infertile patients with varicocele than in healthy controls in the present study. We used MiOXSYS for ORP measurements; this method has been recently used for the evaluation of male infertility and its validity was confirmed to provide an accurate measure of oxidative stress in semen (Agarwal, Arafa, et al., 2017; Agarwal, Henkel, Sharma, Tadros, & Sabanegh, 2018). The results of the present study highlight the importance of routine incorporation of advanced sperm function tests in the evaluation of infertile men, especially during the evaluation of varicocele. ORP and

DFI tests would be useful not only for patients with varicocele but also for patients with idiopathic male infertility. The combination of conventional sperm analytical indices with advanced sperm function tests like ORP and DFI would help to identify sperm with greater reproductive potential and could be used for novel sperm selection techniques during assisted reproductive technology (Agarwal, Wang, Tadros, & Sabanegh, 2017; Arafa, Henkel, Agarwal, Majzoub, & Elbardisi, 2019). Treatment efficacy could be potentially predicted by the monitoring of ORP and DFI levels in varicocele patients because higher ORP and DFI levels are indicative of the progression of infertility. Future research will test our preliminary findings. It has become easier and cheaper in modern clinical practice to measure ORP and DFI values. These tests may be essential for predicting male infertility as well as the therapeutic effects of assisted reproductive technology.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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