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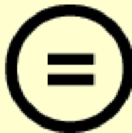

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Relationship between human semen parameters and phospholipase C zeta level after swim-up

스윔업 처리 후 측정한 정자의 포스포리파제 C 제타치와 정액 변수와의 연관성

2014 년 08 월

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이 논문을 의학석사학위논문으로 제출함

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박주희의 석사학위논문을 인준함
2014년 5월

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Abstract

Relationship between human semen parameters and phospholipase C zeta level after swim-up

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Objective: To investigate the relationship between immunoreactivity of phospholipase C zeta (PLCz) and other parameters in donor sperms after swim-up.

Materials and methods: Semen samples were obtained from participants (n = 44) and processed by the conventional swim-up method. Sperm concentration, motility, strict morphology, DNA fragmentation index, immunofluorescence assay for 8-

hydroxy-2' -deoxyguanosine (8-OHdG), and PLCz level were assessed.

Results: Immunoreactivity for PLCz had a negative correlation with immunoreactivity for 8-OHdG ($r = -0.404$, $p < 0.05$). Immunoreactivity for 8-OHdG had a negative correlation with concentration and total motile sperm count ($r = -0.326$, $p < 0.05$). Multivariate analysis revealed that immunoreactivity for PLCz had a marginal significant negative correlation with immunoreactivity for 8-OHdG ($r = -0.317$, $p = 0.056$), regardless of sperm DNA fragmentation index and concentration.

Conclusion: The PLCz level was only correlated with the 8-OHdG level. Higher expression of PLCz in sperms was associated with lower oxidative stress.

Keywords: phospholipase C zeta (PLCz), 8-hydroxy-2' -deoxyguanosine (8OHdG), oxidative stress, smoking, sperm analysis, Assisted Reproductive Technology (ART)

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Introduction

The sperm−specific phospholipase C zeta (PLCz) exhibits the expected properties of a sperm−associated oocyte−activating factor (1). PLCz is composed of four elongation factor−hand domains in the N−terminus, catalytic X and Y domains, and a C2 domain in the C−terminus. Each of the individual PLCz domains appear to play an essential role in conferring the distinct biochemical characteristics and the unique mode of regulation of this gamete−specific PLC isozyme (2). The PLCz, a gamete−specific 70−kDa protein, is predominantly localized to the equatorial region of the human sperm head, with relatively smaller populations in the acrosomal and post−acrosomal regions. PLCz is also considered the endogenous agent of oocyte activation in mammals (3).

Previous studies have demonstrated that PLCz is the physiological agent responsible for inositol 1,4,5−trisphosphate pathway−mediated Ca^{2+} release in activating oocytes, a process known as Ca^{2+} oscillations (1,4). This striking Ca^{2+} signaling phenomenon is necessary for the completion of oocyte activation processes such as cortical granule exocytosis, the resumption and completion of meiosis, and the formation of

pronuclei (2). Given the fundamental role of PLCz in activating an oocyte after gamete fusion, sperm from patients who display either reduced PLCz protein levels or who express mutated forms of PLCz are correlated with higher rates of failed fertilization after intracytoplasmic sperm injection (ICSI) treatment. Known as oocyte activation deficiency, this is due to the inability of sperm to initiate critical Ca^{2+} oscillations required for oocyte activation (2, 5, 6).

The ICSI technique is of great importance for the field of assisted reproductive technology (ART) to address male infertility, and has been developed increasingly. ICSI has resulted in a mean fertilization rate of 80%. However, 2 3% of ICSI cycles fail due to problems with oocyte activation (7, 8). The etiology underlying the poor success of ICSI is likely to be multifactorial and may involve oocyte factors (9). A very low rate of fertilization by ICSI is notable in cases of globozoospermia, a rare but severe disorder in male infertility characterized by round-headed, acrosomeless sperm cells (10). An absence or reduced level of PLCz has been demonstrated in cases of globozoospermia (11). Separately, oocyte activation failure following ICSI may result from one or more of the

following factors: a deficiency in PLCz, absent or reduced PLCz expression, incorrect pattern of localization, or abnormally low molecular weight of PLCz (12).

Microinjection of human cRNA of PLCz can initiate Ca^{2+} oscillations and oocyte activation in human oocytes, thus stimulating embryonic development to the blastocyst stage (6, 13). Immunodepletion of endogenous PLCz from sperm protein extracts either eliminates their ability to release Ca^{2+} or causes premature termination of Ca^{2+} oscillations (1, 2, 5). Therefore, in cases of globozoospermia, barriers to fertilization can be overcome through artificial oocyte activation (10), even without inducing Ca^{2+} oscillations at the time of fertilization. ICSI along with a Ca^{2+} ionophore in globozoospermic sperm devoid of PLCz expression can result in a high rate of fertilization and pregnancy (14). Therefore, injection of PLCz protein during ICSI may be an important new therapeutic strategy in cases of previously failed fertilization following ICSI treatment. However, it remains unknown whether the wild-type human PLCz protein can physiologically activate oocytes in the presence of mutant PLCz, and if this would successfully lead to normal embryo development.

In addition to its therapeutic role, PLCz is thought to represent a prognostic biomarker of sperm quality in male infertility (8). One previous study found that density-gradient washing positively selects spermatozoa with detectable PLCz, suggesting that PLCz may be useful in the identification of sperm with the greatest ability to activate oocytes (15). Indeed, these studies suggest that PLCz may represent a useful diagnostic tool to investigate sperm quality thus contributing towards the improvement of current ART and greater success of conception (6, 15). Another study reported that sperm from fertile men exhibit significant variance in total levels of PLCz protein (16), thus quantitative PLCz immunofluorescence might not be a good prognostic indicator of sperm quality. However, it should be noted that these observations were based on a study with a relatively small sample size. Finally, No study has focused on the relation between PLCz level and other sperm parameters. The primary aim of the present study was to investigate the relationship between the level of PLCz protein in donor sperm and other sperm parameters. We also compared PLCz level between smokers and non-smokers.

Materials and methods

Study subjects

Semen samples were obtained from male participants (n = 44) between April 2013 and February 2014. Informed consent for enrollment into the study and for the use of semen in analysis was obtained from all participants. The Institutional Review Board at Seoul National University Bundang Hospital reviewed and approved the study (B−1205−155−003). The mean age of the participants was 32.0 ± 5.5 years (range: 23 49 years). No participant had a history of genital inflammation or genital surgery. No subjective symptoms or self−reported medical risk factors were identified. No participant had taken prescription at the time of study enrollment. Sixteen participants were smokers. While 47% (21/44) of the study participants were married, fertility potential was proven in only 5/21 married participants.

Conventional semen analysis

Semen samples were collected by masturbation after 3 days of sexual abstinence. After liquefaction for 30 min at room

temperature, sperm quality was assessed using computer-assisted semen analysis (CASA) (SAIS-PLUS 10.1; Medical Supply Co., Seoul, Korea) and classified according to World Health Organization (WHO) guidelines published in 2010. Strict criteria for the definition of normal spermatozoa were used during morphological assessment. Baseline semen characteristics were as follows: volume, 2.9 ± 1.4 mL (range: 1.0 6.0 mL); concentration 99 ± 82 million/mL (range: 18 460 million/mL); motility, 54.6 ± 15.3% (range: 10.1 75.8%); total motile sperm, 177 ± 238 million (range: 17 1,485 million); and strict morphology, 11.3 ± 5.6% (range: 2.3 25.0%). All semen samples contained motile sperm and no sample had significant numbers of round cells or leukocytospermia in accordance with WHO guidelines (<1 million round cells/mL).

Conventional swim up

The semen was processed by the conventional swim-up method. After centrifuging the semen (300 ×g for 5 min), a pellet was obtained via removal of seminal plasma. The pellet was suspended in fresh Ham' s F10 (1.5 mL) supplemented

with 10% serum substitute supplement (SSS; Irvine Scientific, Santa Ana, CA, USA). After centrifugation (300 ×g for 5 min), the supernatant was discarded and Ham' s F10 with 10% SSS media (0.5 mL) was gently layered on the pellet and incubated at 37° C in a 5% CO_2 atmosphere for 1 h. The supernatant (0.5 mL) was then transferred to a conical tube. The processed samples were re-assessed using CASA prior to use in subsequent experiments.

TUNEL Assay

Nuclear DNA integrity was measured by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay as described previously by Jee et al. (17). The samples were smeared on a silane-coated slide (DAKO) and air-dried. Sperm samples were fixed with 4% paraformaldehyde for 1 h at 15 25° C and then washed with phosphate-buffered saline (PBS). Sperms were permeabilized with 0.1% Triton™ X-100 in 0.1% sodium citrate (Sigma-Aldrich Corporation, St. Louis, MO, USA). A commercial apoptosis detection kit was used (In Situ Cell Death Detection Kit; Roche Diagnostics GmbH, Mannheim, Germany). The remaining procedures were

performed as per manufacturer's instructions. Counterstaining was performed using a mounting medium with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA). Sperms with fragmented DNA had nuclei stained green, whereas the nuclei of other cells were blue. Sperm heads with >50% of the area stained green were considered positive. At least 500 sperm were counted per experimental set, with fragmented DNA sperm percentage determined using a DNA fragmentation index.

Immunofluorescence Assay for 8-Hydroxy-2'-Deoxyguanosine

This method was used for the detection of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a known biomarker for oxidative stress. A specific antibody (Argutus Medical OxyDNA Test, BD Biosciences, Franklin Lakes, NJ, USA) conjugated to fluorescein isothiocyanate (FITC) was used. The intensity of FITC fluorescence was then assessed under fluorescent microscopic evaluation (Fig. 1). Briefly, sperm samples were fixed by 4% paraformaldehyde and permeabilized. A specific antibody was then added for 1 h, according to the

manufacturer’s instructions. At least 500 sperms were counted in different areas of each slide. Sperm heads with >50% of the area stained green were considered positive.

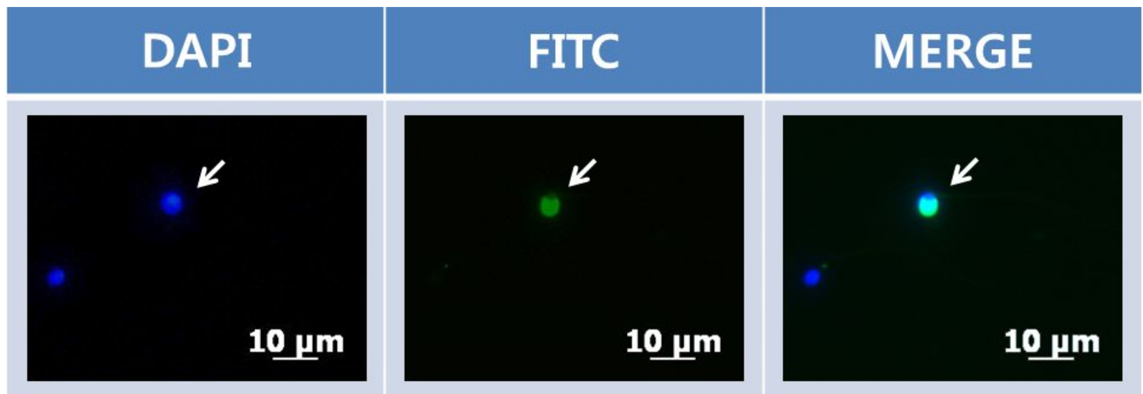

Fig. 1. A representative microphotograph showing immunofluorescent sperm for 8-hydroxy-2'-deoxyguanosine (x1,000); 4,6-diamidino-2-phenylindole (DAPI), fluorescein isothiocyanate (FITC) and merged capture (MERGE).

PLCz Western Blotting

Sperm samples from six participants were used for preliminary detection of PLCz1 protein as described by Yoon et al. (18). Sperms were diluted to appropriate concentrations in ×2 sample buffers and stored at −20° C until use. Thawed samples were boiled for 3 min and then loaded onto 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Resolved polypeptides were transferred onto polyvinylidene difluoride membranes (Millipore) using a Mini Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were blocked in 5% nonfat dry milk in Tris-buffered saline-0.1% Tween and incubated overnight at 4° C with PLCz1 antibody (1:1000 dilution, Abcam, Cambridge, UK), followed by 1 h of incubation with rabbit horseradish peroxidase-labeled secondary antibody (1:1000 dilution, Bio-Rad). Immunoreactivity was detected using chemiluminescence according to the manufacturer's instructions (PerkinElmer) using a Kodak Image Station 440CF (Fig. 2). Western blotting procedures were repeated at least twice per sample.

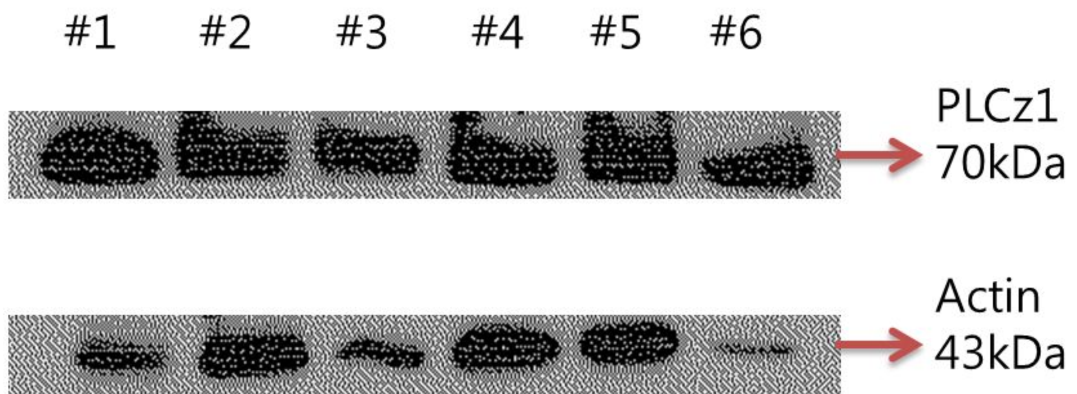

Fig. 2. Phospholipase C zeta 1 (PLCz1) protein was detected in six donors' sperm after swim up. Western blot assay demonstrates a 70 kDa of PLCz1 and a 43 kDa of actin in sperm lysates.

PLCz immunostaining

PLCz was detected by immunofluorescent staining with a polyclonal anti-PLCz antibody as described by Grasa et al. and Heytens et al. (3, 12). Sperm samples were fixed with 4% paraformaldehyde/PBS, permeabilized with 0.5% (v/v) Triton X-100/PBS and stored at 4° C until use. Sperm smears were created on pre-coated glass slides (Menzel-Gläser, Braunschweig, Germany), incubated in 3% bovine serum albumin (BSA)-PBS, and labeled with a polyclonal anti-PLCz antibody (25 μg/mL, Santa Cruz Biotechnology, Dallas, TX, USA) in 0.05% BSA-PBS overnight at 4° C. After three washes with PBS, samples were labeled with Alexa Fluor 555 goat anti-rabbit IgG (1:400 Invitrogen, Merelbeke, Belgium), counterstained with 5 μg/mL Hoechst 33258 and 5 μg/mL FITC (Alexa 488, Invitrogen, Grand Island, NY, USA), mounted in glycerol-DABCO. Sperms (n = 400), and examined using a microscopy research fluorescence (Axioskop40, Carl Zeiss Microscopy, Germany) (Fig. 3). Non-specific binding in the tail has been reported previously by Grasa et al. (3). PLCz immunostaining was performed twice in each donor sample, with consistency assessed using Pearson' s correlation test.

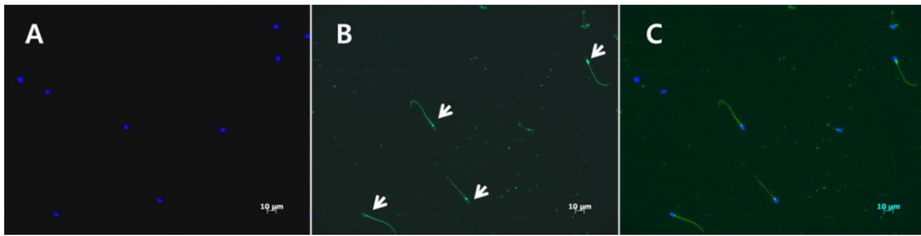

Fig. 3. A representative microphotograph showing immunofluorescent staining with a polyclonal anti-phospholipase C zeta antibody. Four immunofluorescent 'green' sperms were noted (B; white arrows, x400). Non-specific binding in the tail has been reported previously by Grasa et al. (3). (A: Hoechst 33258, B: FITC, C: merged capture).

Statistical analysis

Statistical analysis was performed using SPSS (Version 18.0,SPSS, Inc. Chicago, IL, USA). While several parameters were not normally distributed, data are expressed as the mean ± standard deviation (SD). Nonparametric Spearman' s correlation test was used to assess an association between different numerical parameters. Immunoreactivity of PLCz was averaged using two measurements. Correlation between the two measurements of PLCz was assessed by the Pearson' s correlation test because this variable revealed a normal distribution. Intergroup differences were assessed by nonparametric Wilcoxon test. A p-value of <0.05 (two-tailed) was considered statistically significant.

Results

Table 1 details the basic sperm characteristics of participant samples including DNA fragmentation index and immunoreactivity for 8-OHdG and PLCz sperms after swim-up.. As expected, motility was greatly enhanced after swim-up. DNA fragmentation index and immunoreactivity for 8-OHdG did not reveal a normal distribution due to its high variance. PLCz immunoreactivity was averaged from two measurements. When duplicate PLCz tests were performed on the same sperm samples from each of the 44 participants, similar results were obtained (74.1 ± 9.4% versus 75.4 ± 9.7%). Two measurements of PLCz were found to be highly correlated with one another ($r = 0.759$, $p < 0.001$; Fig. 4). Thus, the PLCz test was highly reproducible within the same sample. The mean intra-assay coefficient of variation for PLCz was 3.4%.

Table 1. Basic sperm parameters, DNA fragmentation index and immunoreactivity for 8−OHdG and phospholipase C zeta (PLCz) in donor sperms after swim up

	Mean ± SD	Range	Normal distribution
Concentration (million/mL)	23 ± 31	0.8−160	No
Motility (%)	93.7 ± 6.4	72.1−99.9	No
Total motile sperms (million)	10 ± 13	0.4−67	No
Strict morphology (%)	13.9 ± 7.4	3.4−47.6	Yes
DNA fragmentation index (%)	8.5 ± 9.3	0.2−34.6	No
Immunoreactivity for 8−OHdG (%)	16.8 ± 12.9	0.6−44.0	No
Immunoreactivity for PLCz (%)*	74.6 ± 9.0	47.2−92.4	Yes

*averaged from two−times measurement.

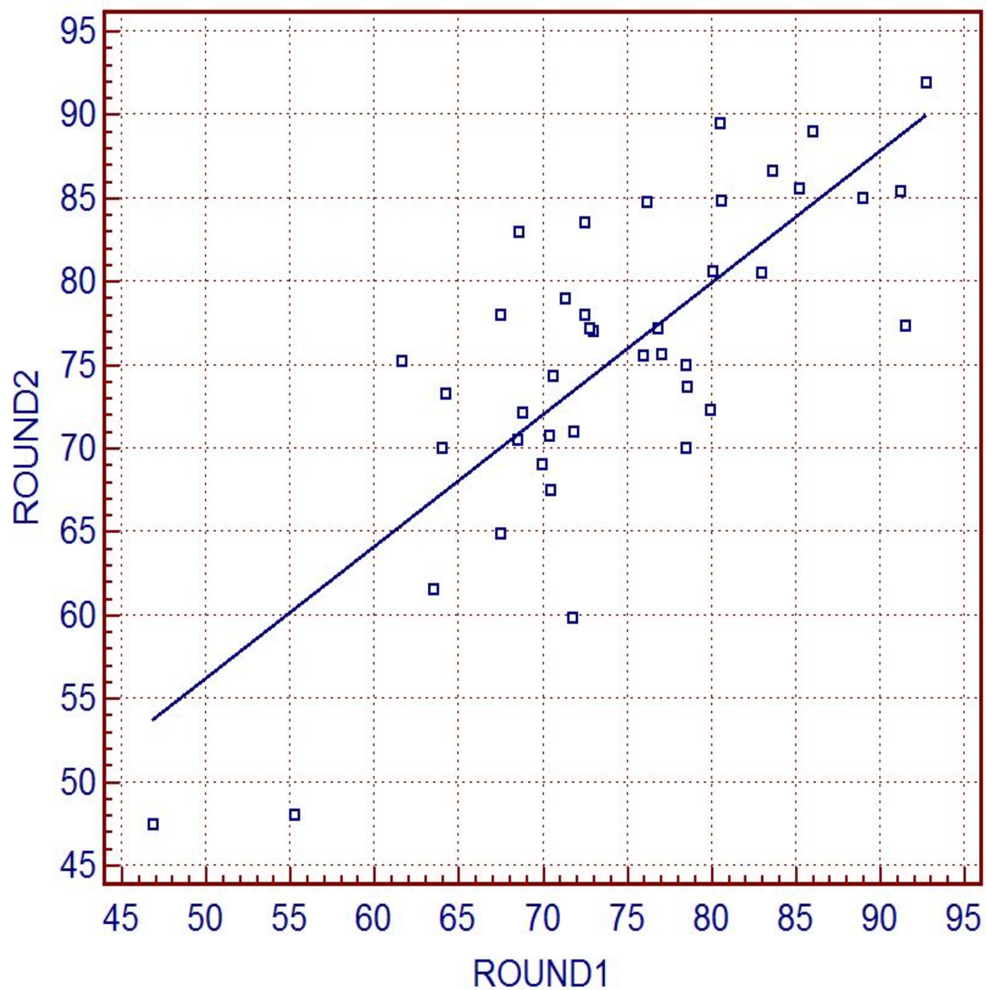

Fig. 4. Two times measurements of phospholipase C zeta immunoreactivity (round1 and round2) were highly correlated each other ($r = 0.759$, $p<0.001$, by the Pearson correlation test).

Table 2 depicts the correlations among variable parameters. Immunoreactivity for 8-OHdG was negatively correlated with sperm concentration or total motile sperm. It was also negatively correlated with immunoreactivity for PLCz ($r = -0.404$, $p < 0.05$). Multivariate analysis revealed that immunoreactivity for PLCz had a marginally significant negative correlation with immunoreactivity for 8-OHdG ($r = -0.317$, $p = 0.056$) regardless of sperm DNA fragmentation index and concentration.

Table 2. Correlation between various sperm parameters

	Conc	Mot	TMC	SM	DFI	8-OHdG	PLCz
Age	−0.104	−0.010	−0.079	−0.150	0.155	−0.128	0.075
Conc		−0.068	0.953*	0.161	−0.095	−0.387*	0.271
Mot			−0.007	0.156	−0.256	0.292	−0.221
TMC				0.159	−0.038	−0.326*	0.217
SM					−0.168	−0.039	0.055
DFI						0.216	−0.029
8-OHdG							−0.404*

*P<0.05 by Spearman correlation test.

All values represent correlation coefficient (r).

Conc: sperm concentration, Mot: sperm motility, TMC: total motile sperm count, SM: strict morphology, DFI: DNA fragmentation index, 8-OHdG: immunoreactivity for 8-hydroxy-2'-deoxyguanosine, PLCz: immunoreactivity for phospholipase C zeta.

Sixteen participants (36.4%) were smokers and no differences were found in the various sperm parameters before or after swim–up between this group and the non–smoking participants (Table 3). In smokers, cigarette exposure was not correlated with various sperm parameters before or after swim–up (Table 4). However, age was significantly correlated with smoking dose.

Table 3. Comparison of various sperm parameters between smokers and non-smokers

		Smoker (n = 16)	Non-smoker (n = 28)	P
	Age	31.6 ± 4.7	32.3 ± 6.0	NS
Before swim up	Volume (mL)	3.0 ± 1.6	2.9 ± 1.3	NS
	Concentration (million/mL)	89 ± 63	104 ± 92	NS
	Motility (%)	59.5 ± 11.4	51.8 ± 16.7	NS
	Total motile sperms (million)	171 ± 136	181 ± 282	NS
	Strict morphology (%)	11.9 ± 6.3	10.9 ± 5.2	NS
After swim up	Concentration (million/mL)	21 ± 22	24 ± 35	NS
	Motility (%)	94.5 ± 4.5	93.3 ± 7.2	NS
	Total motile sperms (million)	10 ± 10	11 ± 15	NS
	Strict morphology (%)	13.6 ± 7.1	14.2 ± 7.7	NS
	DNA fragmentation index (%)	9.5 ± 9.9	7.9 ± 9.1	NS
	Immunoreactivity for 8OHdG (%)	21.1 ± 15.9	14.6 ± 10.7	NS
	Immunoreactivity for PLCz (%)*	72.9 ± 8.4	75.5 ± 9.4	NS

By the Wilcoxon test.

*averaged from two-times measurement.

Table 4. Correlation between smoking dose and various sperm parameters in sixteen smokers

		Pack per Year	P
	Age	0.584	0.018
Before swim up	Volume (mL)	−0.003	NS
	Concentration (million/mL)	−0.421	NS
	Motility (%)	−0.313	NS
	Total motile sperms (million)	−0.358	NS
	Strict morphology (%)	−0.441	NS
After swim up	Concentration (million/mL)	0.044	NS
	Motility (%)	0.239	NS
	Total motile sperms (million)	0.212	NS
	Strict morphology (%)	−0.069	NS
	DNA fragmentation index (%)	0.010	NS
	Immunoreactivity for 8OHdG (%)	−0.127	NS
	Immunoreactivity for PLCz (%)*	−0.388	NS

By the Spearman correlation test.

*averaged from two−times measurement.

Discussion

In this study, we demonstrated that PLCz immunoreactivity has a negative relationship with the immunoreactivity of 8-OHdG. Univariate analysis suggests that PLCz has a significant negative correlation with 8-OHdG, sperm concentration, and total motile sperm. To eliminate the confounding effect of concentration and total motile sperm, we performed multivariate analysis. As a result, PLCz level has a marginal significant negative relation to 8-OHdG level, which suggests that oxidative stress may interfere with PLCz expression and that oxidative damage to sperm DNA may hinder oocyte activation.

While potential functional roles of PLCz have been proposed for infertile populations (6, 8, 12), reference values among the general population remain unknown. Numerous factors are involved in inducing sperm DNA damage, which may then result in male-factor infertility. One of these factors is the overproduction of reactive oxygen species (ROS). For example, cigarette smoking induces leukocytospermia and ROS overproduction (19, 20, 21). It appears that leukocytes can generate high levels of ROS in semen. This in turn may overwhelm the antioxidant capacity of semen and result in

oxidative stress, which could disturb sperm function (22). Sperm DNA damage can occur through ROS processes, including hydroxyl radicals or nitric oxide. Sperm DNA damage may also occur through the activation of sperm caspases and/or endonucleases by physicochemical factors such as high temperature as well as environmental factors (22). The attack of ROS on sperm DNA could first result in the formation of 8-OHdG, followed by single-stranded DNA fragmentation (23). Due to its high specificity, strong mutagenicity, and relative abundance in DNA, 8-OHdG is a known biomarker of oxidative damage in sperm chromatin. The levels of 8-OHdG in sperm analyzed by high-performance liquid chromatography are significantly higher in infertile men than in fertile controls, and have an opposite relation with sperm concentration (24). Other studies have shown that 8-OHdG levels in human sperm DNA have a significant value in predicting clinical pregnancy after intrauterine insemination but not with ICSI treatment (25). The 8-OHdG level in sperm DNA has also been shown to increase in smokers (26).

Although the predictive value of sperm DNA fragmentation testing in ART is debatable, the TUNEL method is considered

the optimal test with a high predictive value (27). One controversy is about the relationship between DNA fragmentation and ART outcomes. Host et al. reported that DNA strand breaks in human sperm impair fertilization in IVF. However, after ICSI, DNA strand breaks were not seen (28). In contrast, other studies have found a significant negative correlation between sperm DNA fragmentation and ICSI results (29, 30). Benchaib et al. found a negative correlation between sperm parameters and sperm DNA fragmentation. Furthermore, a high proportion of sperm with fragmented DNA was found to be a pejorative factor for achieving pregnancy with ICSI; however, this relationship was not observed when conventional IVF was performed (31). Likewise, the current data suggest that sperm DNA fragmentation level cannot provide independent information about embryo quality, fertilization, and pregnancy rates for infertile patients undergoing ART (32).

Our analysis shows that sperm DNA fragmentation level was unrelated to other sperm parameters including immunoreactivity of 8-OHdG and PLCz. A previous report showed that the level of 8-OHdG determined by liquid chromatography was related to the degree of damage to sperm

DNA (24). This discordance might come from the study subject; however, in previous reports, 8-OHdG level was measured in infertile men and fertile donors. In our study, we included unselected men without considering fertility status. We found a significant relation between 8-OHdG level and semen concentration and total motile sperm. Our findings concur with those of Kodama et al. (24) who reported that 8-OHdG level in male infertility has an inverse relationship with sperm concentration.

In the present study, we also found that immunoreactivity of PLCz is not associated with smoking status. Cigarette smoking is a serious health problem in most countries. However, conflicting data exist in published reports on the adverse effect of cigarette smoking on semen parameters. Although some studies report that cigarette smoking is associated with abnormal semen parameters (33, 34), others have found no relationship between smoking and sperm characteristics such as concentrations, motility, and/or morphology, which are reduced compared with those in non-smokers that often remain within the normal range (35, 36).

Although the semen quality of men with idiopathic infertility

seems not to be significantly affected by cigarette consumption, heavy smokers show significantly lower sperm concentration (37). This is assumed to be an adverse effect of ROS on sperm quality that could justify the observed sperm damage, since elevated levels of ROS have been found in infertile smoking men who showed concomitantly decreasing levels of antioxidants in seminal plasma (37). In our study, there were no differences in sperm parameters including 8-OHdG and PLCz levels between smokers and non-smokers before/after sperm preparation. In addition, they were not related to cigarette consumption (pack years).

Standard semen analysis by CASA is the first step in the assisted reproduction process and the most popular laboratory test for the diagnosis of male fertility. WHO classified semen analysis results were made according to sperm concentration, motility, morphology, and vitality. However, it is well known that routine semen analysis cannot predict the sperm-fertilizing potential, thus methods for the functional assessment of sperm quality have been developed.

In the present study, we did not examine whether PLCz level can be used as a diagnostic predictor of fertilizing potential. At

present, sperm DNA fragmentation level and 8-OHdG level are considered indicators for selection of healthy sperm. Our results suggest an agreement with those findings because immunoreactivity of 8-OHdG has a negative correlation with PLCz immunoreactivity. Therefore, men with a low proportion of PLCz may have poor IVF outcomes. However, larger cohort studies are needed to validate the association between PLCz level and fertilization or subsequent embryogenesis.

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요약(국문초록)

난자 활성화는 수정 직후 세포내 칼슘이온의 급격한 증가(칼슘진동)에 의해 유발되는데 이 과정에 정자 내 포스포리파제C 제타가 결정적인 역할을 한다고 알려져 있다. 포스포리파제 C 제타가 수정에 필수 조건이라는 여러 연구결과가 발표되고 있지만, 불임 남성의 정자에서 그것을 측정하는 것이 체외수정의 진단적 도구 또는 예후적 인자로서의 가치가 있는지는 아직 정립되지 않았다. 본 연구에서는 공여 정자를 대상으로 면역형광염색법을 통하여 포스포리파제C 제타치를 측정하고, 그것이 다른 정액변수들과 어떤 상관관계를 가지는지를 알아보았다. 44명의 공여자에서 얻은 정자 샘플을 swim up 방법으로 처리 후 농도, 운동성, 정자정밀형태를 측정하였고 TUNEL 방법으로 DNA 분절 지수를 측정하였으며, 8-하이드록시-2-데옥시구아노신 염색을 통하여 oxidative stress 정도를 간접적으로 측정하였다. 그 결과 포스포리파제C 제타치는 농도, 운동성, 정자정밀형태, DNA 분절 지수와는 상관이 없었으나 8-하이드록시-2-데옥시구아노신과 음의 상관관계를 보였다 ($r = -0.404$, $p<0.05$). 즉 정자에서 oxidative stress 정도가 높으면 포스포리파제C 제타치 발현이 낮았다. 또한 흡연군과 비흡연군 간에 포스포리파제C 제타치는 차이를 보이지 않았다. 비록 불임남성에서 측정한 것은 아니지만 포스포리파제C 제타치의 일반인에서의 분포 양상을 확립하였으며 oxidative stress 정도와 반비례 관계를 보였다.

주요어 (6단어): 포스포리파제C 제타, 8-하이드록시-2-데옥시구아노신, 산화 스트레스, 난자 활성화, 흡연, 정자검사, 보조생식술

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