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ORIGINAL ARTICLE

Sexual Function

Relation of size of seminal vesicles on ultrasound to premature ejaculation

Zhi-Wei Hong[1,*], Yu-Ming Feng[1,*], Yi-Feng Ge[1], Jun Jing[1], Xue-Chun Hu[1], Jia-Ming Shen[1], Long-Ping Peng[2], Bing Yao[1], Zhong-Cheng Xin[3]

Myriad biological factors have been proposed to explain premature ejaculation (PE). However, data correlating PE with seminal vesicles (SVs) are sparse. The study aimed to evaluate the relationship between the size of SV and PE. The cross-sectional study included 44 outpatients with PE and 44 volunteers without PE, and the size of SV was compared. Self-estimated intravaginal ejaculatory latency time, the Premature Ejaculation Diagnostic Tool (PEDT), the International Index of Erectile Function-15, and the National Institutes of Health-Chronic Prostatitis Symptom Index were used for assessment of symptoms. Compared to the control group, the PE group had significantly higher mean anterior-posterior diameter (APD) of SV ($P < 0.001$). The optimal mean APD of SV cutoff level was 9.25 mm for PE. In the PE group, PEDT was also higher with a mean APD of SV ≥9.25 mm compared with mean APD of SV <9.25 mm. PEDT was significantly correlated with the mean APD of SV ($r = 0.326$, $P = 0.031$). The seminal plasma proteins were compared between six PE and six matched control cases by mass spectrometry and it was shown that 102 proteins were at least 1.5-fold up- or down-regulated. Among them, GGT1, LAMC1, and APP were significantly higher in the PE group. These results indicated that men with a larger mean APD of SV might have a higher PEDT score. Transrectal ultrasound of SV should be considered in the evaluation of patients with premature ejaculation. SV might be a potential target for the treatment of patients with PE and ultrasound change in SV.

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INTRODUCTION

Premature ejaculation (PE) is probably the most prevalent sexual complaint in men, affecting 20%–30% of sexually active men.[1–3] According to the International Society for Sexual Medicine (ISSM), PE is defined as male sexual dysfunction characterized by (i) ejaculation that always or nearly always occurs prior to or within about 1 min of vaginal penetration from the first sexual experience (lifelong PE) or a clinically significant and bothersome reduction in latency time, often to about 3 min or less (acquired PE); (ii) the inability to delay ejaculation on all or nearly all vaginal penetrations; and (iii) negative personal consequences such as distress, bother, frustration, and/or the avoidance of sexual intimacy.[4]

Myriad biological factors have been proposed to explain PE including hypersensitivity of the glans penis,[5] disturbances in central serotonergic neurotransmission,[6] erectile difficulties[7,8] and other sexual comorbidities, prostatitis,[9] chronic pelvic pain syndrome,[10] and thyroid disorders.[11,12] However, data correlating PE with seminal vesicles (SVs) are sparse.

The ejaculatory progress is typically subdivided into three phases: emission, ejection (or penile expulsion), and orgasm.[13] Emission consists of contractions of epididymis, vas deferens, SVs, and prostate, with expulsion of sperm and seminal fluid into the posterior urethra. Ejection involves closure of bladder neck, pulsatile contractions of the bulbocavernosus and pelvic floor muscles, together with relaxation of the external urinary sphincter. The disorders of these organs may result in ejaculation disorder. Moreover, a higher frequency of sexual dysfunction was detected in male patients with change in ultrasound in male accessory gland.[14] Thus, we are curious about whether the size of SV on ultrasound is related to PE.

Nowadays, many factors are considered to be related to SV volume: age and sexual abstinence,[15] PRL,[16] smoking,[17] testosterone,[18] thyroid hormones,[19] and inflammation.[20] We aimed to avoid the effect of these confounders on SV to evaluate the relationship between the size of SV and PE and to explore the preliminary mechanism of the relationship.

MATERIALS AND METHODS

Subjects

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Jingling Hospital. From November 2014 to May 2015, patients who sought treatment for the complaints of premature ejaculation were enrolled from andrology clinics. Individuals who met the following criteria were included in the study, as reported in previous studies, if they[21–23] (i) were aged ≥18 years in a heterosexual, stable, and monogamous

[1]Center for Reproductive Medicine, Jinling Hospital, Medical School of Nanjing University, Nanjing 210002, China; [2]Center for Reproductive Medicine, Jinling Hospital, Southern Medical University, Nanjing 210002, China; [3]Andrology Center, Peking University First Hospital, Peking University, Beijing 100009, China.
[*]These authors contributed equally to this work.
Correspondence: Dr. B Yao (yaobing@nju.edu.cn) or Dr. ZC Xin (xinzc@bjmu.edu.cn)
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sexual relationship with the same female partner for at least 6 months; (ii) had erectile function domain of the International Index of Erectile Function-15 (IIEF-15) ≥26, indicating normal erectile function; (iii) were not consuming any drugs that affect sexual function or psychological status (e.g., selective serotonin reuptake inhibitors and phosphodiesterase type 5 inhibitors); (iv) were without any major psychiatric or somatic diseases; (v) did not have prostatitis-like symptoms such as complaints of perineal and/or ejaculatory pain or discomfort and their total index pain score was <4;[24] and (vi) were without infections caused by *Chlamydia trachomatis*, *Mycoplasma urealyticum*, or *Mycoplasma hominis* in semen.

PE was defined according to the ISSM. Patients who ejaculated always or nearly always prior to or within about 2 min of vaginal penetration from the first sexual experience were also included as lifelong PE.[25] A consecutive series of 44 patients was included. Among them, 28 had lifelong PE and 16 had acquired PE. Another 44 male healthy volunteers without complaints of PE or other sexual dysfunction from our andrology clinics with healthy physical examination were enrolled as a control group. All control cases had a regular sexual relationship and reported good control of ejaculation. Other exclusion measures were the same as those for the patients with PE.

Demographic characteristics and questionnaires

Before their participation, all participants provided written informed consent. Later, all patients underwent a complete andrological and physical examination. The participants were also asked to complete questionnaires including demographics, self-estimated intravaginal ejaculatory latency time (IELT), the Premature Ejaculation Diagnostic Tool (PEDT), the IIEF-15, and the National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI). Participants were categorized as current smokers if they had smoked at least 1 year, past smokers if they had smoked at least 1 year and were not current smokers, and never smokers if they had never smoked or smoked <1 year, according to a previous study.[26] Both past smokers and never smokers were counted as nonsmokers.[17]

Transrectal ultrasound imaging

All patients and control cases underwent ultrasonography following 2 to 7 days of sexual abstinence. The prostate-vesicular region was assessed at rectal ultrasonography using a 6 MHz monoplane and linear transducer through transverse, longitudinal, and oblique scans, with patients placed in the left lateral decubitus (nemio-XG580, Toshiba, Tokyo, Japan). The prostate-vesicular region was assessed at rectal ultrasonography before ejaculation as reported by Lotti *et al*.[15] Both seminal vesicle and prostate were calculated according to previous report.[15,18] To prevent bias on the part of the examiner, the ultrasonographer was unaware of the clinical data.

Serum lipids and hormonal evaluation

Blood samples were drawn in the morning (between 8:00 and 10:00 a.m.) after an overnight fast. The biochemical analyses included the triglycerides, the total cholesterol (TC), the low-density lipoprotein cholesterol, and the high-density lipoprotein cholesterol (HDL-C) level. Total testosterone, follicle-stimulating hormone, luteinizing hormone, and prolactin levels were evaluated using chemiluminescent immunoassay system (Beckman Coulter, Inc., Brea, CA, USA). The intra- and inter-assay coefficients of variation for progesterone, estradiol, testosterone, and prolactin were 8.18% and 7.89%, 5.13% and 6.23%, 2.56% and 5.19%, and 5.52% and 3.53%, respectively.

Detection of differently expressed seminal plasma proteins

All semen specimens were collected by masturbation into sterile containers after 2–7 days of sexual abstinence. The semen was tested for *C. trachomatis* using immunochromatography and cultured for *M. urealyticum* and *M. hominis*. The samples were centrifuged at 1600 ×*g* for 5 min and supernatant seminal plasma was immediately stored at −80°C. Tandem mass tag (TMT) method followed by mass spectrometry analysis was used to compare the relative expression levels of seminal plasma proteins between the PE and control groups.[27,28] Seminal plasma samples (six cases for acquired PE and six matched cases for control) were extracted and digested using trypsin. Later, the proteins were labeled with the TMT reagent. Peptide analysis was performed using the LTQ-Orbitrap instrument (Thermo Finnigan, San Jose, CA, USA) connecting to a Nano AUQUITY UPLC system via a nanospray source. Raw files of proteomics data were processed using MaxQuant (version 1.2.2.5). The false discovery rate (FDR) of the identification was estimated by searching against the databases with the reversed protein sequences. The site, peptide, and protein FDR were all set to 0.05. One-way analysis of variance was used to calculate significant differences in abundance among groups.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± s.d. when normally distributed and as median (quartiles) for parameters with nonnormal distribution. Differences of means of normally distributed parameters between two groups were assessed using unpaired Student's *t*-test or one-way analysis of variance with *post hoc* least square differences. In all other cases, the Mann–Whitney U-test was used for comparisons between the groups. Comparison of proportions was performed using the Chi-square test. Receiver operating characteristic (ROC) curves were plotted and the area under the curve (AUC) of ROC was calculated for the anterior-posterior diameter (APD) of SV before ejaculation. The optimal cutoff values for the mean APD of SV for detecting PE (according to the patients with PE and control cases) were identified using the maximum of the Youden index [(sensitivity + specificity) − 1]. Correlations were assessed using Spearman's or Pearson's method whenever appropriate. Multiple linear regression analyses were used for those factors considered to be related to SV parameters, whenever appropriate.

RESULTS

Sociodemographic, clinical, and color Doppler ultrasound characteristics of the patients with PE and control cases before ejaculation

The sociodemographic, clinical, and color Doppler ultrasound characteristics of the whole sample before ejaculation are summarized in **Table 1**. No statistically significant differences in serum lipids and hormonal levels were observed between these two groups. The PEDT score in patients with PE was significantly higher than that in the control group (12.7 ± 3.0 *vs* 2.6 ± 2.2, $P < 0.001$). The mean APD (10.6 ± 2.7 mm *vs* 8.8 ± 1.9 mm, $P < 0.001$) and SV volume ($P < 0.001$) in the PE group were significantly higher than that in the control group. Patients with PE also had significantly higher incidence of areas of endocapsulation and of wall thickening and septa than the control cases ($P = 0.031$ and $P = 0.029$, respectively). The ultrasound image of control cases and PE patients is shown in **Figure 1**. In addition, mean APD of SV was not significantly different between the acquired PE group and lifelong PE group.

Table 1: Sociodemographic, clinical, and CDU characteristics of the patients with PE and control cases before ejaculation

	PE (n=44)	*Control (n=44)*	*P*
Age (years)	30.2±6.4	29.3±4.1	0.420
Height (cm)	173.6±4.7	174.5±5.2	0.391
Weight (kg)	71.2±11.1	72.3±8.4	0.585
Smoking			0.574
Current smokers	25 (56.8)	23 (52.3)	
Past smokers	0	1 (2.3)	
Never smokers	19 (43.2)	20 (45.5)	
Sexual abstinence (days)	3.4±2.0	4.1±1.6	0.099
Total testosterone (nmol l^{-1})	14.1±5.1	13.7±2.9	0.704
PRL (mIU l^{-1})	222.3±128.7	197.7±64.8	0.312
LH (IU l^{-1})	4.0±1.7	3.9±1.6	0.811
FSH (IU l^{-1})	5.2±3.0	5.1±2.1	0.935
TC (mmol l^{-1})	4.4±0.9	4.3±0.7	0.849
TG (mmol l^{-1})	1.3±1.3	1.4±0.9	0.584
HDL (mmol l^{-1})	1.2±0.2	1.3±0.3	0.459
LDL (mmol l^{-1})	2.5±0.7	2.5±0.7	0.700
PEDT	12.7±3.0	2.6±2.2	<0.001
IIEF-15 erectile function score	27.7±1.3	28.1±1.1	0.163
NIH-CPSI pain domain score	0 (0–2.5)	0 (0–0)	0.509
NIH-CPSI voiding domain score	1.5 (0–3.0)	1.0 (0–2.0)	0.064
NIH-CPSI quality of life domain score	2.0 (0–3.0)	1.0 (0–2.0)	0.078
Self-estimated IELT (min)			<0.001
≤1	16 (36.4)	0	
1–2	25 (56.8)	0	
2–3	3 (6.8)	0	
3–4	0	1 (2.3)	
4–5	0	2 (4.5)	
≥5	0	41 (93.2)	
Mean self-estimated IELT (min)	1.2±0.6	9.3±5.4	
Type of PE			<0.001
Lifelong	27 (38.6)		
Acquired	17 (61.4)		
Duration of PE (years)	3.4±2.9		
CDU parameters			
Prostate			
Prostate volume (ml)	16.6±3.9	15.7±3.9	0.265
Prostate calcifications (%)	7 (15.9)	6 (13.6)	0.764
Inhomogeneous prostatic texture (%)	14 (31.8)	1 (18.2)	0.140
Utricular cyst (%)	0	1 (2.3)	0.315
Seminal vesicles			
Mean LD before ejaculation (mm)	36.3±4.2	35.2±4.1	0.206
Mean APD before ejaculation (mm)	10.6±2.7	8.8±1.9	<0.001
Total volume (ml)	4.2 (2.4–6.4)	2.5 (2.0–3.3)	0.001
Areas of endocapsulation (%)			0.031
Unilateral	2 (4.5)	0	
Bilateral	12 (27.3)	5 (11.4)	
Wall thickening and septa (%)			0.029
Unilateral	1 (2.3)	0	
Bilateral	9 (20.5)	2 (4.5)	
Ejaculatory ducts			
Dilated ejaculatory duct (%)			0.360
Unilateral	1 (2.3)	4 (9.1)	
Bilateral	0	0	

Data are expressed as mean±s.d. or as median (quartiles) when appropriate and as number (percentage) when categorical. A statistical analysis comparing patients with PE to control cases was performed. APD: anterior-posterior diameter; CDU: color Doppler ultrasound; FSH: follicle-stimulating hormone; HDL: high-density lipoprotein; IELT: intravaginal ejaculatory latency time; IIEF-15: International Index of Erectile Function-15; LD: longitudinal diameter; LDL: low-density lipoprotein; LH: luteinizing hormone; NIH-CPSI: National Institutes of Health-Chronic Prostatitis Symptom Index; PE: premature ejaculation; PEDT: premature ejaculation diagnostic tool; PRL: prolactin; s.d.: standard deviation; TC: total cholesterol; TG: triglyceride

ROC curve analysis of the mean APD before ejaculation for PE

AUC of ROC analysis of the mean APD of SV before ejaculation for diagnosing PE was 0.697 (95% confidence interval [95% CI]: 0.586–0.808) (**Figure 2**). The optimal mean APD of SV cutoff level as identified by the maximal Youden index was 9.25 mm for PE with modest sensitivity (61.4%) and specificity (77.3%). AUC of ROC curve analysis of SV volume for PE was the same as that of APD (data not shown). Thus, we preferred APD to volume for the following analysis.

Comparison of SV size between patients with PE with APD cutoff of 9.25 mm before ejaculation

Table 2 shows the comparison in patients with PE between the mean APD of SV ≥9.25 mm and the mean APD of SV <9.25 mm. The PEDT scores were 13.5 ± 3.0 for patients with PE with the mean APD of SV ≥9.25 mm and 11.4 ± 2.4 with the mean APD of SV <9.25 mm (P = 0.016). The mean LD (37.7 ± 4.0 mm *vs* 34.1 ± 3.6 mm, P = 0.004) and SV volume (P < 0.001) before ejaculation with the mean APD of SV ≥9.25 mm were higher than the mean APD of SV <9.25 mm. Compared with the mean APD of SV <9.25 mm group, patients with PE with the mean APD of SV ≥9.25 mm had significantly higher incidence of areas of endocapsulation and of wall thickening and septa (P < 0.001 and P = 0.003, respectively). Patients with PE with the mean APD of SV ≥9.25 mm also had significantly lower TC and LDL-C and significantly higher HDL-C (P = 0.014, P < 0.001, and P = 0.041, respectively). No statistically significant differences between the two groups with regard to self-estimated IELT and type of PE were observed.

Relationships between the PEDT and age, mean APD of SV, IIEF-15, and NIH-CPSI

The statistical analysis revealed that PEDT was significantly correlated with mean APD of SV before ejaculation (r = 0.326, P = 0.031) and IIEF-15 erectile function score (r = −0.413, P = 0.005) (**Table 3**). After adjusting for age, IIEF-15 erectile function score, NIH-CPSI pain domain score, NIH-CPSI voiding domain score, sexual abstinence, smoking, testosterone, and prolactin, PEDT was found still to be significantly correlated with mean APD of SV before ejaculation (r = 0.442, P = 0.009) (**Supplementary Table 1**).

Detection of differentially expressed proteins in seminal plasma of patients with PE and control cases

The sociodemographic, clinical, and color Doppler ultrasound characteristics of the six patients with acquired PE with mean APD of SV ≥9.25 mm and six matched control cases for TMT are summarized in **Supplementary Table 2**. No differences were found except PEDT and the mean APD of SV (12.7 ± 2.9 *vs* 2.0 ± 1.0 and 12.3 ± 0.6 mm *vs* 8.2 ± 0.6 mm, respectively). Examination of the mass spectrometry data using the MaxQuant 1.2.2.5 software revealed that 102 proteins were at least 1.5-fold up- or down-regulated (P < 0.05).

Table 2: Sociodemographic, clinical, and CDU characteristics of the patients with PE with mean APD of SV ≥9.25 mm and mean APD of SV <9.25 mm before ejaculation

	Mean APD of SV ≥9.25 mm (n=27)	*Mean APD of SV <9.25 mm (n=17)*	*P*
Age (years)	29.7±6.3	31.1±6.7	0.490
Height (cm)	174.3±4.8	172.5±4.3	0.209
Weight (kg)	72.4±12.4	69.3±8.6	0.381
Smoking			0.402
Current smokers	14 (51.9)	11 (64.7)	
Never smokers	13 (48.1)	6 (35.3)	
Sexual abstinence (days)	3.6±2.2	3.2±1.7	0.440
Total testosterone (nmol l^{-1})	15.0±6.0	12.6±2.9	0.188
PRL (mIU l^{-1})	251.9±150.0	174.5±64.7	0.089
LH (IU l^{-1})	4.2±2.0	3.7±1.2	0.400
FSH (IU l^{-1})	5.1±3.3	5.2±2.5	0.951
TC (mmol l^{-1})	4.1±1.0	4.9±0.5	0.014
TG (mmol l^{-1})	1.4±1.6	1.1±0.4	0.633
HDL-C (mmol l^{-1})	1.3±0.2	1.1±0.2	0.041
LDL-C (mmol l^{-1})	2.2±0.7	3.1±0.3	<0.001
PEDT	13.5±3.0	11.4±2.4	0.016
IIEF-15 erectile function score	27.7±1.2	27.8±1.3	0.694
NIH-CPSI pain domain score	0 (0–3.0)	0 (0–1.5)	0.656
NIH-CPSI voiding domain score	2.0 (0–3.0)	1.0 (0–2.5)	0.179
NIH-CPSI quality of life domain score	3.0 (1–5.0)	1.0 (0–3.0)	0.069
Self-estimated IELT (min)			0.504
≤1	8 (29.6)	8 (47.1)	
1–2	17 (63.0)	8 (47.1)	
2–3	2 (7.4)	1 (5.9)	
3–4	0	0	
4–5	0	0	
≥5	0	0	
Mean self-estimated IELT (min)	1.3±0.6	1.1±0.6	0.308
Type of PE			0.784
Lifelong	17 (63.0)	10 (58.8)	
Acquired	10 (37.0)	7 (41.2)	
Duration of PE (years)	3.1±2.2	4.0±3.9	0.403
CDU parameters			
Prostate			
Prostate volume (ml)	17.0±3.7	16.0±4.2	0.404
Prostate calcifications (%)	5 (18.5)	2 (11.8)	0.689
Inhomogeneous prostatic texture (%)	10 (37.0)	4 (23.5)	0.349
Utricular cyst (%)	0	0	
Seminal vesicles			
Mean LD before ejaculation (mm)	37.7±4.0	34.1±3.6	0.004
Total volume (ml)	5.7 (4.4–8.0)	2.2 (1.8–2.7)	<0.001
Areas of endocapsulation (%)			<0.001
Unilateral	2 (7.4)	0	
Bilateral	12 (44.4)	0	
Wall thickening and septa (%)			0.003
Unilateral	1 (3.7)	0	
Bilateral	9 (33.3)	0	
Ejaculatory ducts			
Dilated ejaculatory duct (%)			1.0
Unilateral	1 (3.7)	0	
Bilateral	0	0	

Data are expressed as mean±s.d. or as median (quartiles) when appropriate and as number (percentage) when categorical. APD: anterior-posterior diameter; CDU: color Doppler ultrasound; FSH: follicle-stimulating hormone; HDL-C: high-density lipoprotein cholesterol; IELT: intravaginal ejaculatory latency time; IIEF-15: International Index of Erectile Function-15; LD: longitudinal diameter; LDL-C: low-density lipoprotein-cholesterol; LH: luteinizing hormone; NIH-CPSI: National Institutes of Health-Chronic Prostatitis Symptom Index; PE: premature ejaculation; PEDT: premature ejaculation diagnostic tool; PRL: prolactin; s.d.: standard deviation; SV: seminal vesicle; TC: total cholesterol; TG: triglyceride

Among them, 101 proteins were upregulated and 1 protein was downregulated (**Supplementary Table 3**). Ingenuity® Pathway Analysis (IPA; Ingenuity® Systems, www.ingenuity.com) was used to further analyze the networks among PE, SVs, and the 102 differentially expressed proteins. Among them, GGT1, LAMC1, and APP were significantly higher in the PE group. A pathway map can be drawn for better visualization and understanding (**Figure 3**). Additional enzyme-linked immunosorbent assay was performed to verify key proteomic differences discovered using mass spectrometry analysis. It was confirmed that APP in seminal plasma was altered and the result was in agreement with mass spectrometry analysis (data not shown).

DISCUSSION

The results of this study indicate that the ultrasound change in SVs is associated with PE. A recent study demonstrated that in patients with male accessory gland infection (MAGI), patients with PE showed a mean value of the SVs APD detected before ejaculation, which was significantly higher compared to those without PE.[29] These results here represented the higher mean APD of SV in the patients with PE compared to healthy volunteers and confirmed that the peculiar ultrasound phenotype of SV may be associated with PE. In addition, these results revealed a positive linear relationship between PEDT score and the APD of SV, which was consistent with their results despite the difference in the correlation coefficient.[29] Thus, the ultrasound change in SV is possibly associated with PE.

This study demonstrated that the mean APD of SVs in the patients with PE was significantly higher than that in the control cases, with higher incidence of areas of endocapsulation before ejaculation and of wall thickening and septa. These changes in morphology may be associated with premature ejaculation. SV dilation was defined as a SV anterior-posterior diameter >14 mm,[30] suggestive of male accessory gland infection or ejaculatory duct obstruction.[30,31] In our study, the mean APD of SV in the PE patients was larger than that in the control group although the mean APD of SV in most PE patients was no more than 14 mm. In addition, both areas of endocapsulation and wall thickening and septa of SV were associated with male accessory gland infection.[30,32–34] The aim of this study was to evaluate the relationship between the size of SV on ultrasound and PE. Thus, we tried to avoid other causes for PE. In this study, patients with PLS and infection of *Chlamydia* or *Mycoplasma* were excluded to avoid the impact of infection. Since transrectal ultrasound is a valuable diagnostic technique for evaluating indirect signs of ejaculatory duct obstruction, such as dilatation, and the volume of all semen samples were more than 1.5 ml, we assumed that these patients did not have distal seminal tract obstruction. In addition, patients with other disorders that were associated with PE such as ED and hormonal disorders were excluded.

Therefore, the changes in SV are associated with PE and might be the idiopathic cause for PE in the patients with ultrasound change in SV. It needs further study to explore the cause of the changes of SV in the patients with PE. It was notable that APD of SV in this study was not that large as previous studies. This may be due to the different races and inflammatory status.

These results demonstrated that patients with PE had larger mean APD of SV. We proposed that the enlargement of SV might be related to a stronger SV contraction with ejaculation, resulting in a more likely tendency to propel the seminal fluid into the posterior urethra, thus accelerating the phase of emission. Therefore, at equal stimulus, cases with large SV may ejaculate more quickly. It was reported that SV pressure response to electrical nerve stimulation could be inhibited by selective serotonin reuptake inhibitors or alpha-adrenergic blocker in rat,[35–37] which could be the potential mechanism of the treatment for PE. Thus, it is speculated that the SV pressure plays an important role in ejaculation and the larger mean APD of SV could result in the change in SV contraction, which further causes PE. The detailed mechanism needs to be studied in the future.

Seminal plasma proteins are secreted from SVs (~65% of semen volume), prostate (~25%), testis and epididymis (~10%), and bulbourethral and periurethral glands (~1%).[38] Thus, the change in SV may be associated with different expression of seminal plasma proteins. Later, the seminal plasma proteins were determined and several differentially expressed proteins associated with SV and PE were found.

Among the 102 differentially expressed proteins, the expression of GGT1 and LAMC1 was significantly higher in the seminal plasma of the PE group compared with the control group. These two genes may be associated with SV structure. A previous study showed that homozygous mutant mouse *GGT1* gene (knockout) in mouse decreases the size of SV in male mouse.[39] Mutant mouse *LAMC1* gene (homozygous knockout) increases lack of SV in mouse.[40] Thus, *GGT1* and *LAMC1* may be associated with SV structure, and overexpression of these two genes may result in the enlargement of SVs, which could result in the change in SV pressure. In this study, the expression of APP was significantly higher in the seminal plasma of the PE group compared to the control group. A previous study showed that 3xTgAD mice treated with paroxetine significantly reduced the levels of amyloid beta-peptide (Aβ) and numbers of Aβ immunoreactive neurons in the hippocampus of male and female mice compared to 3xTgAD mice treated with saline.[41] Dysfunction and death of neurons in brain regions resulted in behavioral abnormalities in Alzheimer's disease, which are associated with extracellular accumulations of Aβ (plaques).[42] It was noticeable that paroxetine was effective in delaying ejaculation.[43] Therefore, it seems that paroxetine may delay ejaculation by reducing the levels of APP, and the higher expression of APP could be associated with the neural dysfunction, which could result in the change in SV pressure. The mechanism needs further exploration.

Several limitations of the present study should be considered. First, the cross-sectional nature of the dataset makes causal inferences problematic. Second, SV diameters were not evaluated both before and after ejaculation. Third, the NIH-CPSI was used to measure PLS in this study. However, the NIH-CPSI was not specifically developed to diagnose CP although the index had a significant power to distinguish and identify men with PLS.[21] No laboratory examinations such as Meares-Stamey four-glass test were performed in the present study to exclude bacterial prostatitis. Therefore, prostatitis was not excluded strictly. Forth, we just excluded hypothyroidism and hyperthyroidism according to the physical examination and did not measure thyroid hormones in this study. Thyroid hormones had a positive effect on SV[19] and this could be a potential bias. Fifth,

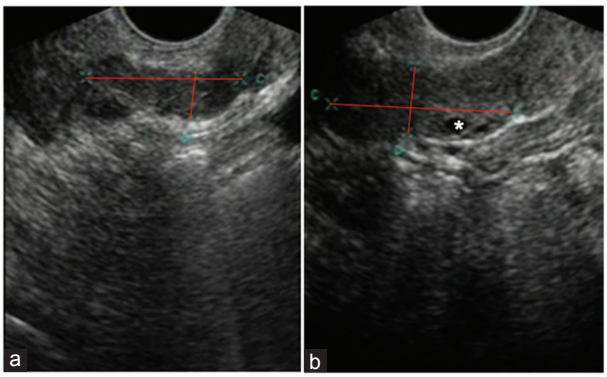

Figure 1: Ultrasound images of SVs before ejaculation. **(a)** SV of control case. **(b)** SV of patient with PE. Areas of endocapsulation (*). The red line indicates the LD and the APD. APD: anterior-posterior diameter; LD: longitudinal diameter; PE: premature ejaculation; SV: seminal vesicle.

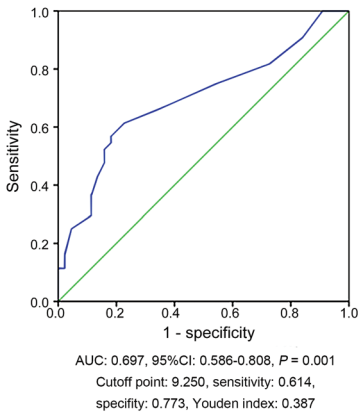

Figure 2: ROC curve analysis for the ability of mean APD of SV before ejaculation to predict PE in all patients. The optimal mean APD of SV cutoff level as identified by the maximal Youden index was 9.25 mm for PE with modest sensitivity (61.4%) and specificity (77.3%). APD: anterior-posterior diameter; AUC: area under the curve; CI: confidence interval; PE: premature ejaculation; ROC: receiver operating characteristic; SV: seminal vesicle; Youden index: (sensitivity + specificity) – 1.

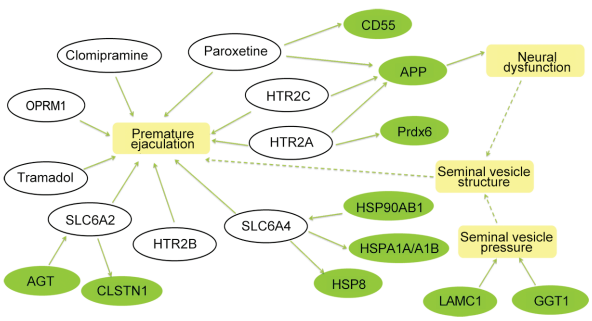

Figure 3: The networks among PE, seminal vesicles, and differentially expressed proteins in seminal plasma. The squares represent functions or diseases and the ovals represent the genes of differentially expressed proteins. Green is indicative of genes going up. The solid line with an arrow represents what was found in the IPA and the dotted line with arrow represents what was speculated.

Table 3: Spearman's correlations between PEDT and age, mean APD of SV before ejaculation, IIEF-15, and NIH-CPSI

	Mean APD of SV before ejaculation	*Age*	*IIEF-15 erectile function score*	*NIH-CPSI pain domain score*	*NIH-CPSI voiding domain score*
PEDT	0.326 (0.031)	0.080 (0.605)	–0.413 (0.005)	–0.108 (0.486)	0.043 (0.784)

Data are presented as a correlation coefficient (*P*). APD: anterior-posterior diameter; IIEF-15: International Index of Erectile Function-15; NIH-CPSI: national Institutes of Health-Chronic Prostatitis Symptom Index; PEDT: premature ejaculation diagnostic tool; SV: seminal vesicle

lifelong PE might be related to several other confounders, including psychological status. We did not take them into consideration because it is difficult during clinical practice. Sixth, this study included data from a single institution; hence, a potential selection bias may have occurred. To address this limitation, a well-designed, larger-scale assessment of the relationship of SVs and PE is warranted to validate the results of this study.

CONCLUSIONS

This study demonstrated the relationship between the size of SV and PE. Men with a larger mean APD of SV might have a higher PEDT score. Transrectal ultrasound of SV should be considered in the clinical evaluation of patients with PE. The treatment for SV might be useful in the treatment for the patients with PE and with ultrasound change in SV. SV might be a potential target for the treatment of patients with PE and ultrasound change in SV. Further studies are needed to explore the mechanism.

AUTHOR CONTRIBUTIONS

ZWH, BY, and ZCX participated in the design of the study. ZWH, YMF, XCH, and JMS participated in data collection. ZWH, YFG, JJ, and LPP performed the statistical analysis. ZWH drafted the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing financial interests. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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REFERENCES

1 Montorsi F. Prevalence of premature ejaculation: a global and regional perspective. *J Sex Med* 2005; 2 Suppl 2: 96–102.
2 Waldinger MD. Premature ejaculation: state of the art. *Urol Clin North Am* 2007; 34: 591–9, vii–viii.
3 Laumann EO, Nicolosi A, Glasser DB, Paik A, Gingell C, *et al*. Sexual problems among women and men aged 40-80 y: prevalence and correlates identified in the Global Study of Sexual Attitudes and Behaviors. *Int J Impot Res* 2005; 17: 39–57.
4 Serefoglu EC, McMahon CG, Waldinger MD, Althof SE, Shindel A, *et al*. An evidence-based unified definition of lifelong and acquired premature ejaculation: report of the second international society for sexual medicine ad hoc committee for the definition of premature ejaculation. *Sex Med* 2014; 2: 41–59.
5 Xin ZC, Choi YD, Rha KH, Choi HK. Somatosensory evoked potentials in patients with primary premature ejaculation. *J Urol* 1997; 158: 451–5.
6 Waldinger MD, Berendsen HH, Blok BF, Olivier B, Holstege G. Premature ejaculation and serotonergic antidepressants-induced delayed ejaculation: the involvement of the serotonergic system. *Behav Brain Res* 1998; 92: 111–8.
7 Jannini EA, Lombardo F, Lenzi A. Correlation between ejaculatory and erectile dysfunction. *Int J Androl* 2005; 28 Suppl 2: 40–5.
8 Chia S. Management of premature ejaculation – A comparison of treatment outcome in patients with and without erectile dysfunction. *Int J Androl* 2002; 25: 301–5.
9 Screponi E, Carosa E, Di Stasi SM, Pepe M, Carruba G, *et al*. Prevalence of chronic prostatitis in men with premature ejaculation. *Urology* 2001; 58: 198–202.
10 Gonen M, Kalkan M, Cenker A, Ozkardes H. Prevalence of premature ejaculation in Turkish men with chronic pelvic pain syndrome. *J Androl* 2005; 26: 601–3.
11 Corona G, Petrone L, Mannucci E, Jannini EA, Mansani R, *et al*. Psycho-biological correlates of rapid ejaculation in patients attending an andrologic unit for sexual dysfunctions. *Eur Urol* 2004; 46: 615–22.
12 Carani C, Isidori AM, Granata A, Carosa E, Maggi M, *et al*. Multicenter study on the prevalence of sexual symptoms in male hypo- and hyperthyroid patients. *J Clin Endocrinol Metab* 2005; 90: 6472–9.
13 Rowland D, McMahon CG, Abdo C, Chen J, Jannini E, *et al*. Disorders of orgasm and ejaculation in men. *J Sex Med* 2010; 7: 1668–86.
14 La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. High frequency of sexual dysfunction in patients with male accessory gland infections. *Andrologia* 2012; 44 Suppl 1: 438–46.
15 Lotti F, Corona G, Colpi GM, Filimberti E, Innocenti SD, *et al*. Seminal vesicles ultrasound features in a cohort of infertility patients. *Hum Reprod* 2012; 27: 974–82.
16 Lotti F, Corona G, Maseroli E, Rossi M, Silverii A, *et al*. Clinical implications of measuring prolactin levels in males of infertile couples. *Andrology* 2013; 1: 764–71.
17 Lotti F, Corona G, Vitale P, Maseroli E, Rossi M, *et al*. Current smoking is associated with lower seminal vesicles and ejaculate volume, despite higher testosterone levels, in male subjects of infertile couples. *Hum Reprod* 2015; 30: 590–602.
18 Lotti F, Maggi M. Ultrasound of the male genital tract in relation to male reproductive health. *Hum Reprod Update* 2015; 21: 56–83.
19 Lotti F, Maseroli E, Fralassi N, Degl'Innocenti S, Boni L, *et al*. Is thyroid hormones evaluation of clinical value in the work-up of males of infertile couples? *Hum Reprod* 2016; 31: 518–29.
20 Lotti F, Maggi M. Interleukin 8 and the male genital tract. *J Reprod Immunol* 2013; 100: 54–65.
21 Tang D, Zhang X, Hao Z, Zhou J, Liang C. Prevalence of prostatitis-like symptoms in outpatients with four premature ejaculation syndromes: a study in 438 men complaining of ejaculating prematurely. *Int J Clin Exp Med* 2014; 7: 1829–36.
22 Zhang X, Gao J, Liu J, Xia L, Yang J, *et al*. Distribution and factors associated with four premature ejaculation syndromes in outpatients complaining of ejaculating prematurely. *J Sex Med* 2013; 10: 1603–11.
23 Gao J, Zhang X, Su P, Peng Z, Liu J, *et al*. The impact of intravaginal ejaculatory latency time and erectile function on anxiety and depression in the four types of premature ejaculation: a large cross-sectional study in a Chinese population. *J Sex Med* 2014; 11: 521–8.
24 Nickel JC, Downey J, Hunter D, Clark J. Prevalence of prostatitis-like symptoms in a population based study using the National Institutes of Health chronic prostatitis symptom index. *J Urol* 2001; 165: 842–5.
25 Waldinger MD. Recent advances in the classification, neurobiology and treatment of premature ejaculation. *Adv Psychosom Med* 2008; 29: 50–69.
26 Corona G, Mannucci E, Petrone L, Ricca V, Mansani R, *et al*. Psychobiological correlates of smoking in patients with erectile dysfunction. *Int J Impot Res* 2005; 17: 527–34.
27 Yang X, Liu F, Yan Y, Zhou T, Guo Y, *et al*. Proteomic analysis of N-glycosylation of human seminal plasma. *Proteomics* 2015; 15: 1255–8.
28 Gao W, Xu J, Wang F, Zhang L, Peng R, *et al*. Plasma membrane proteomic analysis of human Gastric Cancer tissues: revealing flotillin 1 as a marker for Gastric Cancer. *BMC Cancer* 2015; 15: 367.
29 La Vignera S, Condorelli RA, Vicari E, Favilla V, Morgia G, *et al*. Acquired premature ejaculation and male accessory gland infection: relevance of ultrasound examination. *Asian J Androl* 2015. Doi: 10.4103/1008-682X.155539. [Epub ahead of print].
30 Vicari E. Seminal leukocyte concentration and related specific reactive oxygen species production in patients with male accessory gland infections. *Hum Reprod* 1999; 14: 2025–30.
31 Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, *et al*. European Association of Urology guidelines on Male Infertility: the 2012 update. *Eur Urol* 2012; 62: 324–32.
32 Kim B, Kawashima A, Ryu JA, Takahashi N, Hartman RP, *et al*. Imaging of the seminal vesicle and vas deferens. *Radiographics* 2009; 29: 1105–21.
33 Lotti F, Corona G, Mancini M, Filimberti E, Degli Innocenti S, *et al*. Ultrasonographic and clinical correlates of seminal plasma interleukin-8 levels in patients attending an andrology clinic for infertility. *Int J Androl* 2011; 34: 600–13.
34 La Vignera S, Calogero AE, Arancio A, Castiglione R, De Grande G, *et al*. Transrectal ultrasonography in infertile patients with persistently elevated bacteriospermia. *Asian J Androl* 2008; 10: 731–40.
35 Hsieh JT, Chang HC, Law HS, Hsieh CH, Cheng JT. *In vivo* evaluation of serotonergic

agents and alpha-adrenergic blockers on premature ejaculation by inhibiting the seminal vesicle pressure response to electrical nerve stimulation. *Br J Urol* 1998; 82: 237–40.
36 Kim SW, Paick JS. Peripheral effects of serotonin on the contractile responses of rat seminal vesicles and vasa deferentia. *J Androl* 2004; 25: 893–9.
37 Kang KK, Ahn GJ, Sung JH, Kim SH, Kim H, *et al*. Ejaculatory responses are inhibited by a new chemical entity, DA-8031, in preclinical rodent models of ejaculation. *Urology* 2013; 81: 920.e13–8.
38 Drabovich AP, Saraon P, Jarvi K, Diamandis EP. Seminal plasma as a diagnostic fluid for male reproductive system disorders. *Nat Rev Urol* 2014; 11: 278–88.
39 Kumar TR, Wiseman AL, Kala G, Kala SV, Matzuk MM, *et al*. Reproductive defects in gamma-glutamyl transpeptidase-deficient mice. *Endocrinology* 2000; 141: 4270–7.
40 Willem M, Miosge N, Halfter W, Smyth N, Jannetti I, *et al*. Specific ablation of the nidogen-binding site in the laminin gamma1 chain interferes with kidney and lung development. *Development* 2002; 129: 2711–22.
41 Nelson RL, Guo Z, Halagappa VM, Pearson M, Gray AJ, *et al*. Prophylactic treatment with paroxetine ameliorates behavioral deficits and retards the development of amyloid and tau pathologies in 3xTgAD mice. *Exp Neurol* 2007; 205: 166–76.
42 Selkoe DJ, Schenk D. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol* 2003; 43: 545–84.
43 Waldinger MD, Hengeveld MW, Zwinderman AH. Paroxetine treatment of premature ejaculation: a double-blind, randomized, placebo-controlled study. *Am J Psychiatry* 1994; 151: 1377–9.

Supplementary Table 1: Multiple linear regression test of the relationship between PEDT and mean APD of SV before ejaculation

	PEDT		
	β	*Standard error*	*P*
Mean APD of SV before ejaculation			
Model 1	0.343	0.166	0.044
Model 2	0.395	0.151	0.013
Model 3	0.423	0.147	0.007
Model 4	0.442	0.159	0.009

APD: anterior-posterior diameter; IIEF-15: International Index of Erectile Function-15; NIH-CPSI: national Institutes of Health-Chronic Prostatitis Symptom Index; PEDT: premature ejaculation diagnostic tool; SV: seminal vesicle. Model 1: adjusting for age; Model 2: adjusting for age; IIEF-15 erectile function score; NIH-CPSI pain domain score; and NIH-CPSI voiding domain score; Model 3: adjusting for age; IIEF-15 erectile function score; NIH-CPSI pain domain score; NIH-CPSI voiding domain score; sexual abstinence and smoking; Model 4: adjusting for age; IIEF-15 erectile function score; NIH-CPSI pain domain score; NIH-CPSI voiding domain score; sexual abstinence; smoking; testosterone and prolactin

Supplementary Table 2: Sociodemographic, clinical, and CDU characteristics of the PE patients with PE and matched control cases for TMT

	PE (n=6)	*Control (n=6)*	*P*
Age (years)	31.3±10.5	35.0±4.4	0.606
Height (cm)	174.0±4.4	176.0±9.2	0.756
Weight (kg)	64.0±10.6	79.3±16.2	0.252
Sexual abstinence (days)	4.7±2.5	5.3±1.5	0.715
Total testosterone (nmol l^{-1})	11.3±3.6	9.6±2.7	0.549
PRL (mIU l^{-1})	317.7±241.0	176.3±46.8	0.377
LH (IU l^{-1})	5.3±2.8	4.6±1.3	0.687
FSH (IU l^{-1})	4.5±1.9	5.7±1.1	0.412
TC (mmol l^{-1})	4.9±0.3	4.7±0.5	0.592
TG (mmol l^{-1})	1.5±1.2	1.5±0.2	0.962
HDL (mmol l^{-1})	1.3±0.3	1.1±0.2	0.308
LDL (mmol l^{-1})	2.6±0.5	2.9±0.4	0.372
PEDT	12.7±2.9	2.0±1.0	0.004
IIEF-15 erectile function score	27.3±1.5	29.0±1.0	0.189
NIH-CPSI pain domain score	0±0	0±0	
NIH-CPSI voiding domain score	2.7±0.6	1.0±1.0	0.067
NIH-CPSI quality of life domain score	2.3±0.6	0.7±1.2	0.089
CDU parameters			
Prostate			
Prostate volume (ml)	17.4±2.4	20.3±1.3	0.143
Prostate calcifications (%)	0	0	
Inhomogeneous prostatic texture (%)	0	0	
Utricular cyst (%)	0	0	
Seminal vesicles			
Mean LD before ejaculation (mm)	34.0±3.5	32.3±2.8	0.552
Mean APD before ejaculation (mm)	12.3±0.6	8.2±0.6	0.001
Total volume (ml)	5.5±0.8	2.3±0.5	0.004
Areas of endocapsulation before ejaculation (%)			
Unilateral	0	0	
Bilateral	0	0	
Wall thickening and septa (%)			0.002
Unilateral	0	0	
Bilateral	100	0	
Ejaculatory ducts			
Dilated ejaculatory duct (%)			
Unilateral	0	0	
Bilateral	0	0	

Data are expressed as mean±s.d. or as median (quartiles) when appropriate and as number (percentage) when categorical. A statistical analysis comparing patients with PE to control cases was performed. PE: premature ejaculation; TMT: tandem mass tag; APD: anterior-posterior diameter; CDU: color Doppler ultrasound; FSH: follicle-stimulating hormone; HDL: high-density lipoprotein; IELT: intravaginal ejaculatory latency time; IIEF-15: International Index of Erectile Function-15; LD: longitudinal diameter; LDL-C: low-density lipoprotein-cholesterol; LH: luteinizing hormone; NIH-CPSI: national Institutes of Health-Chronic Prostatitis Symptom Index; PEDT: premature ejaculation diagnostic tool; PRL: prolactin; s.d.: standard deviation; TC: total cholesterol; TG: triglyceride