Int J Clin Exp Med 2018;11(7):7088-7095
www.ijcem.com /ISSN:1940-5901/IJCEM0074955

Original Article

Study of chromosome detection and influencing factors in infertile patients with varicocele

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Received February 25, 2018; Accepted April 4, 2018; Epub July 15, 2018; Published July 30, 2018

Abstract: Objective: To study the chromosome characteristics of infertile patients with varicocele (VC) and the pathogenic factors and influencing factors of VC infertility. Methods: A total of 198 infertile men with VC diagnosed by male infertility specialist in Maternal and Child Health Hospital of Zibo City from March 2014 to March 2017 were divided into three groups: VC degree I (n=61), degree II (n=66), and degree III (n=71), according to the degree of VC. Fifty-two males of normal fertility without VC were chosen as the control group. Chromosome nuclear technique analysis, semen routine examination, and Y chromosome micro-deletion examination were performed, and the level of serum neutral hormone was measured. Results: The abnormal rates of chromosome karyotype (15.15%) and the percentage of abnormal chromosome number in total abnormality (83.33%) of VC patients were all higher than those of normal control group (both P<0.001), and the abnormal rate of chromosome increased in proportion to the degree of VC (P=0.018). Compared with the control group, in VC patients, the sperm density, the sperm motility rate, and the ratio of sperm a + b decreased. As degree of VC increased, sperm density, sperm motility rate, and the ratio of sperm a + b decreased (all P<0.05). Compared with the normal control group, the sperm deformity rate increased in VC patients (P=0.011), and the liquefaction time was not statistically significant (P=0.083). In the range of VC I to VC II, the total loss rate of VC azoospermia factor went up (VC II, P<0.05) with the increase of the degree of VC, and the total rate of three groups were higher than that of the control group (VC II and VC III, both P<0.05). With the increase of the degree of VC, a positive correlation was observed between the value of follicle stimulating hormone, luteinizing hormone, and pituitary prolactin in the blood and the degree of VC (all P<0.05). There was no significant correlation between testosterone value, estradiol, and the degree of VC. Conclusion: The degree of VC can influence the quality of semen and the levels of reproductive hormones, and it has a certain correlation with the total loss of Y chromosome abnormalities.

Keywords: Varicocele infertility, chromosome detection, screening

Introduction

Varicocele (VC) is caused by the backflow or valve failure of the internal spermatic venous plexus. Blood stasis caused by blood reflux lead to the tortuosity, dilatation or extend of pampiniform plexus. The symptoms include increase of scrotal temperature, change of local blood microcirculation, oxidative stress reaction, and other pathological phenomena. It affects the semen quality of the male and therefore, it is a common cause of male infertility [1, 2]. The incidence of VC in the general population is 10-15%, while in male infertility patients, the incidence is as high as 40% [3]. Related scholars have done a lot of research on VC infertility, but currently the mechanism of VC causing infertility is not clear yet, clinical trial data of chromosome detection and analysis of related factors are insufficient [4-7]. From the perspective of the relationship between the degree of VC and the semen quality of the patients, the cause of VC sterility was investigated by chromosome nuclear technology analysis, chromosome microdeletion detection and analysis, and routine detection. This study provides some basis for the study of the mechanism of VC infertility.

Materials and methods

Research subjects

This study was approved by the Medical Ethics Committee of Maternal and Child Health Hospital of Zibo City, and all the patients signed

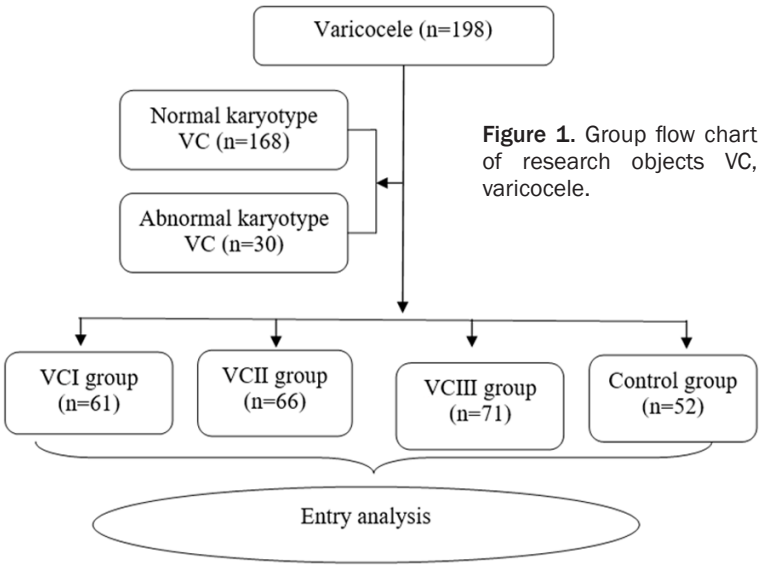

Figure 1. Group flow chart of research objects VC, varicocele.

the informed consent. The data of 198 infertile males with VC admitted to our male infertility clinic from March 2014 to March 2017 were collected in this study. The patients' average age was 27.6 ± 3.7 years. The diagnostic standard for VC: The diameter of unilateral or bilateral spermatic vein tested by color Doppler ultrasound was ≥0.2 cm. Exclusion criteria: Infertility patients caused by immune factors, cryptorchidism, reproductive hormone abnormalities, reproductive tract infections, or obstructive azoospermia. The control group consisted of 52 cases with no VC, normal reproductive history or verified normal fertility. Karyotype analysis was done in all patients, including 30 cases of abnormal chromosome karyotype and 168 cases of normal karyotype. Azoospermia factor (AZF) gene was detected in 198 patients with VC. According to the results of physical examination and color Doppler ultrasound examination, 198 cases of VC infertile patients were grouped according to the degree of VC: VC degree I (61 cases): positive clinical palpation and the maximum inner diameter (DR) of the spermatic vein on natural breath was ≤2.5 mm, and the duration of reflux (TR) was 2-4 s; VC degree II (66 cases): positive clinical palpation, DR=2.5-3.5 mm, TR=4-6 s; VC degree III (71 cases): positive clinical palpation, DR ≥3.5 mm, TR ≥6 s [8]. See **Figure 1**.

Research methods

Cytogenetic examination: In the aseptic condition, the peripheral blood lymphocytes of the patients were cultured for 72 h under 37°C. Conventional production and chromosome karyotype analysis of G-banding were performed. Chromosome karyotype analysis showed that six karyotypes out of 30 metaphases in each case were analyzed, and the chromosomes were named according to the naming principles of human genetic ISCN.

Semen routine examination: After semen centrifugation, the sedimentation smear microscopic examination was performed. The detection indexes mainly included the sperm density, sperm viability, sperm a + b level, sperm deformity rate, and liquefaction time.

DNA extraction, PCR and electrophoresis detection: Peripheral blood (2 mL) was drawn from patients and stored in EDTA anticoagulation tube. Routine extraction of DNA was performed for PCR amplification according to the instruction of blood DNA extraction kit. After multiplex PCR, the products were detected with 2% agarose gel electrophoresis. DNA electrophoresis bands were observed with gel scanner and the results were recorded. Six STS and Sex determining gene (SRY) loci in 3 regions (AZFa, AZFb and AZFc) on the Y chromosome were selected for primer setting. SRY was the internal control, and the water was the blank control. The SRY loci were amplified, and no bands were amplified in the blank control. Six spermatogenic sites in the normal male control group were amplified. If all the loci were amplified, and the fragment size met the design requirements, then it could be judged as no deficiency. If the internal control (SRY locus) of the pending inspection sample was amplified, and the site points of the six sites of AZF weren't amplified, it was judged as deficiency [9, 10].

Determination of reproductive hormones: Peripheral venous blood (4 mL) were drawn, and the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), pituitary prolactin (PRL), estradiol (E_2) and testosterone (T) were measured by chemiluminescent microparticle immunoassay. The normal values of male hormones in Maternal and Child Health Hospital of Zibo City were: FSH (1.37-13.58 mIU/mL), LH

Table 1. Abnormal chromosome karyotype and performance of 30 infertile men with varicocele

Abnormal type	Karyotype	Case	Performance
Abnormal chromosome number	46, XX	1	Azoospermia
	47, XXY	21	Azoospermia, small testicle disease
	47, XYY	3	Severe oligozoospermia, sperm deformity
Abnormal chromosome structure	46, XY, inv (Y) (p11q12)	3	Azoospermia
	46, XY, inv (9) (p11q13)	1	Severe oligozoospermia
	46, XY, del (Y) (q11)	1	Azoospermia

Table 2. Incidence comparison of the of abnormal chromosome karyotype in varicocele patients with different degree in each group

Type of patient	VC group (n=198)	VC I group (n=61)	VC II group (n=66)	VC III group (n=71)	Control group (n=52)
Abnormal chromosome number					
46, XX	1			1	
47, XXY	21	2	4	15	
47, XYY	3	1	1	1	
Abnormal chromosome structure					
46, XY, inv (Y) (p11q12)	3			3	
46, XY, inv (9) (p11q13)	1	1			
46, XY, del (Y) (q11)	1			1	
Total abnormal rate	15.15%	6.56%	7.58%	29.58%	0
P	0.003*	0.060*	0.043*, 0.823#	0.000*, 0.001#, 0.000$	

Note: VC, varicocele. *Compared with control group, #compared with VC I group, $compared with VC II group.

Table 3. Linear trend test of the degree of varicocele and chromosome abnormality ratio

Degree of varicocele	Abnormal chromosome number (n, %)	Abnormal chromosome structure (n, %)	Total chromosome abnormality rate (n, %)
VC I (n=61)	3	1	4
VC II (n=66)	5	0	5
VC III (n=71)	17	4	21
LLA	0.184	5.221	0.192
X^2	5.69	15.44	7.80
P	0.021	0.640	0.018

Note: VC, varicocele.

(1.14-8.75 mIU/mL), PRL (2.58-18.12 ng/mL), E_2 (11.00-44.00 pg/mL), T (1.56-8.77 ng/mL).

Statistical methods

SPSS17.0 software was used for analysis. The measurement data are expressed by $\bar{x} \pm sd$; the comparison between groups was made with one-way ANOVA and Bonferroni test. Counting data were expressed by rate and compared Chi-square test was adopted and $P< 0.05$ was considered statistically significant. And the relationship between the degree of VC and chromosome abnormality, semen quality (sperm viability, sperm a + b ratio, sperm deformity rate) was examined with chi-square linear trend test. Correlation between the degree of VC and the level of reproductive hormones, sperm density and semen liquefaction time were analyzed with Pearson correlation analysis.

Results

Chromosome karyotype examination

One hundred and ninety-eight infertile patients with VC in Maternal and Child Health Hospital

Table 4. Comparison of the degree of varicocele with analysis results of semen ($\bar{x} \pm sd$)

Group	Total VC group	VC I group	VC II group	VC III group	Control group
Case	198	61	66	71	52
Sperm density (10^6/mL)	27.31 ± 6.16*	39.54 ± 9.73*	25.84 ± 8.78*,#	16.31 ± 6.29*,$,^	56.26 ± 10.23
Sperm survival rate (%)	34.12 ± 10.03*	41.23 ± 12.17*	36.58 ± 11.22*,#	23.66 ± 10.12*,$,^	65.43 ± 10.68
a + b scale of sperm (%)	21.66 ± 4.42*	28.19 ± 4.21*	24.86 ± 2.63*,#	18.35 ± 3.32*,$,^	58.85 ± 4.32
Sperm abnormality rate (%)	14.13 ± 5.56*	6.68 ± 2.13*	12.17 ± 6.26*,#	25.56 ± 4.02*,$,^	0.18 ± 0.12
Liquefying time (min)	26.12 ± 2.42*	21.02 ± 1.95*	23.15 ± 1.48*	28.74 ± 2.36*	14.93 ± 1.65

Note: VC, varicocele; *compared with control group; #compared between VC II and VC I groups; $P compared between VC III and VC II groups; ^compared between VC III and VC I group; sperm density (*P=0.002/0.011/0.000/0.013, #P=0.001, $P=0.022, ^P=0.004), sperm survival rate (*P=0.001/0.000/0.021/0.003, #P=0.002, $P=0.013, ^P=0.011), a + b scale of sperm (*P=0.003/0.001/0.024/0.001, #P=0.011, $P=0.002, ^P=0.015), sperm abnormality rate (*P=0.011/0.003/0.012/0.013, #P=0.024, $P=0.013, ^P=0.003), there was no significant difference in liquefying time (*P=0.083/0.061/0.130/0.092).

Table 5. Linear trend test or Pearson correlation analysis of the degree of varicocele and semen parameters

Degree of varicocele	Sperm density (10^6/mL)	Sperm viability (%)	a + b scale of sperm (%)	Sperm abnormality rate (%)	Liquefying time (min)
~LLA/*r	-0.970*	-0.965~	-0.963~	0.972~	0.995*
P	0.010	<0.001	0.006	0.021	0.021

Note: *Pearson correlation analysis; ~linear trend test.

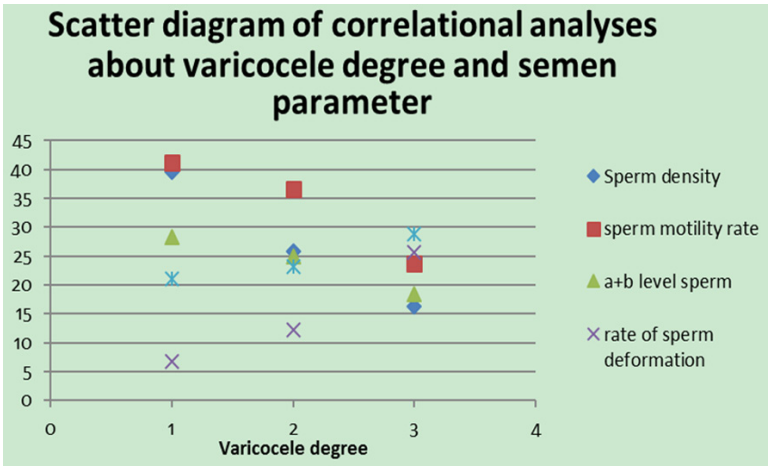

Figure 2. Scatter diagram of correlation analysis between the degree of varicocele and the semen parameters.

of Zibo City underwent the karyotype examination. The abnormal karyotype was found in 30 cases with a total abnormal rate of 15.15%. The patients mainly were diagnosed as azoospermia or severe oligozoospermia (**Table 1**). A total of 25 cases were diagnosed as abnormal chromosome number, accounting for 83.33% of the total abnormalities, and 5 cases as abnormal chromosome structure. The number of patient with normal karyotype was 168 and normal karyotype was 46, XY. No abnormal karyotype was observed among 52 normal men in the control group, and the difference of the incidence of abnormal karyotype between the control group and the control group showed statistically significant (X^2=8.95, P=0.003). In addition, analysis results of chromosomal abnormalities in patients with varying degrees of VC showed that the abnormal rates of chromosome in VC I, II and III groups were 6.56%, 7.58% and 29.58%, respectively. Compared with the control group, the VC I group showed no statistical significance (P=0.060), while the statistical differences were observed in VC II and III group (P=0.043, P<0.001). Between three VC groups, there was no significant difference between the VC II group and VC I group (P=0.823), and the rest had significant difference (both P<0.01). See **Table 2**. With the increase of VC, the total abnormal rate of chromosomes increased (P=0.018, **Table 3**).

Effect of the degree of VC on semen and correlation analysis

Sperm density of VC patients was lower than that of the control group (P=0.002) and the

Table 6. Comparison of the types and incidence of Y chromosome microdeletion in each group

Type of patient	n	AZFa	AZFb	AZFc	AZFb + c	AZFa + b + c	Total loss rate	P
VC	198	1	1	17	3		11.11%	0.012*
VC I group	61					1	1.64%	0.860*
VC II group	66			13	1		21.21%	0.004*
VC III group	71	1	1	4	2		11.27%	0.012*
Control group	52						0	

Note: *Compared with control group. VC, varicocele; AZF, azoospermia factor.

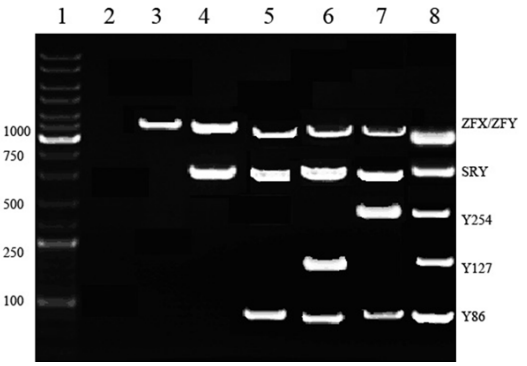

Figure 3. Multiple PCR electrophoresis. 1: Marker for molecular weight reference. 2: Blank control. 3: Normal females (negative control). 4: Patients with AZFa + b + c deletion. 5: Patients with AZFb + c deletion. 6: Patients with AZFc deletion. 7: Patients with AZFb deletion. 8: Patients with AZFa deletion.

sperm density decreased gradually with the increase in the degree of VC (P=0.010). The sperm viability decreased significantly with the increase in the degree of VC (P<0.001). Compared with the control group, the ratios of sperm a + b in the groups of VC patients were less than half of the normal value in the control group (all P<0.05). The rate of sperm deformity increased gradually with the increase in degree of VC (P=0.021). The above parameters were compared with those of the control group and the differences were statistically significant (all P<0.05). Liquefaction time increased with the increase in the degree of VC (P=0.021, **Tables 4**, **5** and **Figure 2**).

AZF microdeletion detection

AZF microdeletion was carried out on 198 patients, among which 1 case of AZF microdeletion was found in the VC I group, and the deletion rate was 1.64%; AZF microdeletion was found in 14 of 66 patients in VC II group, and the deletion rate was 21.21%, it mainly concentrated on AZFc microdeletion; the detection rate of AZF microdeletion in VC III group was 11.27%, compared with the control group, the VC II group had significant statistical significance (P=0.004). The VC III group had statistically significant (P=0.012), while the VCI group was compared with the control group. There was no statistical significance (P=0.860). AZF microdeletion was not found in the control group. See **Table 6** and **Figure 3**.

Comparison and correlation analysis of reproductive hormones in each group

The detection of reproductive hormone in 198 patients with VC showed that: compared with the normal control group, FSH, LH and PRL in VCIII group were significantly higher than that of the control group (P<0.01). T also showed a significant decrease (P=0.012) and no significant change in E_2 (P=0.053). The VC I group and the VC II group were compared with the control group. The value of FSH, LH and PRL also increased in varying degrees, and the T value decreased, but the degree of the increase or decrease was smaller than that of VC III group. See **Table 7**.

The correlation study between the level of reproductive hormone and the degree of VC in patients with VC infertility showed that the relationship between the value of FSH, LH, and PRL and the degree of VC was positive (P<0.05); while comparing T value and E_2 with the degree of VC, there was no significant correlation (P=0.560). See **Table 8**.

Discussion

At present, about 15% of couples at childbearing age in the world are infertile, of which about 50% are caused by male infertility [11]. Many factors can affect male infertility, and VC is a common cause of male infertility [12-15]. According to different spermatogenic functions, the semen quality of VC patients can be graded as normal, oligospermia, severe oligozoospermia, and even azoospermia. In this study, chromosome karyotype and multiplex PCR

Table 7. Comparison of reproductive hormone levels among patients in each group

Group	Total VC group	VC I group	VC II group	VC III group	Control group
Case	198	61	66	71	52
FSH (mIU)	19.88 ± 7.13*	15.34 ± 4.23*	21.34 ± 6.78*,#	39.34 ± 11.78*,$,^	6.14 ± 1.23
LH (mIU)	20.33 ± 8.15*	13.76 ± 8.13*	19.58 ± 9.27*,#	34.28 ± 10.16*,$,^	5.36 ± 0.38
PRL (ng/mL)	13.24 ± 3.14*	8.12 ± 1.37*	14.65 ± 2.67*,#	18.69 ± 4.26*,$,^	5.86 ± 1.35
E_2 (pg/mL)	20.42 ± 11.29*	20.56 ± 11.02*	21.35 ± 12.26*,#	20.06 ± 12.18*,$,^	20.18 ± 10.27
T (ng/mL)	3.13 ± 1.52*	3.74 ± 1.36*	3.15 ± 1.48*,#	3.02 ± 1.95*,$,^	8.93 ± 2.11

Note: VC, varicocele; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, pituitary prolactin; E_2, estradiol; T, testosterone. *compared with the control group; #compared with VC I group; $compared with VCII group; ^P was compared with VC I group; FSH (*P=0.001/0.001/<0.001/0.023, #P<0.001, $P=0.013, ^P=0.003), LH (*P=0.001/0.001/0.011/0.003, #P=0.004, $P=0.023, ^P=0.034), PRL (*P=0.003/0.021/0.025/0.000, #P=0.021, $P=0.012, ^P=0.005), E_2 (*P=0.001/0.002/0.062/0.053, #P=0.004, $P=0.011, ^P=0.001), T (*P=0.003/0.031/0.21/0.012, #P#=0.002, $P=0.001, ^P=0.001).

Table 8. Pearson correlation analysis of the degree of varicocele and reproductive hormone levels in patients with VC infertility

Degree of varicocele	FSH (mIU)	LH (mIU)	PRL (ng/mL)	E_2 (pg/mL)	T (ng/mL)
r	0.862	0.873	0.960	0.494	-0.832
P	0.016	0.015	0.040	0.560	0.070

Note: VC, varicocele; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, pituitary prolactin; E_2, estradiol; T, testosterone.

detection technology were used to analyze the chromosome characteristics of infertile patients with VC, and to analyze correlation between the degree of VC and semen quality and reproductive hormone levels.

Among 198 cases of VC, 30 cases were diagnosed with chromosome abnormality with a total abnormal rate of 15.15%, which was significantly higher than that of the control group, and consistent with the reported chromosomal abnormality rate of 2.2-19.6% in male infertility patients reported at home and abroad [16]. Chromosome abnormalities can affect the patient's sperm production, resulting in infertility. So it's necessary to carry out chromosome karyotype examination in patients with VC. The analysis of semen quality parameters mainly includes semen density, vitality and morphology, which are important basis for the diagnosis of male infertility [17]. It is found in the study that a correlation exists between the degree of VC and the related parameters of semen quality. With the increase in the degree of VC, the sperm density, sperm viability, and sperm a + b ratio decreased and they are negatively correlated (r=-0.970, LLA=-0.965, LLA=-0.963). As the degree of VC increases, sperm deformity rate and liquefaction time increase too and they are positively correlated (LLA=0.972, r= 0.995). This is consistent with Damsgaard's relation research [18].

Infertility of patients with VC is also closely related to Y chromosome microdeletion [19]. As for the analysis of the chromosome microdeletion, many scholars point out that a lot of gene regulation is needed to produce sperm. Particularly, the AZF region Yq11 is very important in spermatogenesis. This region was divided into AZFa, AZFb, AZFc (and AZFd) [20, 21]. In this study, it was found that the total deletion rate of Y chromosome microdeletion in VC II group and VC III group of infertile patients with VC was relatively high, and mainly concentrated in AZFc. AZFc deletion had the highest probability and AZFb and AZFa deletion followed, which was in accordance with some studies of chromosome microdeletion in male infertility of related scholars [9].

Reproductive hormones play a very important role in the formation of spermatozoa. Related studies have shown that FSH and LH with a certain concentration and pulse frequency are necessary in the process of spermatogenesis [22]. Some studies suggest that spermatogenesis is the synergistic effect of FSH acting on supporting cells and LH through stimulating the secretion of T by interstitial cells [8]. The results analysis of reproductive hormone test in 198 patients with VC infertility showed that with the increase in degree of VC, the values of FSH, LH

and PRL were all significantly increased, and T value decreased. While the E_2 value did not change significantly. The results showed that the degree of VC was positively correlated with FSH, LH, and PRL values (r=0.862, r=0.873, r=0.960), but had no significant correlation with T value and E_2 value.

In summary, VC sterility is closely related to chromosomal abnormalities, semen quality level, chromosome microdeletion, and reproductive hormone levels, and its pathogenesis is complex. Therefore, it is necessary to do chromosome karyotype examination, semen routine quality detection, PCR detection of chromosome microdeletion and related reproductive hormone levels in male patients with VC infertility. However, the pathophysiology of VC is complex. This study only focused on the relationship of chromosome microdeletion, semen quality and reproductive hormone levels in VC patients with normal chromosome karyotype. The study of VC patients with abnormal chromosome karyotype was more general, and it will be further explained in the follow-up study.

Disclosure of conflict of interest

None.

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