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ORIGINAL PAPER

Can serum 17-hydroxy progesterone predict an improvement in semen parameters following micro-varicocelectomy? A prospective study

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Summary

Background & objectives: Notably, 17-hydroxy progesterone (17-OHP) (17-OHP) is a precursor for testosterone (T) synthesis, and intratesticular testosterone (ITT) is essential for spermatogenesis. Varicocele (Vx) has an estimated prevalence of 15% in the general population and 35% in those with primary infertility.
We aimed to evaluate the correlation between changes of serum 17-OHP after sub-inguinal micro-varicocelectomy and improvement of semen parameters.
Patients and methods: The current prospective study included 45 infertile men attending the andrology clinic form February 2021 to August 2021. Two semen analyses and hormonal profile were evaluated. Colored duplex ultasonography (CDUS) was done in standing and supine position for accurate measurements of testicular volumes and confirmation of Vx. Patients underwent sub-inguinal micro-varicocelectomy using a surgical microscope HB Surgitech. We followed them prospectively up for three months following micro-varicocelectomy with serum TT and 17-OHP.
Results: Sperm concentration improved significantly from 8.36 ± 5.04 million/ml to 12.52 ± 8.42 million/ml after 3 months following sub-inguinal micro-varicocelectomy (p = 0.001), with normalization of concentration in 15/45 (33%) patients. Total motility did not improve significantly but progressive motility improved significantly from 8.62 ± 8.74% to 16.24 ± 14.45% (p = 0.001). Abnormal forms significantly declined from 96.67 ± 2.03% to 95.75 ± 2.47% (p = 0.009).
Serum 17 OHP and 17 OHP/total testosterone (TT) improved significantly from 1.21 ± 0.45 ng/ml and 0.26 ± 0.09 to 1.42 ± 0.76 ng/ml and 0.3 ± 0.16 (p = 0.013, p = 0.004), respectively, while serum TT did not improve significantly. A significant correlation was found between improvement in sperm concentration and both serum 17 OHP and 17 OHP/TT ratio (p = 0.001, p = 004). Furthermore, change in abnormal sperm forms showed significant correlations with changes in both 17-OHP and 17-OHP/TT.
Conclusions: 17 OHP and 17OHP/ TT ratio can be used as biomarkers to detect improvement in semen parameters following sub-inguinal micro-varicocelectomy.

KEY WORDS: *Sub-inguinal micro-varicocelectomy; 17 hydroxy progesterone; Total testosterone; Sperm count; Progressive sperm motility; Abnormal sperm forms.*

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INTRODUCTION

According to the *World Health Organization* (WHO), infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse (1). In mammals, spermatogenesis is totally dependent upon *testosterone* (T) (2). Androgens are essential for male fertility and maintenance of spermatogenesis and T is the androgen in the testis that is responsible for supporting spermatogenesis (2). In absence of T or functional *androgen receptors* (AR), males become infertile because spermatogenesis rarely progresses beyond meiosis (*Sharpe & Cooper, 1984*). T is produced by Leydig cells and acts upon Sertoli and peritubular cells of the seminiferous tubules and drives spermatogenesis (2).

It is believed that *intratesticular testosterone* (ITT) directly stimulates spermatogenesis in men. However, the amount of ITT required to initiate spermatogenesis has yet to be determined. It is important to note that ITT concentration in healthy men is approximately 100 times greater than T concentration in serum, which suggests that serum T may not be an accurate marker for ITT (3). Additionally, serum T is affected by several factors such as obesity, social environment, age, time of day and infections such as Covid-19 (4). According to *Kelly and Jones* (2015), low serum T levels are associated with increased fat mass particularly central obesity (5). Intratesticular steroids consist of approximately 70% T, 20% 17-OHP, and smaller percentages of other hormones (6). *Amory et al.* (2008) found that serum 17-OHP strongly reflects ITT concentrations in men with normal gonadotropic function receiving gonadotropin suppression and *human chorionic gonadotropin* (HCG) (6). 17-OHP was found to correlate with ITT in the presence of normal or near normal HCG stimulation (7). About 70% of 17-OHP is thought to be of testicular origin, the remainder of 17-OHP production is thought to be of adrenal origin (7).

Notably, 17-OHP is a precursor for T synthesis, and ITT is essential for spermatogenesis but can only be reliably measured with invasive testicular sampling (8). Furthermore, 17-OHP would be useful in conjunction with serum T measurements to assess the ideal dosage of HCG required to treat males with infertility (8). *Varicocele* (Vx) is significantly associated with primary and secondary infertility due to the multifactorial way in which they affect fertility (9-10). Vx is one of the reversible causes and has an estimated prevalence of 15% in the general population and 35% in those with primary infertility (9-10). One of the mechanisms by which Vx can affect the testicles is inducing disturbance of Leydig cell function, resulting in decreased ITT biosynthesis. In a meta-analysis, it was found that surgical repair significantly increased *testosterone* (T) levels in men with Vx (11-14). The negative effect of Vx on male fertility is well studied. However, the relationship between clinical Vx and impaired hormonal production is still vague and requires further analysis (15). Thus, we based our current study on two hypotheses. The first hypothesis assumes that 17-OHP is a reliable serum biomarker that correlates with ITT levels. The second one speculates that Vx is associated with impairment of androgen synthesis. Therefore, we analyzed the correlation between Vx repair and semen parameters, using serum TT and 17-OHP preoperative and postoperative measurements.

Patients and methods

The current prospective study included 45 infertile men attending the andrology clinic form February 2021 to August 2021. The institutional review board approved the current work that conforms to Helsinki declaration 2013 (16). An informed consent was signed by the patients prior to joining the study and after explaining the possible outcomes of sub-inguinal micro-varicocelectomy.

Inclusion criteria

Males suffering from infertility for more than one year (age ranges between 20-51 years old) with at least one abnormal semen parameter (oligoasthnospermia, asthenozoospermia, or teratozoospermia) and a palpable Vx were included.

Exclusion criteria

History of intake of medications that affect androgen synthesis, azoospermia, elevated follicle stimulating hormone (FSH), hypogonadotropic hypogonadism, history of previous testicular disease (torsion, trauma and infection), history of Covid-19 infection, history of previous testicular surgery and patients who received chemotherapy or radiotherapy within the last six months were excluded.

Age and duration of infertility were reported. Sexual history including frequency of intercourse as well as any ejaculation disorder, any past history of testicular disease or previous operation, and any special habits affecting semen parameters including smoking were also reported. Evaluation of testicular size, spermatic cord, vas deferens and grading of Vx were determined. Laboratory evaluation included: two semen analyses (one before the operation and another one three months following the sub-inguinal micro-varicocelectomy) according to the fifth edition of WHO guidelines (2010) (17) and hormonal profile including FSH, *luteinizing hormone* (LH), total testosterone, estradiol, ACTH and blood sampling for assay of 17-OHP by ELISA commercial kits (preoperative and three months postoperative).

Colored duplex ultasonography (CDUS) (Mindray DP-30 portable ultrasound) in standing and supine position for accurate measurements of testicular volumes and confirmation of Vx by detection of venous regurge and measurement of maximum venous diameters was conducted. Patients underwent sub-inguinal micro-varicocelectomy using a surgical microscope HB Surgitech [*5 Step Magnifications (4x, 6x, 10x, 16x & 25x)* 45 degree Inclined Binocular Tubes, 12.5x Wide Field Eye Pieces, F=200 mm Objective Lens, Aadesh Complex, Court Road, Near CJM Court, Ambala-134003, Haryana, India]. We followed patients prospectively up for three months following sub-inguinal micro-varicocelectomy with serum TT and 17-OHP.

Statistical analysis

Results are expressed as mean, standard deviation, minimum and maximum, or number (%). The Kolmogorov-Smirnov test for normality was used to measure the distribution of data measured before and after surgery. Accordingly, in normally distributed variables, comparison of data from before and after surgery was performed using paired t-tests. In abnormally distributed data, comparison between before and after operation data was performed using the Wilcoxon Signed Rank test. To perform correlation between change in both 17-OHP and 17-OHP/TT ratio and different motility parameters, firstly, we calculated the change that occurred between before and after operation from the equation: after operation minus before operation. Secondly, a test of normality was done for these variables. Thirdly, Spearman's rho correlation coefficient was used to correlate between variables. *Statistical Package for Social Sciences* (SPSS) computer program (Version 19 Windows) was used for data analysis. P-value ≤ 0.05 was considered significant.

Results

The history and demographic characteristics of the studied men are shown in Table 1. A significant improvement in sperm count was detected three months following sub-inguinal micro-varicocelectomy (Table 2). A significant improvement of progressive sperm motility three months after sub-inguinal micro-varicocelectomy was observed (Table 2). Abnormal forms significantly declined from 96.67 ± 2.03% to 95.75 ± 2.47% (Table 2). Preoperative mean TT was 4.86 ng/dl and postoperative mean TT was 4.77ng/dl with no significant improvement three months following sub-inguinal micro-varico-

Table 1.
Descriptive statistics of age, special habits, spouse's age and menses and duration of infertility.

		Studied group (n = 45)
Patients' age	Minimum- maximum	20.0-51.0
	Mean ± SD	30.93 ± 7.17
Smoking	No	30 (66.7%)
	Ex-smoker	3 (6.7%)
	Yes	12 (26.7%)
Spouse age	Minimum-maximum	19.0-40.0
	Mean ± SD	26.82 ± 5.61
Duration of infertility in years	Minimum-maximum	1.0-14.0
	- Mean ± SD	4.35 ± 3.44

Table 2.
Sperm count and total and progressive sperm motility and abnormal forms in the studied group before and after microvaricocelectomy.

		Before	After	Z value	P value
Sperm count	Min-max	0.5-18.0	0.0-43.0		
	Mean ± SD	8.36 ± 5.04	12.52 ± 8.42	-3.765	0.001
Total sperm motility	Min-max	0.0-75.0	0.0-67.0		
	Mean ± SD	31.86 ± 20.45	31.78 ± 19.20	t= 0.039	0.969
Progressive sperm motility	Min-max	0.0-30.0	0.0-64.0		
	Mean ± SD	8.62 ± 8.74	16.24 ± 14.45	Z= -3.899	0.001
Abnormal forms	Min-max	79.0-100.0	76.0-100.0		
	Mean ± SD	96.67 ± 2.03%	95.75 ± 2.47%	Z= -1.780	0.009
Total testosterone (TT)	Min-max	2.60-8.30	2.73-7.69		
	Mean ± SD	4.86 ± 1.38	4.77 ± 1.31	Z= -1.383	0.167
17 hydroxy progesterone (17-OHP)	Min-max	0.38-2.10	0.39-4.42		
	Mean ± SD	1.21 ± 0.45	1.42 ± 0.76	t= -2.577	0.013
17-OHP/TT ratio	Min-max	0.06-0.52	0.07-1.02		
	Mean ± SD	0.26 ± 0.09	0.30 ± 0.16	Z= -2.873	0.004

Wilcoxon Signed Ranks Test. t value = paired t test.

Table 3.
Correlations between change in both 17-OHP and 17-OHP/TT ratio and different semen parameters.

	17-OHP		17-OHP/TT ratio	
	r	P value	r	P value
17-OHP	----	-----.	0.853	0.001*
Count	0.477	0.001*	0.417	0.004*
Total motility	0.275	0.068	0.177	0.244
Progressive motility	0.141	0.356	0.034	0.825
Change in abnormal forms	0.331*	0.014	0.320*	0.017

*r- Spearman's rho correlation coefficient. p > 0.05 = not significant; *p ≤ 0.05 = significant.*

celectomy detected (Table 2). On the other hand, preoperative mean 17-OHP was 1.21 ng/ml and it increased significantly to 1.42 ng/ml at 3 months after sub-inguinal micro-varicocelectomy (Table 2).

Consistently, the preoperative 17-OHP/TT ratio had a mean value of 0.26 and significantly increased postoperatively to 0.30 (Table 2). The preoperative means of ACTH, E2, LH and FSH were 33.80 ± 12.73 pg/ml, 18.31 ± 5.73 pg/ml, 4.98 ± 1.81 mIU/ml, 4.42 ± 2.30 mIU/ml, respectively. Preoperative scrotal duplex studies showed that left venous diameter ranged from 2.6 mm to 5.5 mm (mean value = 3.58 ± 0.67 mm), while right venous diameter ranged from 1.4mm to 3.7 mm (mean value = 2.5 ± 0.48 mm) with reflux on the left side in 45 patients (100% of the cases) and reflux on the right side in 32 patients (71.1% of the cases). Right testicular volume ranged from 8.25 ml to 20.28 ml (mean = 12.91 ± 2.85 ml), while left testicular volume ranged from 7.23 ml to 19.15 ml (mean = 11.77 ± 2.78 ml). A positive correlation between changes in 17-OHP and changes in the 17-OHP/ TT ratio was found (Table 3). A significant correlation between postoperative sperm count improvement and a postoperative change in serum 17-OHP was also shown (Table 3).

Moreover, a significant correlation between postoperative sperm count improvement and a postoperative change in 17-OHP/TT ratio was detected (Table 3). Furthermore, change in abnormal sperm forms showed significant correlations with changes in 17-OHP and 17-OHP/TT (Table 3). Twenty-nine patients had sperm count improvement after sub-inguinal micro-varicocelectomy (mean change = 4.15 mil/ml) and 27 patients had semen progressive motility improvement (mean change = 7.62%). Twenty-three patients had an elevation in serum 17-OHP level following sub-inguinal micro-varicocelectomy (mean change = 0.20 ng/ml) and 28 patients had an elevation in serum 17- OHP/TT ratio (mean change = 0.04).

Figure 1.
Mean values of sperm count measured before and after operation.

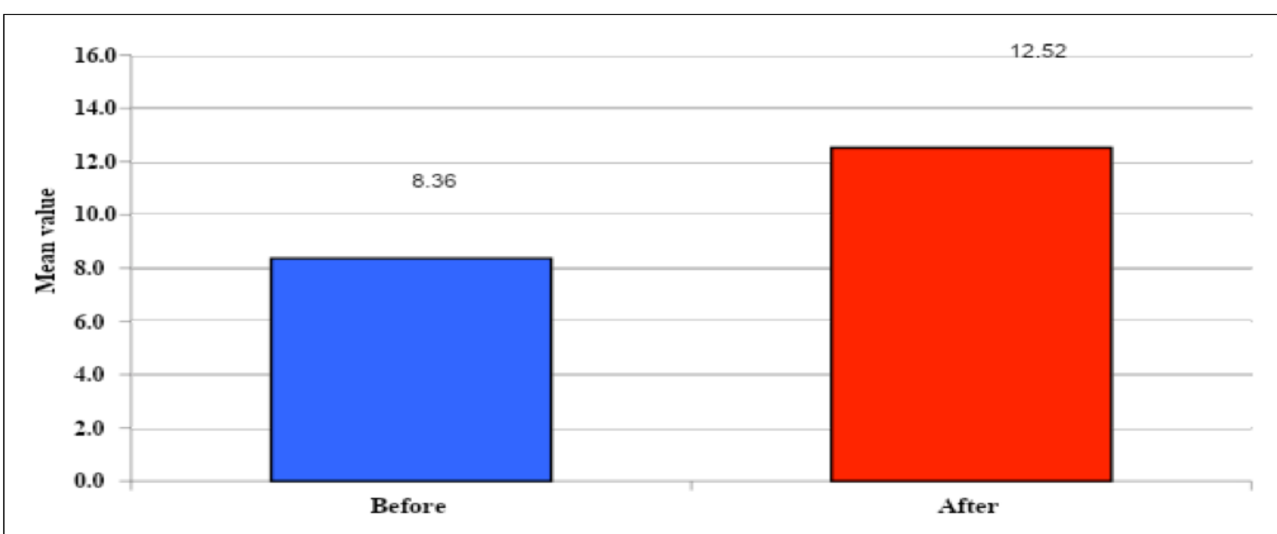
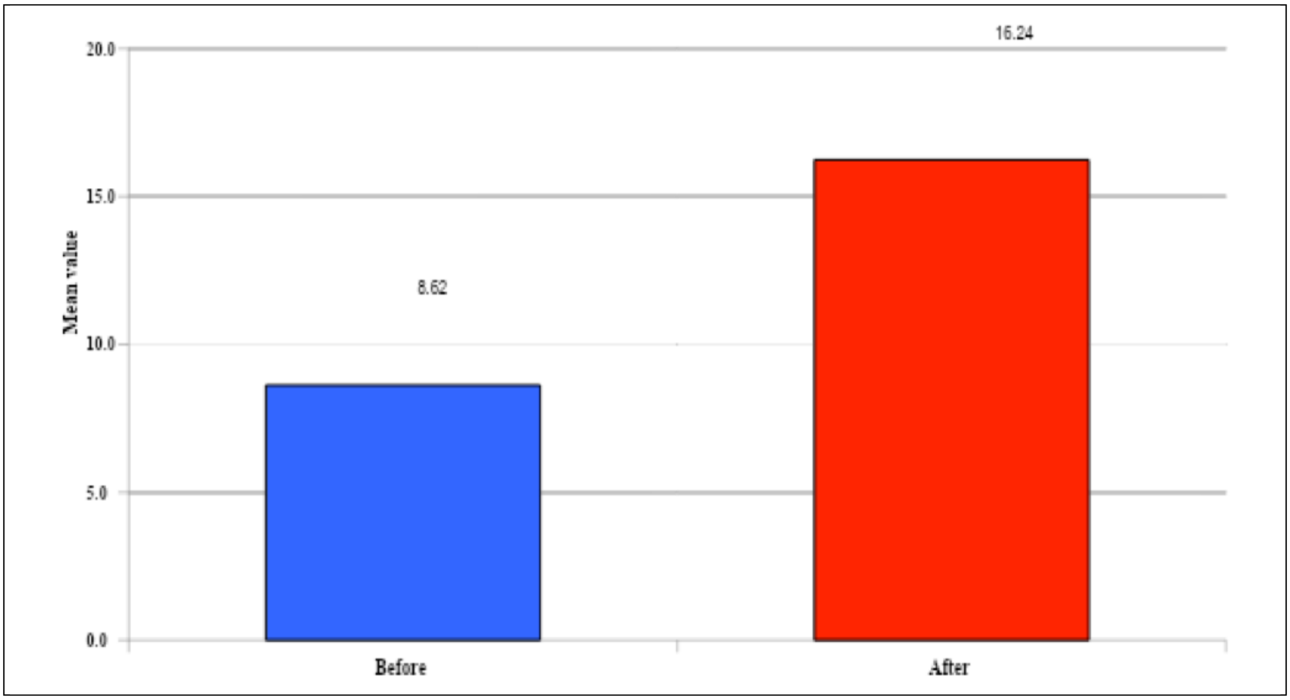


Figure 2.
Mean values of progressive motility measured before and after operation.



Discussion

The current study documented significant improvements of sperm count and progressive motility and sperm normal forms following sub-inguinal micro-varicocelectomy. Out of 45 patients, 29 (64%) had improvement in sperm count and 14 of those had a normal sperm count following Vx repair. However, one patient dropped from severe oligoasthenozoospermia to azoospermia following the operation. Our findings of count improvement after surgery were consistent with other studies (18). Twenty-seven patients (60%) had improvement in sperm progressive motility three months following the operation. Of those twenty-seven patients, only six patients had normal progressive motility following the operation. Before the operation, mean sperm progressive motility was 8.62 % which significantly improved three months following the operation to become 16.24%. Also, abnormal forms significantly improved after sub-inguinal micro-varicocelectomy. Out of 45 patients, 23 (51.1%) had a significant elevation of serum 17-OHP post-operatively.

Twenty-eight patients (62.2%) had a significant elevation of 17 OHP/TT ratio in the follow-up after sub-inguinal micro-varicocelectomy. This came in contrast to another study which found that microsurgical varicocele repair resulted in improvements in all evaluated semen parameters, but not in ITT/17-OHP or serum T levels and percentage of normal sperm forms (14). Conversely, mean preoperative *total motile count* (TMC) was 31.86 %, while 3-month post-varicocelectomy it was 31.78%, with no significant improvement. This could be seen contradictory to *Lima et al.* (2020) who suggested a significant improvement of TMC following varicocelectomy (14).

Furthermore, we did not find a significant improvement in serum TT three months following varicocelectomy. This was consistent with previous studies which showed that men with normal serum T were less likely to improve postoperatively (14, 19). Noteworthy, a positive correlation between changes in 17-OHP and improvement in semen concentration were noticed. Such results came in alignment with those of one study which suggested that serum 17-OHP strongly reflects ITT concentrations (6) as well as another study which suggested that 17-OHP could be a useful biomarker for ITT (9). Additionally, change in abnormal sperm forms showed significant correlations with changes in 17-OHP and 17-OHP/TT. The positive outcome of the semen parameters following the sub inguinal micro-varicocelectomy comes in the same line of a recent study conducted by *Kalantan et al.* (2023) (20).

The strengths of this study include the novelty of associating sperm count and progressive motility improvement and sperm abnormal forms after sub-inguinal micro-varicocelectomy with 17-OHP and 17-OHP/TT ratio. We tried to control variability in this study by performing all serum measurements between 8 and 10 am.

Scrotal duplex scans were performed for all patients to guarantee accurate diagnosis of Vx by venous diameter measurement, assessment of testicular volume and detection of venous reflux with Valsalva maneuver. Furthermore, we performed multiple follow-up assessments. In addition, we potentially identified a serum biomarker that had the ability to characterize men who were deficient in ITT as well as follow up by monitoring these patients for changes in semen parameters. This could be seen in alignment with *Bridges et al.* (2015) (21). Hypothetically, we succeeded in presenting 17-OHP and 17-OHP/TT as potential biomarkers for predicting improvement in semen parameters following sub-inguinal micro-varicocelectomy. On the other hand, there are several limitations of the current study that must be mentioned. Firstly, the small sample size is a major limitation. Secondly, we did not follow up the effect of improvement of semen parameters on pregnancy rates. Finally, we were unable to measure ITT. However, it should be noted that we based the correlation of 17-OHP with ITT on a study conducted by *Patel et al.* (2019) (8).

Conclusions

According to the current study, sub-inguinal micro-varicocelectomy resulted in significant improvements in sperm count and progressive motility that significantly correlated with changes in 17-OHP and 17-OHP/ TT ratio.

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Conflict of interest: The authors declare no potential conflict of interest. The present study has been presented as a poster in the *12th European Congress of Andrology Abstract Book*.