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ORIGINAL PAPER

Correlation between seminal alpha-Glycerylphosphorylcholine and semen parameters in infertile patients pre and post sub-inguinal micro-varicocelectomy: A prospective study

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Summary *Background: Varicocele (Vx) which is the most treatable cause of male infertility, is also associated with low sperm count, decreased sperm motility and increased sperm abnormal morphology. We aimed in the current study to evaluate the correlation between seminal Alpha-Glycerylphosphorylcholine (αGPC) and semen parameters in infertile patients pre and post sub-inguinal micro-varicocelectomy.*

Methods: The current comparative prospective study was carried out on 20 male patients who presented to Kasr Al-Ainy Hospitals from March 2022 to March 2023 as well as 20 healthy controls. The participants were divided into groups as follow: group (1) included fertile normozoospermic men (n = 20) who served as controls. Group (2) included infertile oligoasthenoteratozoospermia (OAT) men with varicocele (n = 20). Patients in group (2) were followed up to 3 months after microsurgical sub-inguinal Varicocelectomy. The examination included assessment of Vx with scrotal Duplex. Semen analysis was done according to the 5th Edition of WHO manual for semen analysis.

Results: The study demonstrates that αGPC level was significantly higher among fertile normozoospermic control group and infertile OAT men post varicocelectomy when compared to infertile OAT men preoperative (p < 0.001). Moreover, it demonstrates that on follow up of infertile OAT group 3 months after sub-inguinal micro-varicocelectomy, all semen parameters showed significant improvement compared to the corresponding semen parameters pre-operatively among Vxs grade II and grade III (p < 0.001, p < 0.001, respectively). A significant positive correlation was found between αGPC level and semen parameters including sperm normal forms, sperm count and sperm motility. Using ROC curve, αGPC protein showed a sensitivity of (100%) and a specificity of (100%) at cut off value (≤ 1.975 pg/ml) in differentiation between infertile OAT patients with Vx and control fertile normozoospermic men (p < 0.001).

Conclusions: αGPC may play an important role in infertility in men with Vx and correction of Vx improves the seminal αGPC level.

KEY WORDS: *Varicocele; Subinguinal micro-vericocelectomy; α-glycerylphosphorylcholine; Semen parameters.*

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BACKGROUND

Infertility is defined as the inability to conceive after at least 12 months of regular unprotected sexual intercourse. Infertility is a worldwide complaint and is projected to involve 8-12% of couples in the fertility period (1). Males are responsible for 20-30% of cases of infertility and are participating in a further 20%. Male subfertility is a wide range problem with almost unknown cause in most cases (1). Although various diagnostic tests are available, their interpretation is imprecise and often subjective. *Varicocele* (Vx) which is the most treatable cause of male infertility with a prevalence of 40%, is also associated with low sperm count, decreased sperm motility and increased sperm abnormal morphology (2-4). The main hypotheses were that hyperthermia, venous pressure, hormonal imbalance, toxic substances and reactive oxygen radicals were involved in the pathophysiology and that varicocelectomy improved the number and motility of sperms (2-4). Vx causes a progressive decline in fertility with upwards of 80% of men presenting with secondary subfertility having a Vx (5). Several studies tried to answer how Vx causes infertility. Factors included increased oxidative stress due to increased pressure on venous walls, scrotal hyperthermia, hypoxia, reflux of renal and adrenal metabolites, hormonal imbalances, the formation of antisperm antibodies and change in the seminal fluid composition including epididymal proteins (6-8). Miyaoka and Esteves found that patients with both clinical and subclinical Vxs benefited from varicocelectomy because their sperm counts went up by a lot (9). *Alpha-glycerylphosphorylcholine* (αGPC), one of the major phosphorus containing-choline compounds of seminal plasma, is secreted mainly by the epididymal epithelium under androgenic control (10). The organic fraction of human seminal plasma contains phosphate esters, particularly αGPC, *phosphorylcholine* (PCh) and inorganic phosphate (11). αGPC is synthesized by the epididymis.

It originated from *phosphatidylcholine* (PC) and broke down into choline and α-glycerophosphate (12). PC synthesis in mammalian tissue occurs by Kennedy pathway with choline as one of the pillar substances that necessitates the removal of fatty acids by phospholipase activity

(12). Also, the synthesis of αGPC entails sequential activity of a phospholipase A or alternatively, activity of a single phospholipase B. Notably, αGPC is water soluble and degraded by hydrolysis to glycerol-3-phosphate and choline catalysed by GPC phosphodiesterase activity (12). Evaluation of αGPC activity may also help find out if the epididymis is open and if sperm isn't normal. Unfortunately, there are different opinions in the literature about how useful the assay is for male infertility (13). The epididymal function in semen analysis has been previously recommended as the epididymis is highly involved in preparing spermatozoa for fertilization. Alpha-glucosidase, αGPC and L-carnitine were measured in sperm-free seminal plasma to determine the exact importance of these proteins in male fertility but with conflicting results (14). The objective of this study was to evaluate the effectiveness of evaluation and assessment of seminal of αGPC in infertile men before and after sub-inguinal micro-varicocelectomy.

Patients and methods

The current comparative prospective study was carried out on 20 male patients who presented to Kasr Al-Ainy Hospitals from March 2022 to March 2023 as well as 20 controls. The institutional ethical committee of Beni suef university approved the work that conforms to Helsiniki declaration 2013 (15) (FMBSREC/08032022).

Inclusion criteria

Any infertile case with oligoasthenoteratozoospermia (OAT) and Vx aged 20 to 30 years old.

Exclusion criteria

Patients suffering from azoospermia and subclinical Vx, smoking, patients with congenital anomalies or leukocytospermia, history of blood transfusion, iron therapy or anemia were excluded from the study

Inclusion criteria of the controls

They were healthy age matched individuals who were companions to the cases.

The participants were divided into groups as follows: group (1) was fertile normozoospermic men (n=20) served as controls. Group (2) was infertile OAT men with Vx (n = 20). Patients in group (2) were followed up to 3 months after sub-inguinal micro-varicocelectomy.

General and clinical examinations were done. The examination included assessment of Vx with scrotal Duplex. Semen analysis was done according to the 5th Edition of WHO manual for semen processing (2010) (16).

Clinical examination was carried out in a warm room at the standing position with/without Valsalva maneuver. Color Doppler Ultrasonography was conducted for assurance of Vx and its grade when one or more veins had a maximal diameter >3 mm with a retrograde flow at rest or under Valsalva maneuver. Vx was classified according to *Chiou et al.* (1997) and *Kim et al.* (2008) characterization (17-18).

The ejaculates were obtained after 4-5 days of sexual abstinence into sterile containers. More than one sample was provided 2 weeks apart. Sub-inguinal micro-varicocelectomy was done under general anaesthesia (19). Also, it was done 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].

After hospital discharge, patients were invited to attend to follow-up visit 3 months after sub-inguinal micro-varicocelectomy. Semen samples were obtained as described above. Fertile men infertile delivered one sample only, while infertile OAT men delivered 2 samples pre and post sub-inguinal micro-varicocelectomy.

Measurement of αGPC

Quantitative detection of seminal αGPC was assayed by *enzyme linked immunosorbent assay* (ELISA) sandwich principle Human PC/CPG (Choline Phosphoglyceride ELISA kit supplied by the American research products, USA (Cat no EELH0730) according to manufacturer's instructions.

Statistical analysis of the data

Data were fed to the computer and analysed using IBM SPSS software package version 20.0 (*Armonk, NY: IBM Corp*). Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean and standard deviation. The significance of the obtained results was judged at the 5% level. F-test (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups with Post Hoc test (*Tukey*) for pairwise comparisons. Pearson coefficient was used to correlate between two normally distributed quantitative variables. Chi-square test was used to examine the relationship between two qualitative variables. T Test was to assess the statistical significance of the difference between two study group means. Finally, receiver operating characteristic curve was used to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups.

The optimum cut off point was defined as that which maximized the AUC value. The area under the ROC curve (AUC) results were considered excellent for AUC values between 0.9-1, good for AUC values between 0.8-0.9, fair for AUC values between 0.7-0.8, poor for AUC values between 0.6-0.7 and failed for AUC values between 0.5-0.6.

Results

The sociodemographic characteristics of the participants are shown in Table 1. The study demonstrates that αGPC level was significantly higher among fertile normozoospermic control group and infertile OAT men post subinguinal micro-varicocelectomy when compared to infertile OAT men preoperative ($p < 0.001$) (Table 2). Moreover, it was demonstrated that on follow up of infertile OAT group 3 months after sub-inguinal micro-varicocelectomy, semen parameters showed significant improvement compared to the corresponding semen parameters pre-operatively among Vxs grade II and grade

		Group I (n = 20)	Group IIa (preoperative) (n = 20)	Group IIb (postoperative) (n = 20)	Test of Sig.	P value
Age (years)	Min-Max	22-26	22 - 26	22-26	F = 1.037	0.361
	Mean ± SD	23.7 ± 1.4	24.15 ± 1.2	24.15 ± 1.2		
	Median (IQR)	23.5 (22.5-25)	24.5 (23-25)	24.5 (23-25)		
Semen volume (ml)	Min-Max	1.4-4.8	0.6-6.9	2- 5.2	F = 3.897 *	0.035 *
	Mean ± SD	2.7 ± 0.9	2.76 ± 1.4	3.3 ± 0.74		
	Median (IQR)	2.4 (2-3)	2 (1.5-3.4)	3.2 (2.8-3.9)		
Sperm count (10^6 ml)	Min-Max	24 -95	1-10	10-55	F = 54.968 *	< 0.001 *
	Mean ± SD	54.6 ± 22.4	5.33 ± 2.7	31.5 ± 12.3		
	Median (IQR)	56 (65-70)	5 (2.6-8.2)	35 (16.3-40)		
Total sperm motility (%)	Min-Max	35-65	5-25	35-65	F = 66.216 *	< 0.001 *
	Mean ± SD	53.5 ± 7.3	14 ± 5.5	49.3 ± 9.1		
	Median (IQR)	55 (50-60)	15 (10-18.8)	50 (41.3-55)		

SD = Standard deviation; IQR = Inter Quartile Range; p value was calculated using ANOVA test.

Table 1.
Descriptive data of the controls and group IIa (pre-operative infertile OAT with varicocele) and group IIb (Infertile OAT after sub-inguinal micro-varicocelectomy).

		Group I (controls) (n = 20)	Group IIa (preoperative) (n = 20)	Group IIb (postoperative) (n = 20)	Test of Sig.	P value
αGPC level (pg/ml)	Min-Max	2.65-9.86	0.3-1.30	2.0-10.86	F = 68.589 *	< 0.001 *
	Mean ± SD	6.66 ± 2.11	0.6 ± 0.31	6.38 ± 2.32		
	Median (IQR)	7.05 (5-8.2)	0.54 (0.33-0.96)	6.20 (3.2-6.9)		
	p_1		< 0.001 *	0.123 *		
	p_2			< 0.001 *		
Sperm normal forms (%)						
	Min-Max	5.0-7.0	1-2	4.0-7.0	F = 49.468 *	< 0.001 *
	Mean ± SD	6.33 ± 0.8	1.7 ± 0.47	5.35 ± 0.93		
	Median (IQR)	6.0 (50-60)	2.0 (1-2)	5.0 (2-4)		
	p_1		< 0.001 *	0.061		
	p_2			< 0.001 *		

SD = Standard deviation; IQR: Inter Quartile Range; F: F for ANOVA test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Tukey); p: p value for comparing between the 3 groups (group I and group II a and group IIb); p_1: p value for comparing between group I and group IIa (pre-operative) and Group IIb (post-operative); p_2: p value for comparing between group IIa (pre-operative) and group IIb (post-operative).

Table 2.
Comparison between controls and groups IIa and IIb regrading αGPC level and normal forms.

Patients with Vx grade II		Group IIa (n = 11) (preoperative)	Group IIb (n = 11) (postoperative)	Test of Sig.	P value
Age (years)	Min-Max	22.0-26.0	22.0-26.0	t = 0.07	0.949
	Mean ± SD	24.33 ± 1.15	24.33 ± 1.15		
Sperm count (10^6 ml)	Min-Max	1-10	10-45	t = 5.41 *	< 0.001 *
	Mean ± SD	5.14 ± 2.99	27.73 ± 14.85		
Total sperm motility (%)	Min-Max	5-25	35-65	t = 9.71 *	< 0.001 *
	Mean ± SD	13.75 ± 5.9	46.82 ± 11.46		
Alpha GPC level (pg/ml)	Min-Max	0.03-1.3	2-6.7	t = 12.18 *	< 0.001 *
	Mean ± SD	0.63 ± 0.24	5.58 ± 1.47		
Sperm normal forms (%)	Min-Max	1-2	4-6	t = 8.38 *	< 0.001 *
	Mean ± SD	1.68 ± 0.49	4.27 ± 0.65		

SD = Standard deviation; t: Student t-test; p: p value for comparing between the studied categories.

Table 3.
Data of infertile men with varicocele grade II pre and post sub-inguinal micro-varicocelectomy.

III ($p < 0.001$, $p < 0.001$, respectively) (Tables 3-4). There were no significant differences among patients with Vx grade II and Vx grade III, pre and post sub-inguinal micro-varicocelectomy regarding age (Tables 3-4).

Furthermore, there was a significant positive correlation between αGPC level and semen parameters including sperm normal forms, sperm count and sperm motility (Table 5). Using ROC curve, αGPC protein showed a sensitivity of (100%) and a specificity of (100%) at a cut off value of ≤ 1.975 pg/ml in differentiation between infertile OAT patients with Vx and control fertile normozoospermic men ($p < 0.001$) (Figure 1).

Patients with Vx grade III		Group IIa (n = 9)	Group IIb (n = 9)	Test of Sig.	P value
Age (years)	Min - Max Mean ± SD	22.0 - 26.0 23.87 ± 1.35	22.0 - 26.0 23.87 ± 1.35	t= 0.20	0.841
Sperm count (10^6 ml)	Min - Max Mean ± SD	5.75 5.75 ± 3.56	20 - 55 33.78 ± 8.93	t=9.33*	<0.001*
Total sperm motility (%)	Min - Max Mean ± SD	5 - 20 14.45 ± 5.6	35 - 65 52.78 ± 8.33	t=11.19*	<0.001*
Alpha GPC level (pg/ml)	Min - Max Mean ± SD	0.18 - 0.98 0.59 ± 0.26	5 - 10.9 9.14 ± 1.62	t=15.28*	<0.001*
Sperm normal forms (%)	Min - Max Mean ± SD	1 - 2 1.75 ± 0.46	5 - 7 6.33 ± 1.0	t=11.10*	<0.001*

SD = Standard deviation; t: Student t-test; p: p value for comparing between the studied categories.

Table 4.
Data of infertile men with varicocele grade III pre and post sub-inguinal micro-varicocelectomy.

		Age (years)	Semen volume (ml)	Sperm count (10^6 ml)	Total sperm motility (%)	αGPC level (pg/ml)	Sperm normal forms (%)
Age (years)	r P	1.0	0.013 0.919	-0.097 0.462	-0.098 0.458	-0.109 0.408	-0.109 0.408
Sperm count (10^6 ml)	r P			1.0	0.691 * < 0.001 *	0.691 * < 0.001 *	0.744 * < 0.001 *
Total sperm motility (%)	r P				1.0	0.831 * < 0.001 *	0.893 * < 0.001 *
Alpha GPC level (pg/ml)	r P					1.0	0.886 * < 0.001 *
Sperm normal forms (%)	r P						1.0

r: Pearson coefficient.

Table 5.
Correlations between different parameters in the current study.

Figure 1.
ROC curve showing α-GPC level (pg/ml) to discriminate between infertile OAT patients with varicocele and controls.

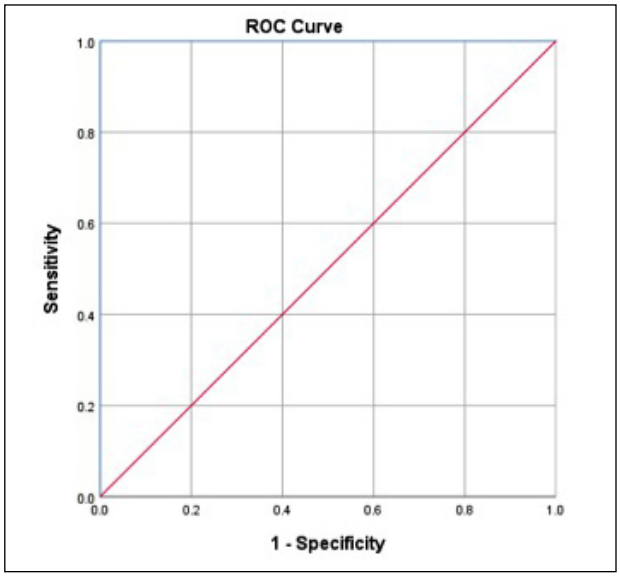
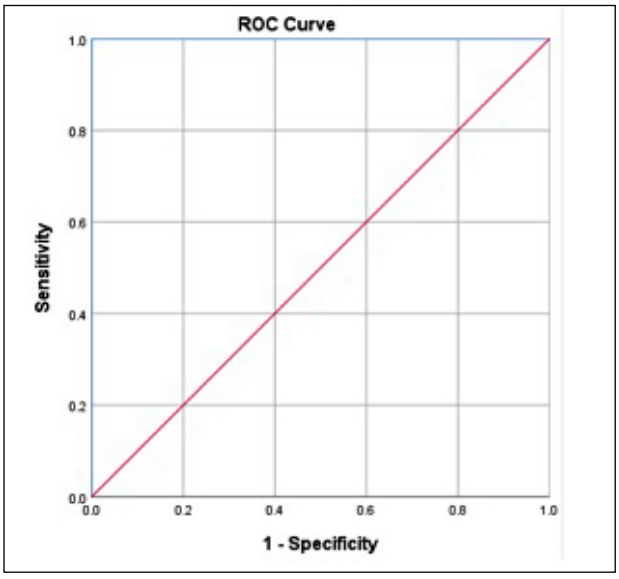


Figure 2.
ROC showing α-GPC level (pg/ml) to discriminate between infertile OAT patients with varicocele before and after varicocelectomy.



+Discussion

In our study, analysis of semen parameters revealed that infertile OAT men with Vx showed statistically significant decrease regarding sperm counts and percentage of motile sperms when compared to fertile normozoospermic men who had normal basic semen paramaters. On follow up of infertile OAT group 3 months after sub-inguinal micro-varicocelectomy, semen parameters

showed significant improvement compared to the corresponding semen parameters pre-operatively. In agreement with our results, *Shabana et al.* reported that sperm count and progressive motility significantly improved after varicocelectomy compared to pre-operative analysis (20). Consistently, *Rehman et al.* reported that sperm count, motility and normal morphology were significantly lower in Vx patients in comparison to normal individual (21). This result agreed with a large-scale study of 7035 healthy young men from general European populations demonstrated that the presence of Vx was associated with poorer semen quality (22). Evidence from both animal and human studies show that varicocele affects sperm quality. Experimental Vx has been associated with impairment of testicular and epididymal endocrine and exocrine function, which may contribute to infertility seen in men with Vx (23). Moreover, we found that normal forms of sperms significantly improved in cases of Vx grade III compared to cases of Vx grade II after sub-inguinal micro-varicocelectomy. Consistently, *Pasqualotto et al.* did a study on 61 men with Vx (24). They found that men with large varicoceles have worse sperm parameters before surgery, but they improve more after surgery than men with small or medium-sized varicoceles (24). *Krishna Reddy et al.* showed that patients with grade III Vx not only have better sperm parameters after surgery than those with grades I and II, but also have a significant increase in testicular volume, which goes along with improvement in sperm parameters (25).

In our current study, on follow up of infertile OAT group 3 months after sub-inguinal micro-varicocelectomy, semen parameters and αGPC showed significant improvement compared to the corresponding semen parameters pre-operatively among Vx grades II and III. In our study, we believe that αGPC levels decrease in infertile men with Vx this may be related to hyperthermia, venous pressure and reactive oxygen radicals which could be involved in the pathophysiology of the devastating impact of Vx on spermatogenesis. In the current study levels of seminal αGPC were significantly higher post sub-inguinal micro-varicocelectomy compared to pre-operative in infertile males with Vx. Seminal αGPC were nearly matching to levels of fertile normozoospermic men after sub inguinal micro-varicocelectomy. Statistically significant positive correlation was found between αGPC level and semen parameters. Also, the current study did not demonstrate a relation between age and αGPC level. Seminal αGPC showed 100% sensitivity and 100% specificity in differentiation between infertile OAT patients with Vx and fertile normozoospermic men. Same values of sensitivity and specificity were obtained when comparing seminal αGPC between infertile OAT patients with Vx and post sub-inguinal micro-varicocelectomy. *Camargo et al.* conducted a study to determine the seminal plasma lipid fingerprints in adults with Vx before and after varicocelectomy (26). They reported that αGPC levels improved in the post-varicocelectomy group (26). Seminal αGPC is one of the three main epididymal markers important for proper spermatogenesis. One of the physiological functions attributed to αGPC is a possible role in respiration and motility of sperm (27). In the same context, *Mieusset et al.* (2020) reported significantly lower levels of αGPC in azoospermic men (28). This finding indicated the possible significant role played by αGPC in male fertility. In contrast, *Mieusset et al.* previously reported (1988) no major difference in the total seminal content of αGPC among fertile and infertile men (29).

Furthermore, *Zhang et al.* reported that levels of αGPC in asthenozoospermic men were significantly higher compared to healthy controls (30). Admittedly, small sample size is considered the main limitation of the current study as well as short follow up period. However, the prospective nature of the study can add strength to the current findings.

Conclusions

Seminal αGPC may play an important role in infertility in men with Vx and correction of Vx improves seminal αGPC level.

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Declarations

Ethical approval: *This study was approved by the institutional ethical committee of Beni Suef University. Additionally, informed consent was obtained from the patients.*

Availability of data and material: *All inquiries can be directed to the corresponding author.*

Competing interests: *The authors declare no competing interests.*

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Authors' contributions: *SFG drafted the initial manuscript; SFG revised the article critically; SFG reviewed and edited the article; NN designed the study; SAA performed the lab work of the study; AO, AE, AZ, AAS collected and analyzed the data. All authors reviewed the manuscript.*

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