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Expression of S100 and CD34 markers can predict post-varicocelectomy success; a quasi-experimental trial

Rohollah Valizadeh[1], Saman Farshid[2*], Ata Abbasi Eslamloo[3], Behzad Abyar[2]

[1]Student Research Committee, Epidemiology Department, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[2]Clinical Research Development Unit of Imam Khomeini Hospital, Urmia University of Medical Sciences, Urmia, Iran
[3]Department of Pathology, School of Medicine, Imam Khomeini Hospital, Urmia University of Medical Sciences, Urmia, Iran

***Correspondence to**
Saman Farshid, Email: samanf63@gmail.com, farshid.s@umsu.ac.ir

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Abstract

Introduction: Varicocele is a dilatation of the pampiniform venous plexus and the internal spermatic vein. Varicocele is the most modifiable common cause of infertility in men with a general prevalence ranging from 15% to 45%.
Objectives: This study aimed to determine the expression of S100 and CD34 markers in the spermatic vein in patients undergoing.
Patients and Methods: This quasi-experimental (before-after) study was performed on 30 patients undergoing varicocelectomy surgery referred to a tertiary care medical center in Urmia. The expression of S100 and CD34 markers in spermatic veins were examined after surgery. According to the definition of the World Health Organization (WHO), we arranged the data of semen analysis to normal and abnormal. Data collecting forms were collected and analyzed using SPSS version 18 software.
Results: The mean age of patients was 27.66 ± 6.94 years. Regarding the S100 factor, 48.3% of patients were positive and 51.7% were negative. Regarding CD34 factor, 79.3% of patients were positive and 20.7% were negative. There was no significant correlation between count, morphology, and motility variables before and after varicocele surgery with expression of the S100 and CD34 markers in spermatic vein wall. There was a statistical relationship between S100 and motility before surgery showing the high normal motility in patients who have S100. Regarding improvement, two patients (6.9%) in count, one patient (3.49%) in motility, five patients (17.2%) in morphology, seven patients (24.1%) in count and motility, four patients (13.8%) in count and morphology, five patients (17.2%) in motility and morphology and two patients (6.9%) in all factors had improvement.
Conclusion: In this study we found, varicocelectomy leads to improvement in count, morphology, and motility variables. In high grade varicocele, S100 and CD34 markers are damaged. According to the WHO definition, varicocelectomy can increase the value of count, motility and morphology parameters. It is recommended that a large randomized control trial and cohort study be conducted on S100 and CD34 markers to understand the relation between spermatic vein structural changes and semen analysis disorders.
Trial Registration: The trial was registered in Iranian Registry of Clinical Trials (identifier: IRCT20180625040232N1, https://en.irct.ir/trial/32142).

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Introduction

Varicocele is an abnormal dilatation and enlargement of the pampiniform plexus of testicular veins within the spermatic vein (1,2). It is the most modifiable common cause of infertility in men, with a prevalence ranging from 15% to 45% of the male population. The age-specific prevalence of varicocele for pre-puberty, adolescence, and after 15 years of old is 1%, 2-16% and 20%, respectively (3). Approximately, 90% of the varicocele is on the left side, bilateral in 30%-50%, and uncommon in the isolated right side (3-5). The main diagnosis of varicocele has been based on clinical examinations, therefore the diagnosis of moderate to severe varicocele (grade II and III) can easily be made by ordinary physical examinations (6, 7).

Key point

In a quasi-experimental (before-after) study on 30 patients undergoing varicocelectomy surgery referred to a tertiary care medical center in Urmia, we found, varicocelectomy leads to improvement in count, morphology, and motility variables.

The most common disorder of sperm parameters in patients with varicocele is reduced sperm motility, which occurs in 90% of cases, followed by a decrease in the number of sperm. However, there is more than 20 million per ml in up to 20%

of sperm counts. Morphological disorders may appear as the sole sperm abnormality (8). The most common indication of varicocele surgery is male infertility (9). Venous endothelial, muscular, and nervous tissues are important factors in preventing blood reflux facilitating the varicocele. The S100 and NF-200 factors are used to examine vascular nerves, while the CD31 is used for examining vascular endothelial tissue. The use of IHC staining (imunohistochemistry) for the presence of the S100, NF200, CD31 and CD34 factors in the vein wall can indicate endothelial and vascular changes in the vascular wall (10). In a study by Sobhy et al, a significant correlation has been shown between histopathological and immunological changes in the spermatic venous wall and changes of the NF-200 and CD31 factors with varicocele progression (11).

Objectives

Considering the lower costs of S100 and CD34 IHC in comparison with NF200 and CD31 respectively, we employed these factors to study changes in endothelial and nervous tissues inside the spermatic vein walls of patients with varicocele before and after varicocelectomy, investigating their potential role as predictors of improvement in semen analysis indexes.

Patients and Methods

Study design and patients

This quasi-experimental study was conducted on patients with high-grade varicocele accompanied with impaired semen analysis, infertility or refractory scrotal pain in a tertiary care medical center in Urmia. Thirty patients who were candidates for varicocele surgeries were selected and written informed consents were obtained. The data were collected in the two phases (before and after the intervention).

Semen analysis and abstinence period

Before surgery all patients had semen analysis tests done with consideration of standards of the World Health Organization (WHO) semen analysis criteria (12) (semen samples were given with an abstinence period of 2-7 days, and all of the semen samples were analyzed by computer-assisted sperm analysis (CASA) in a single laboratory) (13).

Inclusion and exclusion criteria

Inclusion criteria included age between 18 to 60 years, of American Society of Anesthesiologists (ASA) physical status of class I or II, and having no congenital genitourinary problems. Exclusion criteria were ASA> II, having emergency surgery, or congenital genitourinary problems.

Surgical technique

After induction of general or spinal anesthesia in supine position, a 2 cm horizontal incision was made inferior and medial to anterior superior iliac crest. Peritoneum was swept medially; after dissecting the fascia and muscle layers, spermatic vein(s) were observed. Tedious dissection of spermatic vein was done. Finally, the vein was ligated with 3-0 silk at two ends, and after ligation, a small piece of vein was cut off for pathologic examination. All surgical procedures were performed by a single surgeon.

Laboratory

After fixation, thin sections with a thickness of 5 µm from the block were prepared and IHC staining for S100 and CD34 markers was performed. Staining of these factors in the vessel walls was considered as a positive reaction. No staining in four high power fields was considered negative IHC for S100 Marker and CD34.

Semen analysis improvement criteria

According to meta-analysis performed by Agarwal et al to evaluate semen analysis improvement, sperm concentration rising ≥9.71×10^6/mL, motility rising ≥9.92% and sperm morphology rising ≥3.16% was considered an improvement.

Primary outcome

Primary outcome was the relationship between the changes of semen analysis parameters with the expression of S100 and CD34 markers before and after varicocele surgery.

Statistical analysis

The results of the study were analyzed with SPSS version 18. Chi-square test and paired *t* test were used for qualitative and quantitative variables respectively. The significance level is considered less than 0.05. All data are presented as a mean ± standard deviation.

Results

In this study, 29 patients were selected. The mean age was 27.66 ± 6.94 years. The mean abstinence period was 4 ± 1.18 days (ranging from 3 to 6 days). Mean spermatic counts before and after surgery were 58.65 ± 48.52 and 74.57 ± 53.15 million per ml, respectively. The mean motility was 44.62 ± 18.58% and 57.56 ± 19.76%, respectively. The mean abnormal morphology before and after surgery were 53.75± 23.08% and 44.66 ± 22.03%, respectively (Table 1, Figure 1).

Eleven patients (37.9%) had grade II and 18 patients (62.1%) grade III varicocele. (Table 2) The frequency of S100 and CD34 negatives in varicocele in patients with grade III was higher than patients with grade II (Table 3, Figure 2A-2D). Regarding the improvement status, two patients (6.9%) in count, one patient (49.3%) in motility, five patients (17.2%) in morphology, seven patients (24.1%) in count and motility, four patients (13.8%) in count and morphology, five patients (17.2%) in motility and morphology, and two patients (6.9%) had a significant

Table 1. Abstinence period and semen analysis parameters (before and after surgery)

Variable		Mean	SD	Minimum	Maximum	*P* value
Semen volume (mL)	Before	2.6	1.01	1.7	3.6	0.094
	After	2.5	1.42	1.8	3.8	
Count (million/mL)	Before	58.65	48.52	6.00	184.00	0.072
	After	74.53	77.15	5.00	382.00	
Motility (%)	Before	44.62	18.58	13.00	80.00	0.001
	After	57.56	19.76	18.00	100.00	
Abnormal morphology (%)	Before	53.75	23.08	22.00	97.10	0.013
	After	44.66	22.03	12.00	90.00	

improvement in all factors (Table 4, Figure 3).

According to the definition of WHO, we arranged the data of semen analysis to normal and abnormal (16). In this regard, we can find visually and numerically difference between before and after parameters (Table 5). Additionally, there was a statistical relationship between S100 and motility before surgery in patients studied in the study showing the high normal motility in patients who have S100. The frequencies of S100 and CD35 in semen factors before and after surgery were assessed as well (Tables 6 and 7). Table 8 presents the frequency of improvement in semen factors by CD34 and S100 after surgery in patients studied in the study. Regarding improvement, two patients (6.9%) in count, one patient (3.49%) in motility, five patients (17.2%) in morphology, seven patients (24.1%) in count and motility, four patients (13.8%) in count and morphology, five patients (17.2%) in motility and morphology and two patients (6.9%) in all factors had improvement (Table 8).

Discussion

Varicocele is an abnormal dilatation and enlargement of the pampiniform plexus of testicular veins within the spermatic veins and is the most modifiable common cause of infertility in men, with a general prevalence ranging from 15% to 45% of the male population (1-3). One of the reasons for varicocele development is a disruption of the antireflux mechanism of vessel walls, which is related to the nature of the smooth muscles of the vessel walls and its density between the adventitia and media layers. The reduction in number of vasa vasorum in vein walls has been related to the atrophy of smooth muscles of venous walls thus loss of the contractile force of the vessel. NF200 factor CD34 has been used in the assessment of presence of the vasa nervosa and endothelial changes accordingly. We used S100 instead of NF200 and CD34 instead of

Table 2. Frequency of varicocele grade in patients studied in the study

Variable		Frequency (n)	Percent
Grade	Grade II	11	27.9
	Grade III	18	62.1

Table 3. Frequency of S100 and CD34 by varicocele grade in patients studied in the study

Variable		Grade II	Grade III	Statistics
S100	Negative	5	10	χ^2=0.279
	Positive	6	8	*P* =0.442
CD34	Negative	2	4	χ^2=0.068
	Positive	9	14	*P* =0.592

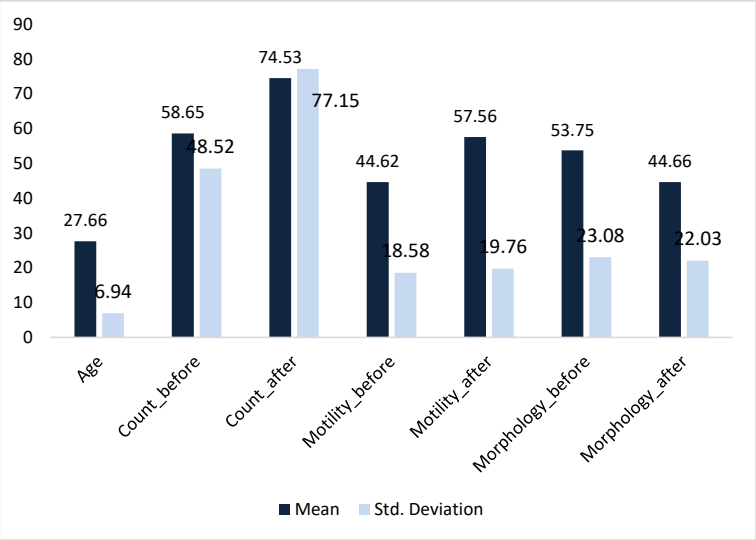

Figure 1. Abstinence period and semen analysis parameters before and after surgery.

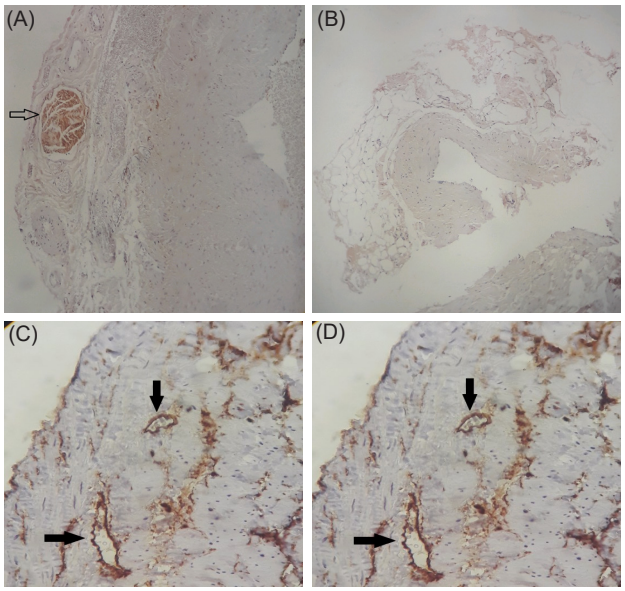

Figure 2. (A) Sections of spermatic venous wall showing focal S100 staining demonstrating nerve bundles (arrow) (IHC, 20×). **(B)** Spermatic venous wall with no evidence/decreased of S100 staining (IHC, 10×). **(C)** CD34 staining of venous wall demonstrating small vasculatures (arrows) (IHC, 20×). **(D)** Venous wall with negative CD34 staining for microvaculature. Endothelial lining of the large vein (arrows) is stained by CD34 and considered as internal positive control measure (IHC, 20×)

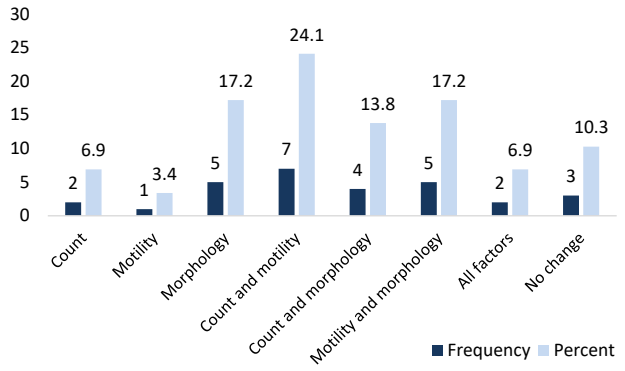

Figure 3. The frequency distribution of improvement in patients with varicocele.

Table 4. The frequency distribution of improvement according to improvement Values; sperm concentration rising ≥ 9.71×10⁶/mL, motility rising ≥ 9.92% and sperm morphology rising ≥3.16% considered improvement

Variable	Frequency (n)	Percent
Improvement		
Count	2 cases	6.9
Motility	1 case	3.4
Morphology	5 cases	17.2
Count & motility	7 cases	24.1
Count & morphology	4cases	13.8
Motility & morphology	5 cases	17.2
All factors	2 cases	6.9
None	3 cases	10.3
Total	29	100

Table 5. The frequency distribution of abnormal and normal value of study parameters (count > 39×10⁶/mL, motility > 40% and morphology > 30% considered as normal)

Variable		Frequency	Percent
Morphology before surgery	Abnormal	5	17.2
	Normal	24	82.8
Morphology after surgery	Abnormal	10	34.5
	Normal	19	65.5
Motility before surgery	Abnormal	12	41.4
	Normal	17	58.6
Motility after surgery	Abnormal	5	17.2
	Normal	24	82.8
Count before surgery	Abnormal	13	44.8
	Normal	16	55.2
Count after surgery	Abnormal	10	34.5
	Normal	19	65.5

CD31 as cost benefit equivalents (14).

In this study, the mean age of patients was 27.66 ± 6.94 years. 14 patients (48.3%) were single and 15 patients (51.7%) were married. Sobhy et al in 2011 found vasa vasorum and nerve fibers to be decreased in the wall of spermatic veins in grades I and II varicocele cases and minimal to absent in grade III cases with the help of immunostaining for CD31 and NF-200 (11).

In the present study, staining was positive in 48.3% of cases for the S100 marker 79.3% for the CD34 marker. Some studies have shown a relationship between time and severity of the varicocele with the loss of smooth muscle of spermatic vein (15). Additionally, we sought to link the absence or existence of semen analysis factors improvement with the absence of CD34 and S100 factors in vein wall. Almost all patients (except three patients) experienced improvement in one of the count, morphology, or motility variables. It should be noted that the S100A12 marker is a family of S100 multigens (16) and it may be considered as a potential inflammatory marker in the serum (17).

A meta-analysis performed by Agarwal et al to evaluate infertile men with palpable varicocele after surgical varicocelectomy (high ligation or inguinal microsurgery) demonstrated that the sperm concentration significantly increased by 9.71×10⁶/mL, motility increased by 9.92% and sperm morphology increased by 3.16% (17). In our study, the mean count improvement was 16×10⁶/mL, morphology improvement was 9%, motility improvement was 13% showing acceptable improvement after varicocele surgery.

In a study conducted by Grasso et al, in 2014, 74 patients with left varicocele were selected. The mean sperm motility before surgery was 13.7% and after surgery was 17.6% (P<0.001). The mean normal sperm morphology before surgery was 6.7% and after surgery was 15.2%. The results showed that surgical treatment of high-grade varicoceles results in improvement of sperm parameters and fertility (18). Motility changes were consistent with

Table 6. Frequency of S100 and semen factors before and after surgery in patients studied in the study

Variable		S100		χ^2 Fisher	*P* value
		Positive	Negative		
Motility before surgery	Abnormal	3	9	4.441	0.041
	Normal	11	6		
Motility after surgery	Abnormal	2	3	0.166	0.535
	Normal	12	12		
Morphology before surgery	Abnormal	2	3	0.166	0.535
	Normal	12	12		
Morphology after surgery	Abnormal	4	6	0.419	0.400
	Normal	10	9		
Count before surgery	Abnormal	7	6	0.293	0.434
	Normal	7	9		
Count after surgery	Abnormal	6	4	0.840	0.300
	Normal	8	11		

our study, which is changed from 44.62% to 56.57% in our study, but did not correlate with our study in terms of post-operative morphology changes, which could be due to the small sample size of our study. Besides, in a study by Sohrabi et al in 2014 on varicocele patients and its effect on sperm parameters, all post-operative sperm parameters were better, in such a way that the mean semen volume, mean sperm count, and sperm motility increased (11).

Table 7. Frequency of CD34 and semen factors before and after surgery in patients studied in the study

Variable		CD34		*P* value
		Positive	Negative	
Motility before surgery	Abnormal	9	3	0.487
	Normal	14	3	
Motility after surgery	Abnormal	4	1	0.731
	Normal	19	5	
Morphology before surgery	Abnormal	4	1	0.731
	Normal	19	5	
Morphology after surgery	Abnormal	8	2	0.669
	Normal	15	4	
Count before surgery	Abnormal	2	11	0.435
	Normal	4	12	
Count after surgery	Abnormal	1	9	0.302
	Normal	5	14	

Table 8. Frequency of improvement in semen factors by CD34 and S100 after surgery in patients studied in the study

Improvement in	No. of patients	S100		CD34	
		Negative	Positive	Negative	Positive
Count	2	1	1	0	2
Motility	1	1	0	0	1
Morphology	5	2	3	0	5
Count and motility	7	4	3	2	5
Count and morphology	4	1	3	1	3
Motility and morphology	5	4	1	3	2
All factors	2	2	0	0	2
No change	3	0	3	0	3
Total	29	15	14	6	23

These results were consistent with the results of our study.

The authors believe that some markers can predict the improvement of semen factors because tissue injury following varicocelectomy can change the level of CD34 and S100. In this regard, immunohistochemistry as an integral component for analysis of tissue status can be applied, and six markers are useful for this target including desmin, epithelial membrane antigen (EMA), CD34, keratin cocktail AE1/AE3, alpha smooth muscle actin and S100 protein. In addition, these markers frequently are used in differential diagnoses of fibroblastic, myoid, and nerve sheath, and perineurial cell tumors, synovial and epithelioid sarcoma, and others (19-21).

Conclusion

Given that the absence of S100 and CD34 are more prominent in patients with varicocele grade III than grade II, it can be predicted that there is a relationship between spermatic vein wall disturbances and varicocele grade. In patients with the absence of these factors in the spermatic vein wall, pre-operative semen analysis was impaired compared to those with positive factors in the vein wall. Although the association was found between the existence of S100 and recovery of motility three months after surgery, no relationship between existence of these markers and recovery of other semen analysis parameters was detected, which could be due to low sample of patients included in the study. However, according to relation between absence of these markers and severity of semen analysis before surgery and relation between existence of S100 in vein wall and recovery sperm motility three months after surgery further investigation was suggested to be done for better evaluation of vein wall factors importance as a prognostic factor of semen analysis recovery after surgery.

Limitations of the study

One of the important limitations of this study was the low sample size, which makes it difficult to decide on a clinical finding. Another limitation of this study was the lack of a control group to compare with normal vein walls.

Suggestions

It is recommended that studies with larger sample size be conducted in the form of cohort design or clinical trials for a more accurate and precise examination of the S100 and CD34 markers.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contribution

FS, AB and VR participated in research design, the writing of the paper and the performance of the research. VR analyzed the data. AA and AB contributed to the study design, preparation of the manuscript and final revision. AB; consultant of study. All authors read and approved the paper.

Conflicts of interest

There is no conflict of interest in this study.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained. Patients were free to leave the study at any time and the research was approved by the ethical committee of Urmia University of Medical Sciences (IR.umsu.rec.1396.212) as a urology residential thesis of Behzad Abyar (Thesis#96-09-63-3029. Before conducting the investigation, the study was registered in Iranian registry of clinical trials (identifier: IRCT20180625040232N1, https://en.irct.ir/trial/32142). Additionally, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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