Markers of accessory sex glands function in men with varicocele, relationship with seminal parameters

Giovanny Vivas-Acevedo, MS,[1] Ricardo Lozano-Hernandez, MS,[1]
Maria I. Camejo, PhD[2]

[1]Centro Diagnostico de Infertilidad y Enfermedades Geneticas, Merida, Venezuela
[2]Universidad Simon Bolivar, Caracas, Venezuela

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Introduction: *Varicocele has been associated with decreased semen quality, not much is known about the effect of varicocele on the accessory sex glands function. The purpose of this study was to evaluate the relationship among varicocele, seminal parameters and biochemical markers of accessory sex glands: neutral alpha glucosidase (NAG, epididymis), fructose (seminal vesicles), prostatic acid phosphatase (PAP) and zinc (prostate).*

Materials and methods: *A clinical study was performed in 190 men with varicocele and 100 men normozoospermic as control group. Semen analysis, hypoosmotic swelling test (HOST), polymorphonuclear (PMN), fructose, zinc, PAP and NAG were determinate. Differences were evaluated by, t test, ANOVA and a Pearson's coefficient correlation.*

Results: *Varicocele group showed a decrease in sperm motility, normal morphology, HOST and vitality. No differences were observed in fructose, PAP and zinc levels between control and varicocele group. The NAG was significantly decreased in varicocele group. A positive correlation was observed between both fructose and PAP with semen volume, sperm concentration, PMN, and zinc levels. Additionally, a decrease of NAG was correlated with a decrease of normal sperm morphology, motility, vitality and HOST.*

Conclusions: *Varicocele does not alter fructose secretion by seminal vesicles and PAP and zinc by prostate. Varicocele is associated with a decrease of NAG activity in seminal fluid, suggesting epididymal dysfunction possibly associated with a detrimental in sperm quality.*

Key Words: fructose, neutral alpha glucosidase (NAG), prostatic acid phosphatase (PAP), sperm, varicocele, zinc

Introduction

The accessory sex glands play an important role in the acquisition of sperm fertilization potential. It has been reported that sperm motility may be influenced by the components of seminal plasma.[1,2] The semen is constituted by the contribution of the testicular-epididymal fluid (< 10%), prostatic (20%-40%); seminal vesicles (50%-80%) and urethral and bulbourethral glands (< 10%).[3] Each gland contributes with different molecules in the ejaculation: carnitine, neutral alpha glucosidase and glycerophosphocholine from epididymis, citrate, zinc and prostatic acid phosphatase from prostate and fructose and prostaglandins from seminal vesicles.[4]

Varicocele has been associated with decreased sperm quality.[5-7] However there is little information

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Address correspondence to Dr. Maria Isabel Camejo, Departamento de Biologia de Organismos, Universidad Simon Bolivar, Caracas 1080 A. Venezuela

about the effect of varicocele on the function and secretion of the accessory sex glands[8,9] and the possible relationship with the biochemical markers composition of semen and seminal parameters.

The purpose of this study was to explore the relationship among varicocele and biochemical markers of accessory sex glands secretion, by measure of fructose levels (seminal vesicles), epididimal neutral alpha glucosidase (NAG), prostatic acid phosphatase (PAP) activity and zinc (prostate) and the effect on the seminal parameters.

Material and methods

Patients

Every analysis has been performed after obtaining institutional board approval and each one of the patients gave their express consent. A clinical cross-sectional analysis has been conducted in patients on infertility evaluation at a diagnostic centre (Centro Diagnóstico de Infertilidad y Enfermedades Genéticas, Mérida), from January 2007 to May 2009.

Men with current or former urogenital-diseases, patients positive to anti-C trachomatis antibodies (EIA kit Monovalent IgA ImmunoComb II ORGENIC, Israel), men with azoospermia or hypogonadism were excluded.

A total of 190 consecutive patients with varicocele were included in the study (grade I: 42; grade II: 66; grade III: 23; bilateral: 59). Varicocele was clinically classified as grade I (palpable with Valsalva manoeuvre), grade II (palpable without Valsalva manoeuvre), grade III (visible through the scrotal skin) or bilateral (some grade of varicocele in both testes). The diagnostic of varicocele was confirmed by Doppler ecography. Additionally, 100 of normozoospermic men without varicocele were selected as control group. All patients were evaluated by two male infertility specialists (medical record, physical examination and criteria of inclusion-exclusion) and a semen analysis was performed.

Semen analysis

Semen samples were collected by masturbation after 3 to 5 days of ejaculatory abstinence. After semen liquefaction, seminal analysis was performed according to WHO criteria.[10] Normal values were: total sperm (rapid and slow progressives; a + b) motility (≥ 50%), sperm concentration ≥ 20 x 10^6 per mL, and normal sperm forms of ≥ 30%.

The hypoosmotic swelling test (HOST) has been performed by mixing 0.1 mL of semen with 1.0 mL of a hypo-osmotic solution prepared as follow: 7.3 g of sodium citrate and 13.5 g of fructose and 1000 mL of distilled water.[11] The mixture has been incubated for 60 minutes at 37°C, the semen samples were examined under phase contrast microscope at 400x. Two hundred spermatozoa were evaluated.

Leukocytes, polymorphonuclear granulocyte neutrophils (PMN), were counted by the method Endtz[12] modified by Politch et al.[13] Briefly, 0.0375% H_2O_2 was added to 4 mL benzidine stock solution (0.0125% w/v benzidine, Sigma Aldricht, in 50% ethanol). Twenty microlitres of ejaculate was mixed with 20 mL fresh benzidine–H_2O_2 solution. After 5 min of incubation, 160 mL of PBS was added and peroxidase-positive (round, brown stained) and peroxidase-negative (unstained) cells were counted in a Mackler chamber using a phase-contrast microscope.

Biochemical analysis

Concentration of seminal fructose has been measure according Mann method colorimetric test,[14,15] using acid resorcinol; 50 µl of deproteinized seminal plasma was mixed with 1.0 mL of acid resorcinol to give a color reaction. Prostatic acid phosphatase activity has been analyzed by means of hydrolysis of p-nitrophenyl phosphate. Seminal plasma was diluted 1:10.000 in distilled water and p-nitrophenyl phosphate has been used as substrate. Colorimetric measurement has been made at 480 nm.[16]

Seminal zinc concentration has been measured by colorimetric method described by Johnsen & Eliasson, modified for Cooper et al.[17] The compound 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)-phenol (5-Br-PAPS) binds with zinc, producing a change in a colour, which absorbs light at a wavelength range of 560 nm.

The neutral alpha glucosidase has been measured in all samples of seminal plasma according to the photometric method described by Guérin et al.[18] Seminal plasma contains both a neutral α-glucosidase isoenzyme, which originates in the epididymis, and an acid isoenzyme provided by the prostate. The latter can be selectively inhibited by sodium dodecyl sulfate (SDS) to allow measurement of the neutral α-glucosidase, which reflects epididymal function. Accounting for non-glucosidase-related substrate breakdown, by using the inhibitor castanospermine, makes the assay more sensitive. The substrate (paranitrophenyl α-D-glucopyranoside) is hydrolysed specifically by a-NAG into paranitrophenyl, during 2 h of incubation at 37°C, pH 6.8. The quantity of paranitrophenyl is measured by spectrophotometer at a wavelength of 405 nm. One international unit (U) of glucosidase activity is defined as the production of 1 µmol product (p-nitrophenyl) per minute at 37°C, pH 6.8.

TABLE 1. **Seminal parameters in control and varicocele group**

	Control (n = 100)	Varicocele (n = 190)	p value
Semen volume (mL)	3.7 ± 1.0	3.6 ± 1.7	ns
Sperm x 10^6/mL	63.9 ± 41.1	71.5 ± 48.5	ns
Motility a + b (%)	68.7 ± 10.2	53.2 ± 20.0	0.0001
Normal morphology (%)	37.1 ± 6.6	31.8 ± 9.7	0.0001
HOST (%)	67.7 ± 2.8	50.4 ± 15.8	0.0001
Vitality (%)	80.8 ± 4.8	66.3 ± 16.1	0.0001
PMN/ejaculate	1.8 ± 0.9	3.7 ± 3.0	0.0001

Statistical analyses

Statistical analysis has been performed using SPSS 10.0 statistical software for Windows (SPSS, Chicago, IL, USA). Basic descriptive statistics (mean ± SD) were calculated to varicocele groups (grades I, II, III and bilateral) and control group. Differences between varicocele total group and control group were determined by t-test. Differences between varicocele (different grades) and control group were determined by analysis of variance (ANOVA) and LSD *pos hoc* comparison for normally distributed values. Pearson´s coefficient correlation has been performed, p values below 0.05 were considered statistically significant.

Results

The average age of the control group was 32.3 ± 7.1 years while in the varicocele group was 28.4 ± 9.4 ($p < 0.05$). Because age may affect the results of the seminal parameters, we performed an ANOVA using age as a covariate. Table 1 shows the seminal parameters in control group and varicocele group. There were no significant differences in the sperm concentration/mL between varicocele total group (grade I+II+III+bilateral) and control group. However, the percentages of following seminal parameters were statistically higher ($p < 0.0001$) in the control group than in the varicocele group: motility a + b, normal morphology, hypoosmotic swelling test and sperm vitality. The concentration of polymorphonuclear (PMN)/ejaculate was increased in varicocele group in comparison with control group.

Table 2 shows the fructose levels, prostatic acid phosphatase activity, zinc levels and neutral alpha glucosidase activity in semen in control group, total varicocele group and varicocele subgroups: grade I, II, III, and bilateral. There was no significant statistical difference between control and varicocele total group in seminal levels of: fructose, PAP and zinc. On the other hand, the activity of the epididymis marker, the neutral alpha glucosidase (NAG), was diminished in patients with varicocele. The ANOVA post hoc analysis (LSD) showed that NAG mU/ejaculate was decreased in all the grades of varicocele.

TABLE 2. **Markers of accessory sex glands in control and varicocele groups**

	Control (n = 100)	Varicocele total group (n = 190)	p value	Varicocele grade I (n = 42)	Varicocele grade II (n = 66)	Varicocele grade III (n = 23)	Varicocele bilateral (n = 59)
Semen volume (mL)	3.7 ± 1.0	3.6 ± 1.7	ns	3.5 ± 1.7	3.5 ± 1.6	3.7 ± 2.0	3.7 ± 1.7
Fructose (mg/ejaculate)	967.8 ± 311.7	1026.4 ± 673.9	ns	1085.8 ± 735.0	944.3 ±575.9	1130.4 ± 919.2	1035.5 ± 625.7
Prostatic acid phosphatase (U/ejaculate)	2360.5 ± 807.9	2531.3 ± 1655.4	ns	2650.3 ± 1503.1	2424.4 ± 18.5	2772.3 ± 1997.2	2472.3 ± 1455.2
Zinc (µmol/ ejaculate)	10.5 ± 8.1	11.8 ± 7.5	ns	10.8 ± 6.7	12.5 ± 7.6	8.9 ± 9.5	13.0 ± 6.8
Neutral alpha glucosidase (mU/ejaculate)	33.0 ± 8.5	15.8 ± 7.7[a]	< 0.0001	16.0 ± 7.0[a]	14.6 ± 6.7[a]	16.3 ± 7.4[a]	16.9 ± 9.3[a]

Values are expressed as mean ± SD
[a]as compared to control group

TABLE 3. **Correlation coefficients between markers of accessory sex glands and sperm parameters in total group of men evaluated (n = 290)**

	Correlation	r Pearson
↑ Fructose (mg/ejaculate)	↑ volume	0.77
	↑ Sperm concentration/ejaculate	0.26
	↑ PMN/ejaculate	0.42
	↑ PAP (U/ejaculate)	0.44
	↑ Zinc (µmol/ejaculate)	0.41
↑ PAP (U/ejaculate)	↑ volume	0.65
	↑ Sperm concentration/ejaculate	0.31
	↑ PMN/ejaculate	0.37
	↑ Zinc (µmol/ejaculate)	0.41
↑ Zinc (µmol/ejaculate)	↑ volume	0.62
	↑ Sperm concentration/ejaculate	0.40
	↑ Motility a + b	0.12
	↑ PMN/ejaculate	0.37
↑ NAG (mU/ejaculate)	↑ Normal morphology	0.14
	↑ Motility a + b	0.23
	↑ Vitality	0.26
	↑ HOST	0.32
	↓ PMN/ejaculate	-0.19

All correlations were statistically significant ($p < 0.05$).
PAP = prostatic acid phosphatase activity; NAG = neutral alpha glucosidase; PMN = polymorphonuclear granulocyte neutrophils; HOST = hypoosmotic swelling test

Table 3 shows the correlation studies between fructose, PAP, zinc, NAG in seminal plasma and sperm parameters in total group of men evaluated (control + varicocele). A positive correlation was observed between both fructose and PAP with the following variables: volume, sperm concentration, PMN, and zinc. Additionally zinc concentration was positively correlated with semen volume, sperm concentration, progressive motility and polymorphonuclear cells and was negatively associated with sperm vitality. On the other hand, NAG was positively correlated with: normal sperm morphology, progressively motility, sperm vitality and hypoosmotic swelling test.

Discussion

In the present study, the markers of epididymal, prostate and seminal vesicles were associated with seminal parameters in normozoospermic men and patients with varicocele, to determinate if varicocele could affect secretion of accessory sex glands. No differences were observed in fructose, PAP and zinc levels between normozoospermic men and patients with varicocele. Similar to our study, Andò et al[8] showed no differences in the concentrations of fructose, however, they found a decrease in PAP and zinc concentration in patients with varicocele; the difference may be explained because in the present study we used modified techniques for zinc and PAP, while they employed the original techniques. Additionally, a recent publication by our group[19] found no significant difference in the concentration of Zn, in another group of men with varicocele or normospermic, by using the equipment "Total Reflection X-Ray Fluorescence".

In the present investigation the percentage of spermatozoa with normal morphology, motility, vitality, and HOST was reduced in patients with varicocele. A previous investigation from our laboratory,[7] showed a detailed analysis of the relationship between degrees of varicocele and seminal parameters, reporting that the percentage of sperm with normal morphology was the unique parameter, that changed between varicocele grades, decreasing in men with varicocele grade III, while other parameters are not affected in relation to the degrees of varicocele, indicating that seminal parameters are affected by the presence or absence of varicocele, regardless of grade.

The activity of NAG was significantly decreased in varicocele total group, similar results were found in a previous study,[9] however, this is the first study which

shows that NAG activity decreased in all grades of varicocele, could be possible that varicocele presence, regardless the grade, affects epididymal functions. NAG is secreted by the epididymal epithelium, mostly in the corpus and cauda.[20] Experimental varicocele in rat produces detrimental effects of the epididymal epithelium with apparition of deformed sperm and macrophages into the epididymal lumen and, also a significant reduction of the NAG activity in the caput, corpus and cauda of epididymis.[21] Previous studies reported a positive association between NAG and sperm motility, as in this study,[9,22] while another study does not show correlation.[23]

The epididymis performs an important role in the maturation of spermatozoa,[24] if the varicocele affects epididymis function, sperm quality could be impaired. Epididymis is located within the scrotum, sharing the same conditions of the testis with varicocele, such as a high reactive oxygen species levels and low total antioxidant capacity, compared with the healthy subjects.[25,26] These conditions could produce failure in the sperm maturation, manifested as poor motility, morphology and sperm membrane quality. Importantly, in this study the concentration of NAG was positively correlated with an increase in normal morphology, motility a + b, vitality and HOST, variables that can be modified by sperm storage in the epididymis.

The HOST evaluates the functional integrity of the sperm membrane: under hypo-osmotic conditions spermatozoa "swell' due to the influx of water and the expansion of the membranes. Jeyendran et al[11] reported a good correlation between the percentage of spermatozoa in a semen sample that were capable of undergoing swelling and the percentage of denuded hamster oocytes that were penetrated by capacitated spermatozoa from the same semen sample. Moreover, a recent publication[27] reported that HOST identifies individual spermatozoa with a minimal DNA fragmentation. Additionally a study reported that a decrease in neutral alpha-glucosidase correlated with an increase in the percentage of DNA fragmentation,[28] these results suggested that HOST, NAG and DNA fragmentation could be associated.

In the present research we conducted correlation coefficients studies between markers of accessory sex glands and sperm parameters in total group of men evaluated, in order to determine whether the concentrations of these markers are related to semen quality and may be linked to the findings for varicocele. It has long been suggested that both prostatic and vesicular fluid affect sperm motility. An investigation reports a negative correlation between number of motile spermatozoa and fructose concentration (mg/mL),[15] while another study shows the opposite [4] and another, similar as our study, found no association.[29] Previous studies reported that a marker of prostatic origin, such as prostate-specific antigen, showed a significant positive association with percentage of sperm progressive motility.[1,30,31] In the present study an increase in zinc concentration was associated with an increase of sperm motility and sperm count, which agrees with previous studies.[18,32] On the other hand, we found an increase of PMN in varicocele group as compared with control group; a similar result has been reported recently.[26] However, future studies are necessary to elucidate the origin of PMN in semen.

In relation to the seminal volume and markers of accessory glands, the results indicate that fructose, zinc and PAP were positively correlated with semen volume, it could be explained by the substantial contribution of volume, to final ejaculate, by the seminal vesicles and prostate. However, NAG was not correlated with semen volume, possibly because the epididymis contributes little to the ejaculate volume.

Finally, it is necessary for future research to investigate the process that could explain the decreasing of epididymal NAG activity during varicocele. Additionally, it would be important to assess whether the varicocelectomy leads to an improvement in epididymal NAG activity. In conclusion, varicocele does not alter fructose secretion by seminal vesicles and PAP and zinc by prostate. Varicocele is associated with a decrease of NAG activity in seminal fluid, suggesting epididymal dysfunction possibly associated with a detrimental in sperm quality. □

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