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ORIGINAL ARTICLE

DOPPLER ANALYSIS OF THE TESTICULAR ARTERY CAN BE USED IN ASSESSMENT OF SPERMATOGENESIS

By

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Aim: *It is quite clear that varicoceles are detrimental to testicular growth and spermatogenesis. However, the majority of men with varicoceles are fertile because the effect is modest or they started with a high spermatogenic potential and remained within the fertile range despite the adverse effect of the varicocele.*

Methods: *The study was conducted between April 2008 and January 2009 and included 62 men of them 7 men with obstructive azoospermia, 9 with unobstructive azospermia, 2 oligoasthenospermia and clinical varicocele, 7 with male accessory gland inflammation (MAGI) and clinical varicocele, 2 with MAGI alone, 3 with unexplained oligoasthenospermia, 2 with clinical varicocele, 12 subjects with normal sperm analysis and recent paternity, and 18 with normal sperm analysis and a varicocele with recent paternity. PSV, EDV, RI and testicular volume were compared among the dyspermic and/or control groups using analysis of variance.*

Results: *The PSV and RI were useful for identifying the different groups of patients. Fertile men with varicocele, men with varicoceles + MAGI and those having UOA + varicocele had the highest PSV and RI. Men with unexplained oligoasthenospermia and men with UOA had the lowest PSV and RI.*

Conclusions: *The PSV and RI are reliable indicators to identify infertile/dyspermic men as they differentiate obstructive from unobstructive azospermia.*

Keywords: *Varicocele, dyspermia, resistive index, testicular volume.*

INTRODUCTION

It is quite clear that varicoceles are detrimental to testicular growth and spermatogenesis. However, the majority of men with varicoceles are fertile because the effect is modest or they started with a high spermatogenic potential and remained within the fertile range despite the adverse effect of the varicocele.(1)

Azospermia (absent sperms in the semen) is an important diagnosis at infertility centers. It is classically divided into obstructive azospermia (OA) and unobstructive azospermia (UOA). Patients with unobstructive azospermia have primary testicular failure.(1-3) The testicular structure in unobstructive azospermia is severely altered and some authors have demonstrated that intratesticular blood flow is also strongly modified showing decreased or absent arterial flow. On the other hand, patients with obstructive azospermia usually have normal spermatogenesis and normal testicular blood flow.(2)

Doppler ultrasonography has arrived as a new evaluation method for intra-testicular blood flow.(4) Authors have tried to associate spermatogenesis with doppler evaluation of intratesticular vascularization.(5)

The intra-testicular arterial blood flow and maximum blood flow velocity were significantly lower in patients with germ cell hypoplasia or maturation arrest.(7)

Arterial impedance of undescended testes in adults may have predictive value about histology and provide more accurate information than the testicular volume.(6) The latter is inversely correlated with the pulsatility index (PI) of the trans-mediastinal artery which has higher

resistances in azoospermic men than in oligospermic (refers to sperm densities less than 20 million sperm per milliliter) and normospermic (mean sperm count between 70 and 80 million per milliliter) men.[1-2] The PI of trans-mediastinal artery was significantly higher in men with obstructive azospermia (OA) than in those with unobstructive azospermia (UOA).[8] A colour Doppler semi quantitative score has been used to distinguish OA from UOA affected by primary testicular pathology Table 1.[1-9] These authors concluded spectral echo Doppler traces from the testicular artery may be related to the amount of spermatogenesis and therefore of help in distinguishing OA from UOA.[10]

Table 1. Doppler semi quantitative score.

Category	Number of intratesticular vessels visible
0	No
1	1-3
2	More than 3 vessels

The diagnosis of male accessory gland infilammation is given when semen classification is azospermic or abnormal spermatozoa and this is considered to result from present or past infection of accessory sex glands or infilammatorv disease of urogenital tract. Infection of accessory sex gland includes epididymitis, vesiculitis and /or prostatitis.[11]

The aim of this study is to assess whether the peak systolic velocity (PSV), end-diastolic velocity (EDV) and resistive index (RI) of testicular arteries as well as testicular volume may be useful in distinguishing the various causes of dyspermia (abnormal semen analysis).[1]

PATIENTS AND METHODS

62 men were included in this study almost all of them were referred from the andrology department in Mansoura University Hospital and the study was conducted in surgery and radiodiagnosis departments between April 2008 to January 2009.

Inclusion criteria: Thirty patients were included in this study complaining from primary infertility (for at least 24 months). They were further classified into 2 groups:

Group I includes 14 patients having oligoasthenospermia, defined as sperm concentration of < 20000/ µL, class A motility of`< 25% and normal morphology < 30% documented on three consecutive semen analysis. Their age range from 27-35 years old. The causes were; left clinical varicocele associated with male accessory gland inflammation (MAGI) (epididymitis, vesiculitis and /or prostatitis) in 7 men (2 grade I varicocele, 3 grade II, 2 grade III), MAGI in 2 men and left clinical varicocele in 2 men (both were grade II), unexplained oligoasthenospermia (3 men).

Group II includes 16 patients having azospermia (mean age 28 , range 26-33 years), 9 of them were unobstructive azospermia and 7 were obstructive azospermia (previous genital inflammation in 3, bilateral absence of vas deferens in 4 was found intraoperative). The type of azospermia was assessed by histological testicular examination. In those with UOA, bilateral testicular histology gave a diagnosis of Sertoli cell syndrome in 3men, tubular sclerosis in 2 men, focal spermatogenesis in 2 men, maturation arrest in 2 men.

Two control groups were evaluated: 30 normal married persons were evaluated as normal control, group 1 comprised 12 married persons with normal sperm analysis with paternity 2-14 months before recruitment (mean age 32 years, range from 26-38 years), group 2 (fertile with varicocele) comprised 18 subjects with normal sperm analysis and clinical left varicocele who had also fathered children5-18 months before (mean age 28 range from 22-35 years). The varicocele was grade I in 7 men, grade II in 6 men and grade III in 3 men. This group was assigned as the control for men with oligoasthenospermia.

Exclusion criteria: Patients were excluded from this study if they were receiving current medical treatments (gonadotropins, steroids or cancer chemotherapy), or had hydrocele, diabetes, hypertension, testicular trauma or history of previous testicle, inguinal or pelvic surgery.

All patients were assessed by medical history, physical examination, three semen analysis, echo-colour Doppler scrotal ultrasonography and grading Table 2.[9] Patients with azoospermia, severe oligospermia (sperm < 5000 µL) or asthenospermia (defects in sperms movement) had their hormonal assay (serum testosterone, prolactine, FSH, LH and progesterone) and chromosomes analyzed and any Y chromosome microdeletion assessed.[1]

Table 2. Doppler varicocele grading.

Grade	Veins>3mm in diameter	Venus reflux
0	Absent	Present at valsalva maneuver
1	Present	Present at valsalva maneuver
2	Present	Present at rest

Patients counseled and fully informed in the outpatient clinic and before the operation about the procedure, postoperative sequelea and possible complications. All patients agreed and signed an informed consent.

All men are operated for varicocele through inguinal approach (Fig. 1).

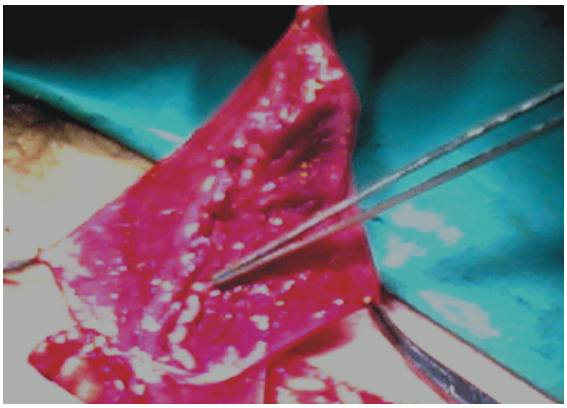

Fig. 1 Congenitally Absent vas deferens diagnosed during varicocelectomy.

Scrotal echo-Doppler ultrasonography: The scanning was undertaken in a warm room with the patient supine, the penis resting on the lower abdomen. Each testicle was measured in three dimensions and the volume was calculated. Doppler flow was measured in each testis using trans-scrotal approach with a 7.5-MHz linear probe (Philips HDI 4000), with 50-Hz filter to eliminate low-frequency signals originating from vessel wall movements. The testicular artery was identified bilaterally in each patient just before the hilum, and colour flow images sampled in a longitudinal plane, the angle of insonation being altered to obtain the maximum colour intensity. Peak systolic velocity (PSV) and end-diastolic velocity (EDV) were calculated by the machine, recorded bilaterally for each patient and expressed in cm/s (Figs. 2,3). The resistive index (RI) was calculated as (PSV-EDV/PSV x 100. For each examination the mean value of three consecutive waveforms was recorded.

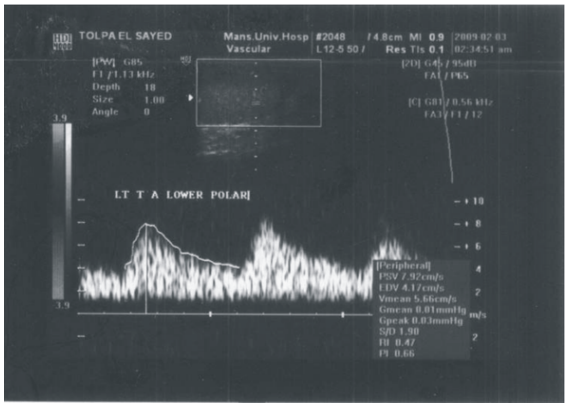

Fig 2. Doppler image of left testicular artery lower polar.

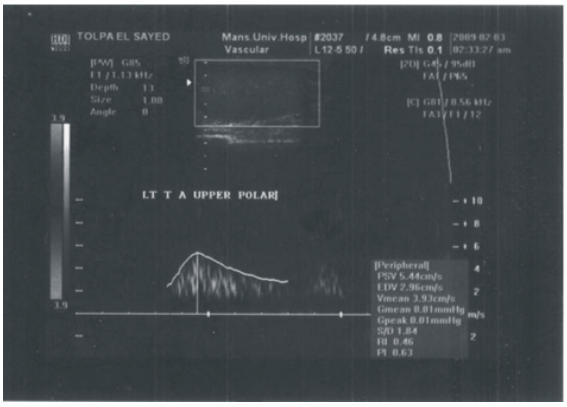

Fig 3. Doppler image of left testicular artery upper polar.

For the sperm samples from each patient or control subjects, the sperm production rate score (SPRS) was calculated as (10):

(sperm concentration x volume of ejaculate) x (WHO A class motile sperm/100) x (morphologically normal sperm/100) / (days of abstinence x bilateral testicular volume).

Sperm was analyzed in three replicates and thus the mean SPRS was used.

Relationships between PSV, EDV, RI or testicular volume (independent variables) as a function of the SPRS (dependant variable) were analyzed using a straight-line regression of data from fertile controls, men with unexplained infertility and patients with UOA. The patients were divided according to the cause of oligoastheno- or azoospermia, then FSH bilateral testicular volume, PSV, EDV and RI were compared between and / or within dyspermic and / or control patient groups using ANOVA of SPSS version 10.

RESULTS

The results of straight–line regression analysis describing the relationship between PSV, EDV, RI or testicular volume as a function of SPRS are presented Table 3. Only PSV and RI were positively and significantly related to SPRS, while EDV and testicular volume were not.

The mean values of PSV, EDV, RI, and bilateral testicular volume are shown in Table 4.

Overall the mean PSV was significantly different among the groups of patients and controls ($P<0.01$); men with varicoceles had the highest value ($P<0.01$) but there were no differences among the three groups with varicocele(associated or not with oligoasthenospermia or MGI). The mean PSV in those with OA, MAGI and in normal controls was not significantly different. Those with unexplained oligoasthenospermia or UOA had significantly lower PSVs than normal controls ($P<0.01$).

Table 3. The relationship of the values from spectral colour Doppler traces of the main testicular artery and bilateral testicular volume, with the functional SPRS. The coefficients a & b are for the equation a+ bx and calculated only for significant relationships.

Variable	R	P	Coefficients a, b	Dxy*
PSV	0.877	<0.01	1.2 x 10,18957	4.41 x10^{22}
EDV	0.048	>0.05		-
RI	0.601	<0.01	4.4 x 10,8297	5.4 x 10
Testicular volume	<0.001	>0.05		

*Deviation from theoretical regression line.

The mean EDV was significantly different among the groups of patients and controls (P<0.05); patients with UOA had a significantly lower mean EDV (P<0.01) than the other groups, but there were no differences among the other groups.

The mean RI was significantly different among the patients and controls (P<0.01), men with varicoceles having the highest RI (P<0.05), but with no differences among the three groups with OA, MAGI and normal controls was not significantly different. Men with unexplained oligoasthenospermia had a significantly lower RI than normal controls (P<0.01) and the RI in men with UOA was significantly lower than in those with unexplained oligoasthenospermia (P<0.01).

The mean testicular volume was also significantly different among the groups of patients and controls (P<0.01); men with varicoceles (associated or not with MAGI or oligoasthenospermia) had significantly lower volumes than those with MAGI, unexplained oligoasthenospermia, OA and normal controls (P<0.01) but there were no significant differences among the three groups with varicocele. Patients with UOA had significantly lower testicular volumes (P<0.01) than those with varicoceles.

DISCUSSION

The aim of this study was to identify a first-line test of spermatogenesis distinct from the sperm count, investigating the PSV, RI, EDV and bilateral testicular volume in different groups of fertile and infertile men. Spermatogenesis is a suitable target as it directly influences sperm function. Indeed, Percoll-gradient selection of male gametes did not increase the fertilization rate for IVF,[12] the fertilization rate after ICSI with UOA testicular sperm was significantly lower than with OA sperm,[13] and the presence of germinal round cells in the ejaculate was associated with a reduced ability of sperm to fertilize oocyte in vitro.[9]

In the present study the SPRS was calculated from routine sperm analysis as the major indicator of spermatogenesis. The high variability of sperm counts and influence of mathematical effect (regression to the mean) casts doubt on the relationship between sperm analysis and in vivo fertilization potential.[14] Thus more sophisticated techniques to understand spermatogenesis have fertilization evaluated. Diverse populations of spermatozoa may be present in semen and this makes such testing problematic because most azospermic infertile patients have fallen into disfavor because they yield no more information than standard semen analysis, but involve much equipment and expense. Thus, in line with most clinicians we used routine semen analysis.

Detecting significant differences among the different groups of infertile men is insufficient to conclude that testicular volume, PSV, EDV and RI should be considered as indicators of spermatogenesis. They should also be assessed as the independent variable of straight-line regression in relation to the SPRS (dependant variable).[15] From histological studies there are three levels of severity in changes of spermatogenesis: men with UOA have severely or totally affected spermatogenesis, those with unexplained infertility are mildly affected, while men with a normal sperm analysis who had recently fathered children are considered to have intact spermatogenesis.[16] These groups were used to assess the straight –line regression with SPRS.

Table 4. The PSV, EDV, RI and bilateral testicular volume (BTV) in the different groups of patients and control.

Group	No	PSV cm/sec	EDV cm/s	RI	BTV ml
MAGI	2	14.3	4.1	0.8	33
OA	7	14.5	3.9	0.78	32
V+MAGI	7	22	4.4	0.78	30
V	2	20.6	4	0.79	31
UO	3	8.6	4.8	0.72	33
UOA	9	2.5	2.7	0.64	18.9
FC	12	14.5	4.3	0.77	33
FV	18	20	4.1	0.8	32

BTV= bilateral testicular volume, V=varicoceles, UO= unexplained oligospermia, FC= fertile controls, FV= fertile varicoceles.

Only RI and PSV were significantly and positively related to SPRS, while EDV and testicular volume were not. Thus PSV and RI could be used as indicators of spermatogenesis. Men with UOA had a significantly higher EDV than the others, probably because of a high resistance to blood flow by the testicular parenchyma, in which connective tissue is more abundant than in other patients.[17] The three varicocele groups had significantly lower testicular volumes than the others (but higher than in UOA), with no difference among them. A case survey indicated that diminished volume is probably linked to the presence of grade III varicoceles, which were found both in infertile and fertile groups.[9]

Men with varicoceles had the highest PSV and RI, with no difference among the three varicocele groups, indicating that in these the PSV and RI cannot predict spermatogenesis. Nor did the EDV differ significantly from that in other patients (exception those with UOA) or controls indicating that a high RI and PSV are not caused by changes in vascular resistance below the artery assessed.[18] Turner (2001)[19] reported that the most important mechanism in producing oligoasthenspermia and venous varicosity in experimental models of left varicocele was increased arterial blood flow. In addition others have already shown an arterial hyperflow in patients with varicocele.[20] These results are coping with those of this study.

Patients with MAGI had PSV and RI values similar to those of the controls. MAGI with oligoasthenospermia is mostly caused by increase in PGs, nitric oxide, reactive oxygen species and interleukins in seminal plasma, rather than by changes in tubular spermatogenesis.[21] These data imply that low sperm count with a testicular volume, PSV, RI, EDV within the normal range may indicate MAGI, thus avoiding more invasive procedures. These findings agree with Biagiotti et al 2002[9] and Vicari & Calogero 2001.[22]

Patients with normal spermatogenesis or OA have a similar number of mature spermatozoa per tubule, while this diminishes markedly in those with unexplained infertility and even more in those with UOA.[15] This could be explained by the fact that patients with obstructive azoospermia have the testicular parenchymal architecture and function preserved. These patients are able to produce spermatozoa and only face problems with sperm transport.[23]

In the present study patients with OA had PSV and RI values similar to those of the controls, but significantly higher than in men with unexplained infertility or UOA. This goes in agreement with Souza et al 2005[24] who reported that obstructive azoospermia is compatible with normal testicular micro-vascularization. These results are consistent with previous reports[2,8,9,25] and indicate that PSV and RI may be useful for distinguishing the two forms of azoospermia. On the contrary EDV and testicular volume are not useful for differentiation of OA from UOA as already noted by other authors.[14,9]

Testicular volume may be confusing; testicles which produce a normal amount of sperm may be small and those which produce no sperm (which have maturation arrest) may often be large. The same is true for ultrasonography of the epididymis; large epididymis do not imply OA, as similarly a normal epididymis does not always indicate UOA.[9]

Some authors use diagnostic testicular biopsy to distinguish OA from UOA; it is invasive and may be associated with potential complications (hematoma, inflammation, testicular devascularization) and patient discomfort.[26] Thus needle biopsy has been regarded as an alternative, but the scattered mosaic arrangement of the various stages of spermatogenesis in the human seminefrous tubule requires = 20 seminefrous tubules foe a good statistical sample of the total range of spermatogenesis in each testicle. Therefore many needle biopsies are needed,[15] thus causing lesions identical in nature and quantity to open biopsy, and being no less painful.[26]

With the aim of bloodless testing of spermatogenesis, many other methods have been used to distinguish OA from UOA. Serum inhibin B was claimed to be positively related sperm retrieval on testicular sperm extraction, and to distinguish UOA from OA,[27] but these data were not confirmed.[28] Nitric acid,[29] transferring and its soluble receptors,[30] and anti Mullerian hormone[31] have been proposed as markers of spermatogenesis but the techniques lack sensitivity and specificity.[9]

The present study showed a clear differentiation of OA from UOA using PSV and RI. No patients with UOA had a PSV > 4.5 cm/s or RI> 0.95. No patients with OA had PSV <11.5 cm/sec or RI < 0.93. Thus we conclude that recording the PSV & RI is an easy, convenient and valuable method for differentiating OA from UOA. Moreover, measuring PSV & PS is inexpensive and quick' as scrotal echo-Doppler scanning is routine for infertility.

Patients with a history of testicular trauma, surgery or clinically detectable diseases were excluded from the present study because it was considered difficult to ascertain whether spectral trace data were related to testicular trauma, disease and surgery or to spermatogenesis.

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