Testicular Contrast Harmonic Imaging to Evaluate Intratesticular Perfusion Alterations in Patients With Varicocele

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Purpose: To determine whether changes in intratesticular microcirculation perfusion affect spermatogenesis in patients with left varicocele we performed testicular contrast harmonic imaging.

Materials and Methods: A total of 90 patients with left varicocele (oligospermia in 50 and normozoospermia in 40) and 36 controls without varicocele (oligospermia in 16 and normozoospermia in 20) were enrolled in the study. Before contrast harmonic imaging all participants were evaluated by clinical examination, hormonal analysis, semen sample and scrotal ultrasound. We calculated contrast material arrival time in the arteriolar circulation (wash-in), time to peak in arterial circulation, arrival time in the venular circulation (washout) and mean transit time in each testis on contrast harmonic imaging.

Results: We found no difference in the distribution rate of varicocele grade in patients with vs without oligospermia. All contrast harmonic imaging parameters were significantly higher in patients with varicocele plus normozoospermia or oligospermia and controls. We found no significant differences in contrast harmonic imaging parameters in patients with lower varicocele grading with respect to the higher grades. In patients with varicocele we found a negative linear correlation between total sperm count and left mean transit time ($r = -0.29$). In a multivariate model left mean transit time was the only independent predicting parameter of oligospermia ($p < 0.05$). Mean transit time greater than 36 seconds predicted oligospermia in patients with left varicocele with 78% sensitivity and 58% specificity.

Conclusions: To our knowledge we report for the first time that testicular contrast harmonic imaging may be a new diagnostic tool able to improve our knowledge about the influence of varicocele on intratesticular microcirculation.

Key Words: testis; fertility, male; varicocele; echocardiography, Doppler, color; contrast media

Abbreviations and Acronyms

CDUS = color Doppler ultrasound

CHI = contrast harmonic imaging

FSH = follicle-stimulating hormone

ISV = internal spermatic vein

LH = luteinizing hormone

MTT = mean transit time

TTP = time to peak arterial circulation

VN = normozoospermia with left varicocele

VO = oligospermia with left varicocele

Submitted for publication April 16, 2009.

Study received hospital ethics committee approval.

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VARICOCELE, that is dilatation of the veins along the spermatic cord with a backup of blood, is the most commonly reported correctable cause of male factor infertility. It is found in approximately 35% of men with primary infertility and in 80% with secondary infertility.[1] However, since varicocele also occurs in 15% of the general population,[2] it is clear that not all varicoceles impair fertility. Why varicocele has a harmful effect on spermatogenesis in only some men is largely unknown and many physiopathological mechanisms have been proposed, such as hypoxia, hyperther-

0022-5347/10/1831-0263/0
THE JOURNAL OF UROLOGY®

Vol. 183, 263-269, January 2010
Printed in U.S.A.
DOI:10.1016/j.juro.2009.08.140

mia, renal-adrenal reflux, hormonal dysfunction, autoimmunity, oxidative stress and apoptosis.[3]

Many of these conditions are due to an impaired testicular venous drainage system. The 1-way valves in the ISV facilitate venous blood flow upward against gravity since there is no active pump in vertical testicular drainage. Without competent 1-way valves the ISVs cease to function as a drainage system and become passive vessels. Several studies show that the destruction of 1-way valves is a bilateral vascular disease.[4,5] Each ISV then contains a vertical blood column (about 40 cm on the left side and 35 cm on the right side) that produces increased hydrostatic pressure (about 31 mm Hg on the left side and 27 mm Hg on the right side) in the testicular venous drainage system. Blood flow is possible into the testis only in the direction of negative pressure, so that when pressure exceeds arteriolar pressure (18 to 20 mm Hg), there is relative hypoxia of the testicular tissues.[6] The hypoxic-ischemic state affects seminiferous tubules, Sertoli's cells, germ cells and later Leydig cells. It impairs sperm quality and decreases sperm quantity, eventually leading to mild, moderate, severe and extremely severe oligoasthenoteratozoospermia.

Usually clinical varicocele is diagnosed by physical examination and graded based on physical findings. In 1985 WHO found that physical examination has only about 50% sensitivity to detect varicocele compared to venography with a 23% false-positive rate.[7] Aside from the issue of sensitivity physical examination is often inconclusive or equivocal, ie in patients with a history of scrotal surgery, concomitant hydrocele, morbid obesity etc. Thus, the American Society of Reproductive Medicine also recommends that imaging must be done to evaluate infertile men with varicocele when physical examination is inconclusive.[8] Of imaging studies CDUS has become the most widely accepted, commonly used modality to evaluate varicocele, although there is no consensus on what CDUS parameters should be used to diagnose varicocele.[9] Furthermore, CDUS provides no information on alterations in intratesticular microcirculation, in particular when hydrostatic pressure exceeds arteriolar pressure, which is considered the principal cause of testicular damage in patients with varicocele. To our knowledge for the first time we evaluated intratesticular microcirculation perfusion alterations by testicular CHI in patients with left varicocele and the possible harmful effect on spermatogenesis.

PATIENTS AND METHODS

After the protocol was approved by the hospital ethics committee from June 2008 to February 2009 we enrolled 126 patients, including 90 with left varicocele (VO in 50 and VN in 40) and 36 men without varicocele (oligospermia in 16 and normospermia in 20) as controls. Before ultrasound the study purpose was explained to each patient and written informed consent was obtained. Each patient was previously evaluated by clinical examination and scrotal CDUS to grade varicocele in 5 groups, as previously described by Sarteschi and Menchini Fabris.[10] Hormonal parameters, including LH, FSH, testosterone, estradiol and prolactin, were measured and a semen sample was obtained by masturbation after 3 days of sexual abstinence. After liquefaction at room temperature semen volume, pH, and sperm concentration, motility and morphology were determined according to WHO guidelines for semen analysis.[11] To exclude infection a microbiological culture was performed in each case.

Study Design

Before beginning CHI patients were asked to stand for 2 hours to improve venous blood flow to the lower limbs. All ultrasound was performed with the patient lying on a bed tilted at 45 degrees. We used a high resolution Aplio™ XV echo color Doppler device with a 6 to 13 MHz multifrequency linear probe (Toshiba, Tokyo, Japan) and predisposed for contrast enhanced imaging. Three experienced sonologists blinded to other imaging data and clinical information performed all examinations. Intra-observer and interobserver variability was estimated to be less than 10%. The scanner was set in CHI mode, the harmonic imaging setting that shows the highest correlation coefficient between contrast concentration and acoustic intensity. The mechanical index was set to less than 0.1 and acoustic power was set to 1%.

To study microcirculation in each testis 2.5 ml contrast agent containing phospholipid stabilized microbubbles filled with sulfur hexafluoride[12,13] were administered as a bolus in the antecubital vein using a 21 gauge intravenous catheter, immediately followed by a 5 ml rapid flush of 0.9% NaCl solution infusion. A 10-minute interval between the 2 contrast injections allowed the contrast agent to be completely removed by breathing.

We recorded a 2-minute video clip at 15 frames per second for each testis after 20 seconds from the injection of the contrast agent. Data were stored on a DVD.

Image Analysis

Digital clips were analyzed and automatically processed by the Qontrast dedicated application (Bracco, Vienna, Austria). Subsequently the tissue region and the perfusion period were defined in a region of interest and the brightness signal was analyzed (fig. 1). The software calculated time-intensity curves on a pixel-by-pixel basis. Numerical data on arrival time in the arteriolar circulation (wash-in), TTP, arrival time in the venular circulation (washout) and MTT of contrast material in each testis were calculated (fig. 2).

Statistical Analysis

Absolute data are expressed as the mean ± SD of the mean and categorical variables are expressed as a percent. Comparison between 2 groups was performed by Student's t test for continuous data after acceptance of normality with the Kolmogorov-Smirnov test and by the chi-square test for categorical data. Linear correlations were ana-