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Role of MR Spectroscopy (H1-MRS) of the Testis in Men with Semen Analysis Altered

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Abstract

Purpose: Proton magnetic resonance spectroscopy (1 H-MRS) has been proposed as a tool to assess male infertility providing metabolic signatures related to the spermatogenesis in the testis. This study sought to identify the role of 1 H-MRS in the diagnosis of infertility in patients with semen analysis altered.

Materials and Methods: 14 patients (27 testicles) with fertility problems and with an altered semen analysis (5 oligospermia, 3 asthenospermia, 6 oligoasthenospermia) and 9 controls (18 testicles) with normal spermatogenesis assessed (men with prior paternity and normal semen analysis) and normal testicles at magnetic resonance (MR) and ultrasonography (US) with colour Doppler (CD) examination were included. All patients underwent testis US and CD investigation, conventional MR examination at 1.5T including T1 and T2 weighted images in three orthogonal planes and proton magnetic resonance spectroscopy (1 H-MRS) with single-voxel PRESS (TR 2000 ms / TE 31 ms). Major metabolites peaks (choline, creatine, lipids, lactate) were calculated and compared between the patients and controls.

Results: Mean choline peak in the semen analysis altered group was statistically significantly lower than the normal group (0.69 vs 1.34, 95% CI: 0.52 - 0.85; $p < 0.001$). 18 testicles within semen analysis altered group (66,7%) had both MR and US examination negative but mean choline peak lower then controls (1.09 vs 1.34, $p < 0.001$). 7 testicles of those presented also varicocele at CD investigation.

Conclusion: 1 H-MRS revealed a significant shift towards lower choline peak in patients with semen analysis altered compared to controls with normal spermatogenesis. Moreover 1 HMRS provided to find out spermatogenesis disorder in patients with normal testis at MR and US examination.

Keywords: Ultrasound-Colour Doppler; MR-Spectroscopy; Magnetic resonance; Molecular imaging; Genital/reproductive system male

Introduction

Male infertility is responsible for about 60% of reproductive impairments within childbearing couples. Approximately 8% of men in reproductive age seek medical attention for infertility problems, 10% of those have a reversible nature: more than one third (40%) have a varicocele [1,2].

Several authors described subfertility and varicocele association but an improvement after treatment is not accepted yet as direct consequence [3].

Nevertheless, infertility represents an indication for the varicocele correction (surgical or percutaneous), even in asymptomatic patients with normotrophic gonads [2,4,5].

Spermogram is the main tool to evaluate the male infertility and some authors address for the treatment whether associated with a varicocele [6,7].

Patients with suspicion of infertility are studied with ultrasonography with color-Doppler (USCD) at first, to evaluate volumetry, inner structure and vascularity of the parenchymal testis. However, USCD cannot directly provide metabolic informations about the spermatogenesis [8].

Magnetic Resonance (MR) of the testis, by means of classic T1 and T2 weighted sequences, is the second level choice to study the parenchymal pattern, the vascularization and for the morpho-volumetric characterization [9-14].

Recently, some authors addressed the application of proton magnetic resonance spectroscopy (1H-MRS) to study the testicular metabolites [15,16].

This technique is already used for the diagnosis and follow up of brain, prostate and breast tumors. 1H-MRS, indeed, allows to measure the concentration of the main metabolites of living organs, providing biochemical variations within the tissues [17-19].

In literature, studies already exists which correlate metabolites variations (mainly choline) with testis cancer; however, to the best of our knowledge, few authors studied the association between parenchymal alterations, metabolites concentrations and spermatogenesis [15,20].

Choline concentration within the testicular tissue is thought to be related with the level of spermatogenesis into the seminiferous tubules. It could be the single evidence of testicular impairment in patients with normal testicular volumetry and tissue [16,21].

Male infertility evaluation is often underestimated or delayed. Therefore, a multiparametric evaluation with lab tests and standardized instruments is crucial to assess the etiology and the proper treatment.

This study sought to identify the role of 1H-MRS in the diagnosis of infertility in patients with semen analysis altered but without morphostructural alterations of the testis at USCD and MR.

Materials and Methods

Exclusion criteria for this study were the common contraindications to perform MR [22]. This study was conducted prospectively and approved by the local institutional review board. Written informed consent was obtained from all patients before inclusion.

Subjects

14 patients (27 testicles: 1 patient orchydectomized for tumor; 7 patients with unilateral varicocele), mean age: 36.2 +/- 11 years (range: 21-58), with fertility problems (at least one consecutive year of reproductive difficulties) and with an altered semen analysis (5 oligospermia, 3 asthenospermia, 6 oligoasthenospermia) and 9 controls (18 testicles) with normal spermatogenesis assessed (men with prior paternity and normal semen analysis) and normal testicles at MR and USCD examination were included.

Imaging technique and data analysis

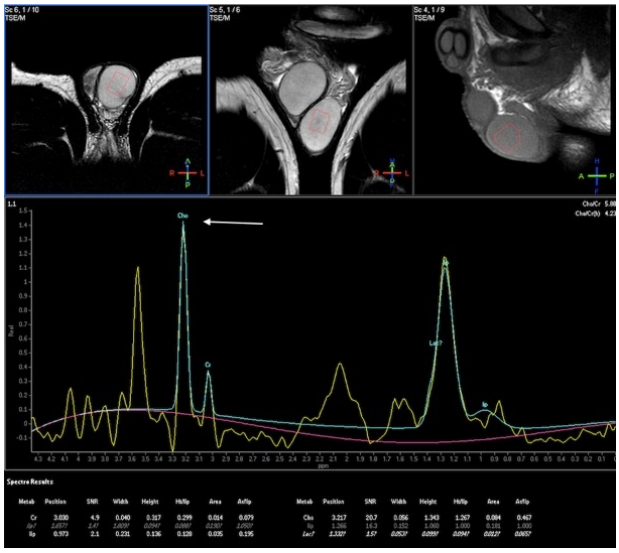

Figure 1: ^{1}H-MRS pattern of normal testis showing significant peaks at 3.21 ppm (choline; arrow), 3.02 ppm (creatine) and at 1.3 ppm (lipids).

All patients underwent USCD performed by two operators (GCP e FA) with 26 and 5 years of experience, who used a high-resolution transducer (7-17 MHz linear-array probe) with B-flow (B-mode flow), color Doppler and power Doppler (GE Healthcare Logic 7, Philips iU22). Automatic settings for testicular examination were used, and the operator modified the pulse repetition frequency (PRF), focal zone, gain and wall filter to obtain optimal color Doppler flow. Those patients underwent, also, conventional MR examination at 1.5 T including fast spin-echo (FSE) sequences, T1 and T2 weighted images in three orthogonal planes. A Philips Achieva 1.5 Tesla system was used with a 14 cm circular surface coil, 12 × 12 cm field of view, 256 × 256 matrix, 3 mm slice thickness and 1 mm of gap. 1H-MRS was also performed: single-voxel Point RESolved Spectroscopy (PRESS, TR 2000 ms / TE 31 ms) with 128 averages during free-breathing pattern. Three chemical-shift-selective radiofrequency wave pulses, each followed by dephasing gradient pulses, suppressed the water peak. Volume of interest (15 × 15 ×15 mm) was placed in the testis parenchyma avoiding contamination from adjacent structures (Figure 1).

USCD and MRI evaluated any parenchymal alteration and morphovolumetry of the testis with the ellipsoid method (length x width x height x 0.52) considering a normal volume 17+/- 5.5 mL [23].

The main metabolites peak (choline, creatine, lipids and lactates), including their ratios (choline+creatine/lipids and choline/lipids), were calculated and compared between the patients and the control group.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 20.0, IBM Corp, Armonk, NY, USA. The 95% bootstrap confidence intervals (CI) for the mean difference and Mann-Whitney U-test was used to compare major metabolites peaks (choline, creatine, lipids, lactates and their ratios) between the patients with semen analysis impaired and the control group [24,25]. A p-value < 0.05 was considered statistically significant.

Results

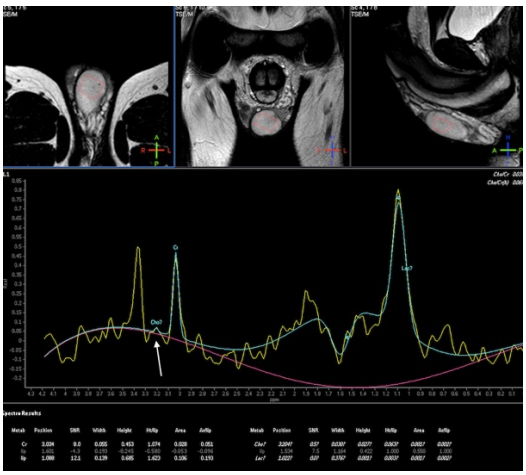

Figure 2: Significant decrease in choline peak (arrow) probably due to failure of spermatogenesis in a patient with altered sperm analysis.

All the 45 testicles presented normal volume at CDUS and no signal alterations at MR. Mean choline peak in the semen analysis altered group was significantly lower than the normal group (0.69 vs 1.34, p < 0.001, 95% CI: 0.52-0.86; Figure 2).

Also the other metabolites and ratios were decreased in the study group compared with the normal group but with less significance (creatine: 0.08 vs 0.31, 95% CI: 0.04-0.2; lipids: 0.77 vs 1.06, 95% CI:

0.64-0.92; lactates: 0.08 vs0.09, 95% CI: 0.01-0.17; choline+ creatine/lipids: 1.04 vs1.56, 95% CI: 0.76-1.32; choline/lipids: 0.9 vs 1.26, 95% CI: 0.69-1.11; $p < 0.05$) as displayed in Table 1.

Metabolites	Spermogram	No of testicles	Mean Peak	SD	Mann-Whitney p-Values
Choline	Normal	18	1.34	0.1	p<0.001
	Altered	27	0.69	0.43	
Creatine	Normal	18	0.31	0.14	p<0.05
	Altered	27	0.08	0.33	
Lipids	Normal	18	1.06	0.13	p<0.05
	Altered	27	0.77	0.35	
Lactates	Normal	18	0.09	0.17	p<0.05
	Altered	27	0.08	0.21	
Choline+Creatine/Lipids	Normal	18	1.56	0.15	p<0.05
	Altered	27	1.04	0.72	
Choline/Lipids	Normal	18	1.26	0.14	p<0.05
	Altered	27	0.9	0.58	

Table 1: Mean peaks of the main metabolites within the controls and altered group. Mann-Whitney U-test for the correlated data (SD, standard deviation).

18 testicles within semen analysis altered group (66.7%) had both MR and US examination negative (normal volumes and no parenchymal signal alterations) but mean choline peak lower then controls (1.09 vs 1.34, $p < 0.001$, Figure 5). 7 testicles of those (67%) presented varicocele at USCD investigation (Figures 3-5).

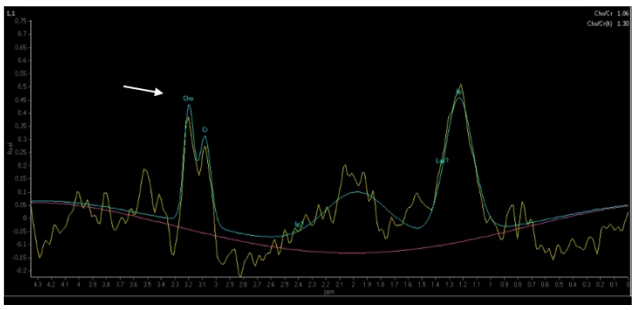

Figure 3: Spectrogram of a patient with varicocele; note the reduction of the choline peak (arrow).

Discussion

The varicocele is one of the most frequent causes of male infertility. USCD represents the most non-invasive and reliable tool to diagnose the varicocele, allowing to evaluate the testicular parenchymal impairment, the ectasia and the incontinence of the pampiniform plexus [8,9,26]. Sometimes, those patients present altered semen analysis with hypoplasia and inhomogeneity of the testicular tissue at US, suggestive for compromised spermatogenesis. However, patients with altered spermiogram and varicocele often are negative at US and MR examination, without any sign of impaired testis.

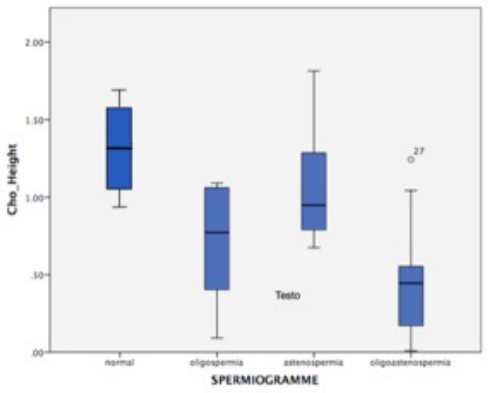

Figure 4: Boxplots show testicular levels of choline and ratios in normal testes and in abnormal testes.

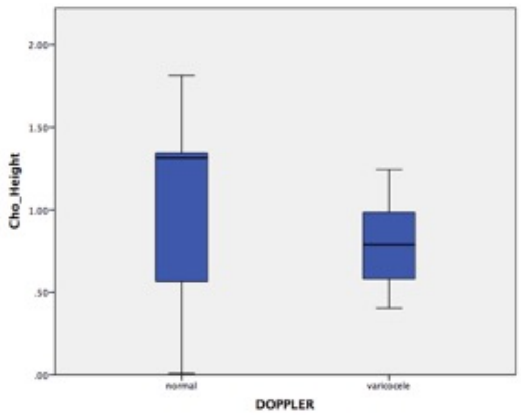

Figure 5: Boxplot shows the different choline peak within normal testes and varicoceles.

In our study, ^{1}H-MRS was the single tool able to assess a suffering testicle in 66.7% of the patients with irregular semen analysis but normal MR and US, and in 100% of patients with varicocele at USCD (7 patients, Figure 5). Choline levels were significantly lower within the testis parenchyma of patients with compromised spermatogenesis. Also lactates and lipids concentrations were altered but with less significance: the former increased probably for the hypossic environment, the latters reduced for alterations of the cells membranes.

^{1}H-MRS is already used in clinical oncology for the diagnosis and follow-up of prostate, breast and brain tumors [17,19,27]. Indeed, choline is the essential part of the phospholipids which compose the cell membrane and its increasement can reflect a higher turn-over as membrane disruption or cell proliferation (e.g., in tumours) [17,28].

In the testes, choline levels reflect phospholipid synthesis in spermatogenesis [26,28]. Sertoli and Leydig cells are also involved in the spermatogenesis but with a slower metabolism [1,29,30]. High levels of choline represent an altered cell metabolism as seen during

oncogenesis or in the neoplastic progression; breast and prostatic cancer are the main examples where increased choline has been correlate with Gleason score.

Aaranson et al. [20] reported higher choline levels in patients with normal spermatogenesis compared with patients with arrested maturation of the spermatozoa. Therefore, 1H-MRS could identify the testicles containing spermatids or spermatozoa.

Gupta et al. found ^{1}H-MRS to be sensitive and reliable in differentiating infertile or azoospermic testicles from normospermic testicles [29].

Gupta [29] and Oates [30] results show choline low levels in patients with testis cancer and varicocele, probably due to the decreased spermatogenesis both the conditions can cause.

Also the study of Baleato-Gonzalez et al. confirms the increased choline levels in testis cancer but with a different behavior compared with other tumors: stratifying their population for tumors and varicoceles, these authors describe a reduction in choline concentration due to altered spermatogenesis [15]. In this case, an integration of clinical analysis and several imaging techniques is required to differentiate between neoplastic and benign hypofertility.

Some authors, on the other hand, found choline high level in testicles after puberty, probably related to an increased spermatogenesis [17].

According to the latest literature, our results confirm ^{1}H-MRS a reliable non-invasive tool in finding and evaluating spermatogenesis alteration into the seminiferous tubules, when semen analysis cannot be performed. Changes in the levels of metabolites (mainly choline), reflect testicular parenchymal alterations [15].

In the present study, conversely to patients with normal semen analysis, patients with varicoceles had lower choline levels, in line with what Sheriff et al. already reported [31].

Patients with varicoceles but without semen alteration not necessarily need to be treated [4]. Even if the sperm analysis still remains the main investigation tool, it is not always available. ^{1}H-MRS, due to its high sensitivity, is able to detect the small metabolic alterations within the testes. This technology could represent an alternative approach to identify which patient will require a treatment for the varicocele. However, the main limitation of this study is the small population included.

Even if our results agree with the literature they need to be confirmed with higher number of patients.

With this drawback, 1H-MRS could be usefull as alternative of the semen analysis, in some targeted cases, for instance:

- In the evaluation of pediatric patients, selected for the treatment of the varicocele;

- To check the improved spermatogenesis during the follow-up;

- In special events such as psychological, moral or religious matters.

Moreover, leading to analyze in detail an area within the testis wider than the commonly used with more invasive testis sperm detection, ^{1}H-MRS has the potential to replace the diagnostic testis biopsy in discriminating between normal and abnormal spermatogenesis in azoospermic men as already stated by Aaranson et al. [20], even if it will not widely substitute the semen analysis.

Conclusions

^{1}H-MRS revealed a significant shift towards lower choline peak in patients with semen analysis altered compared to controls with normal spermatogenesis. Moreover, ^{1}H-MRS provided to find out spermatogenesis disorder in patients with apparently normal testis at MR and US examination. This is particularly useful in patients with varicocele without any other ultra-structural alterations of the testicles, in order to address the correct treatment, especially when the spermogram is not achievable.

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