Proteomic analysis of seminal plasma in adolescents with and without varicocele

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Objective: To compare proteomic profiles of seminal plasma from adolescents with varicocele and changes in semen quality with the plasma from adolescents with varicocele without seminal changes and from adolescents without varicocele.
Design: Observational study.
Setting: Patients in an academic research environment.
Patient(s): Adolescents without varicocele (control group), adolescents with varicocele and normal semen quality (VNS group), adolescents with varicocele and abnormal semen quality (VAS group).
Intervention(s): Two semen collections at 1-week interval. Protein separation by two-dimensional protein electrophoresis, analysis by gel densitometry, and identification by mass spectrometry.
Main Outcome Measure(s): Overexpressed proteins in each group, observed by increased densitometric signal in gels, and exclusively identified proteins in each group.
Result(s): No differences were observed among the three groups regarding clinical parameters. In semen analysis, the VAS group presented lower sperm concentration, motility, and morphology compared with the VNS and control groups. Forty-seven protein spots of interest were submitted to mass spectrometry identification. Apoptosis regulation proteins were overexpressed in the VAS group, whereas spermatogenesis proteins were overexpressed in the VNS group. Controls presented proteins related to homeostasis.
Conclusion(s): Changes in the proteomic profile of adolescents with varicocele and normal semen parameters (VNS group) indicate that normal semen analysis may not reflect alterations in proteins in seminal plasma. Implementation of proteomics will help characterize proteins identified in seminal plasma and will facilitate detection of new proteins associated with spermatogenesis and sperm function. (Fertil Steril® 2013;99:92–8. ©2013 by American Society for Reproductive Medicine.)
Key Words: Varicocele, adolescent, proteomics, semen analysis, seminal plasma

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Varicocele is characterized by dilation of veins of the pampiniform plexus and affects approximately 15%–25% of the male population. It is a time-dependent disease that begins at puberty and is considered the major treatable cause of male factor infertility (1–3). Varicocele accounts for 40% of primary and 80% of secondary causes of male factor infertility (4).

The scrotal temperature required for normal spermatogenesis is 2°–3°C below body temperature. Varicocele impairs the countercurrent heat exchange mechanism, which is the cooling system of the arterial blood supply, resulting in venous stasis and thereby maintaining testicular temperature at body temperature (5, 6). This loss of testicular thermoregulation causes an increase in cellular metabolism, which is not accompanied by an increase in testicular blood supply. The resulting lack of adequate cell oxygenation leads to chronic hypoxia, increased stage-specific apoptosis of germ cells at most susceptible stages of spermatogenesis, and excessive production of reactive oxygen species. Excessive reactive oxygen species production that is not counterbalanced by antioxidant systems results in

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a number of deleterious effects to the sperm, including increased levels of sperm membrane lipid peroxidation (which results in loss of membrane fluidity and changes in membrane permeability), reduced motility, low level of acrosome reaction, decreased mitochondrial activity, increased DNA fragmentation, and apoptosis (6–8).

It is widely believed that clinical varicocele in adult men should be surgically corrected when changes in the results of semen analysis or functional tests are confirmed (9–11). However, this consensus does not apply to adolescents, as the hypothalamic-pituitary-testis axis is still immature during adolescence, thus rendering semen analysis inaccurate. At present, surgical correction is recommended for adolescents with clinical varicocele and reduction in ipsilateral testicular volume of more than 20% compared with the contralateral testis (9). There is a need for more objective criteria for the indication of surgical correction of varicocele in adolescents. Not all adolescents with varicocele will have reduced fertility potential in the future, but many of them may have severely impaired spermatogenesis. Semen analysis is not reliable for evaluating varicocele in adolescents, and current clinical criteria for the indication of surgical treatment are unsatisfactory, as testicular growth retardation would determine impaired spermatogenesis. Studies of varicocele in adolescents are designed to determine which adolescents would benefit from surgical correction and the best time to perform surgery.

With the advent of the human genome project and development of bioinformatics, a new science encompassing omics (e.g., transcriptomics, proteomics, and metabolomics) has emerged and is aimed at improving the analysis of biological systems. Proteomics is a novel approach to the study of protein functions and investigation of metabolic processes to better understand the workings of a cell or tissue at the molecular level (12).

Thus, seminal plasma proteins can serve as potential markers of impaired spermatogenesis and might provide an early indication for varicocelectomy before clinical varicocele is detected by semen analysis or by a reduction in testicular volume. The main objective of the present study was to compare the proteomic profiles of seminal plasma from adolescents with varicocele and changes in semen quality with those from adolescents with varicocele without seminal changes and from adolescents without varicocele.

MATERIALS AND METHODS

The study was approved by the Research Ethics Committee of the Federal University of São Paulo, Brazil, and performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its succeeding revisions. Written informed consent was obtained from all participants or their representatives before their inclusion in the study, and anonymity was assured.

This was an observational study. A cohort of 156 adolescents from 10–19 years of age was initially evaluated in the National Service of Industrial Learning in São Paulo, Brazil, from August 2009–September 2010. The evaluation included the administration of a standardized questionnaire and physical examination using a Prader orchidometer to measure testicular volume. All adolescents were evaluated by the same physician. Inclusion criteria were age 10–19 years and full sexual maturity (Tanner stage V). The exclusion criteria were the presence of systemic disease, Tanner stages ≤ 4, endocrine disease, obesity, congenital malformation of the genitalia, genetic syndrome, prior history of inguinoscrotal surgery, orchitis or epididymitis, testicular torsion, testicular dystopia, absence of masturbation, and other conditions that could affect fertility.

Sixty-seven adolescents entered the study and were allocated to three groups: control group: 21 adolescents without varicocele and normal semen parameters; the VNS group: 28 adolescents with clinical varicocele (grade II or III) and normal semen parameters; and the VAS group: 18 adolescents with clinical varicocele and abnormal semen parameters, as determined by semen analysis.

Semen Analysis

Semen samples were obtained by masturbation after 2–5 days of ejaculatory abstinence and analyzed within 1 hour of collection. After liquefaction, the semen was analyzed according to the World Health Organization 1999 criteria and sperm morphology was assessed using Kruger's strict criteria (13). Semen parameters were considered normal when sperm concentration was $\geq 20 \times 10^6$/mL, motility (% a+b) was $\geq 50\%$, and normal sperm forms were $>14\%$. Two samples were collected with a 7-day interval between collections.

Identification and Quantification of Proteins

For protein identification, an aliquot of semen was processed immediately after centrifugation. Semen was centrifuged at $4{,}000 \times g$, 4°C to separate sperm from seminal plasma. Seminal plasma was submitted to a second centrifugation step, performed at $14{,}000 \times g$, 4°C, to remove cellular debris.

The proteomic profile of seminal plasma was initially determined using two-dimensional polyacrylamide gel electrophoresis. Protein content was quantified using a colorimetric modified Lowry bicinchoninic acid assay (14), and pools from each group were formed, normalized to protein content, and analyzed in quadruplicate. In brief, proteins were separated by two-dimensional polyacrylamide gel electrophoresis in 18-cm pH gradient 3–10 strips on the basis of their isoelectric points by isoelectric focusing. The second dimension separation was performed on gradient (10%–17.5% acrylamide) slab gels. The gels were stained with Coomassie brilliant blue, scanned, and compared using ImageMaster 2D Platinum 7.0 software (GE Healthcare). Protein spots of interest (i.e., those that were significantly different between groups) were excised from the gels and the proteins were identified by tandem mass spectrometry (ESI-Quad-TOF spectrometer; Waters Co.,) and using the Mascot search algorithm (Matrix Science) for searching database. The UniProt (SwissProt) webpage (http://www.uniprot.org/) was used for the final identification of proteins.

Statistical Analysis

The Statistical Package for the Social Sciences for Windows, version 13.0, was used for the statistical analysis of the