American Association of Bioanalysts
Orlando, Florida
May 2006

NON-INVASIVE METABOLOMIC PROFILING OF BIOMARKERS IN SEMINAL PLASMA: EVIDENCE OF OXIDATIVE STRESS USING NMR AND RAMAN SPECTROSCOPY

Ashok Agarwal, Ph.D., HCLD, Rakesh K. Sharma, Ph.D., Anthony J. Thomas, Jr., MD, Cleveland Clinic, Lucy L. Botros, David Burns, McGill University, Canada

INTRODUCTION: Free radicals, such as ROS, exert their effect at the molecular level in all cell types and have a role in both physiological and pathological function. Biomarkers of OS have been found in the male and female reproductive tract. Spermatozoa used for insemination in ART are likely to be exposed to ROS which can cause extensive DNA damage and thus OS has been implicated in the etiology of different forms of male factor infertility. In this study, we hypothesized that semen specimens that are capable of producing a pregnancy may be different in their metabolomic make-up of OS biomarkers compared to those that failed to produce a pregnancy. We utilized a novel, non-invasive method of spectroscopic analysis to determine the metabolomic profile of OS biomarkers in seminal plasma (SP) from different patient groups to explore the possible role of OS in the etiology of male factor infertility.
MATERIALS AND METHODS: SP was collected under Informed Consent from four groups of patients: Group I, Varicocele, N=70; Group II, Idiopathic male factor infertility, N= 15; Group III, Vasectomy reversal, N=9; and Group IV. Female factor, N= 9. In addition, healthy donors (N=30) were included (Group V). Prior to analysis, 5 ul of previously frozen SP was diluted 1:3 in milliQ water and analyzed for specific biomarkers of OS at selected wavelengths by Nuclear Magnetic Resonance (NMR) and Raman spectroscopy. The spectra obtained from each specimen were analyzed separatelyusing a wavelength selective genetic algorithm (GA) to determine regions predictive of biomarkers of OS. The spectral data were quantified using proprietary chemometrics and bioinformatics (Molecular Biometrics, LLC, Chester, NJ), coupled with logistic regression analysis. A leave-one out cross-validation was used to avoid random correlations. Sensitivity and specificity of the analysis were consistently >80-85%.Total analysis time is about one minute.
RESULTS: Each patient group produced unique metabolomic “signatures” as characterized by the presence of OS biomarkers, and their respective differences in CH, NH and OH concentrations. The ratio of the CH to ROH content in the seminal plasma, that is reflective of oxidative stress, was also statistically different among the groups. The individual metabolomic profiles of Varicocele patients were more randomly distributed and did not segregate as a separate population with uniquely identifiable biomarker characteristics. Sensitivity and specificity of the different spectroscopic methods reproducibly exceeded 80%. These observations validate our previous findings of biomarkers of OS in SP using Near Infrared spectroscopy.
CONCLUSIONS: Rapid, non-invasive metabolomic profiling of SP, using NMR and Raman spectroscopy, along with proprietary bioinformatics, can be used to identify different levels of OS in seminal plasma. The ability to quantify differences in the metabolomic profiles observed in different groups of male patients may prove useful as a diagnostic tool to evaluate semen quality and function. Additional studies are planned to further elucidate the role of OS in normal sperm function vs. male factor infertility, and to determine if metabolomic profiling of biomarkers of OS by different forms of spectroscopy can be developed as a routine method for assessing sperm function in ART.