Deciphering the Sperm Proteins Associated With Infertility in Men with Hodgkin’s Disease using Mass Spectrometry and in Silico Methodologies

Ashok Agarwal1, Peter N. Pushparaj, PhD2, Gulafm Ahmad, PhD3,4, Muhammad K. Abu-Elmagd, MD5, Mourad Assadi, Ph.D2, Edmund S. Sabanegh, MD6, and Rakesh Sharma, Ph.D1

1American Center for Reproductive Medicine, Cleveland, OH; 2Center of Excellence in Genomic Medicine Research, Jeddah, Saudi Arabia; 3College of Medicine, Prince Sattam Bin Abdulaziz University, Riyadh, Saudi Arabia; 4Department of Physiology, University of Health Sciences Lahore, Pakistan; 5Department of Urology, Cleveland Clinic

ABSTRACT

Objectives: Hodgkin’s disease (HD) accounts for approximately 11% of all malignant lymphomas. Sperm banking is offered to cancer patients prior to cancer treatment. Low production of male hormones, and disturbed spermatogenesis result in oligospermia, oligoasthenospermia, and azoospermia in patients with HD prior to cancer treatment. There are no proteomic studies identifying alterations occurring at the spermatogenic level in these patients. The objective of this study was to identify the sperm proteins associated with poor sperm quality in HD patients prior to cancer treatment using Liquid Chromatography-LC-tandem Mass Spectrometry (MS) and in silico methodologies.


Materials and Methods: Cryopreserved semen samples from men with HD (n=5) and normal healthy donors (n=5) were prepared for proteomic analysis. Using LC-tandem Foreign LTQ-Orbitrap Elite Hybrid MS system, CID spectra were searched against the human reference sequence database. The relative quantity of these proteins was determined by spectral counts (SC). The in silico analysis was done using the Ingenuity Pathways Analysis (IPA) software to infer DEPs specifically associated with reproductive function. The statistical significance was computed using Fisher’s Exact Test (P<0.05) and the negative log P-value cut-off was fixed ≥ 2.0.

Results: 134 differentially expressed proteins were identified in the HD group. Key sperm proteins associated with fertility were down regulated and included Annexin (AXN), Androgen Receptor (AR), Chromatin- targeting TCP1 subunit 6B (CCT6B), BCL-xL (BCL2L1), Dynamin Associated Intermediate Chain (DANIC1), Fibrinogen gamma chain precursor (FGG), HAF, RPRK2, SH3PXD2A and SORCS1. These proteins were involved in cell migration, cell communication, binding of sperm to the zona pellucida, sperm mobility and regulation of sperm DNA damage and apoptosis.

Conclusions: Down regulation of key proteins involved in spermatogenesis, spermiogenesis, acrosomal reaction, capacitation and sperm mobility function may help explain the poor sperm quality seen in patients with Hodgkin’s disease even prior to cancer therapy. These patients may benefit from assisted reproductive technologies such as intracytoplasmic sperm injection.

INTRODUCTION

Hodgkin’s Lymphoma or Hodgkin’s Disease (HD) accounts for 11% of all malignant lymphomas with an unknown etiology. HD is often exhibited by swollen lymph nodes in the neck or armpits and can potentially metastasize into nearby lymph nodes, bone marrow, lungs and liver. HD affects about 45% of males of reproductive age. HD patients manifest reduced production of male hormones, and disturbed spermatogenesis subsequently leads to oligospermia, oligoasthenospermia and azoospermia prior to treatment. Reduced male fertility often observed even before the initiation of treatment in men with HD and, thus, sperm banking is recommended to preserve the fertility of sperm prior to cancer therapy. Till now, there are no proteomic studies identifying changes occurring at the spermatogenic level in HD patients. The objective of this study was to identify the sperm proteins associated with poor sperm quality in HD patients prior to cancer treatment using Liquid Chromatography-LC-tandem Mass Spectrometry (MS) and in silico methodologies.

RESULTS

1. We identified 134 DEP in the spermatozoa of men with HD compared to NC. These were mainly associated with diseases and biofunctions such as Cell-to-Cell Signaling and Interaction, Reproductive System Development and Function, Cell Assembly and Organization, Protein Degradation, Protein Synthesis, Cancer etc. (Figure 1).

2. Spermatosomal proteins implicated in the canonical pathways such as phagosome maturation and protein ubiquitination were downregulated in HD patients (P<0.05) whereas those implicated in the production of Nitric Oxide (NO) and Reactive Oxygen Species (ROS) were significantly upregulated in HD compared with NC (Figure 2 and 3).

3. Downregulation of ACR, TCP1, CCT2, CCT3, CCT4, CCT6A, CCT7, CCT8, PRSS37 and the up-regulation of ACR lead to a defect in the binding of sperms with zona pellucida. The spermatogenesis was significantly impaired in HD due to the downregulation of TCP1, CCT6B, RUVBL1, GSR, PRSS37 and the up-regulation of ACE, SERPINA5 and NUP210L. The reduced fertility of sperms of men with HD might be due to the downregulation of ACR, ACE, SERPINA5, PRSS37 and the up-regulation of ACE and SERPINA5. Furthermore, the downregulation of NEB8, SPS85 and PRSS37 could cause a defect in the sperm function in men with HD compared to NC (Figure 4).

4. Comparison analysis of DEPs, up-regulated and down regulated proteins in the spermatozoa of men with HD showed that RICTOR, TSC2, 14-3-3-σ, TGFBR1, and DEPs were analyzed using Ingenuity Pathway Analysis (IPA) software to decipher the differentially regulated canonical pathways, upstream regulators, causal networks, novel networks, diseases and biofunctions. The statistical significance was calculated using Fisher’s Exact Test (P<0.05) and the negative log P-value cut-off was fixed ≥ 2.0 whereas SRF, ML1, ML2, RB1, HS1, HS2 and NF212 were inhibited (Z-score ≤ 2.0) in the spermatozoa of men with HD compared to NC (Figure 5).

CONCLUSIONS

1. Proteomic analysis using Finnigan LTQ-Orbitrap Elite hybrid MS coupled with in silico analyses uncovered the downregulation of key proteins involved in spermatogenesis, spermiogenesis, acrosomal reaction, oocyte binding and sperm motility function.

2. Our findings may explain the poor sperm quality seen in patients with HD even prior to cancer therapy.

3. These patients may benefit from assisted reproductive technology procedures such as intracytoplasmic sperm injection.