Mitochondrial disease is characterized by impaired respiratory chain, oxidative phosphorylation, mitochondrial protein import, alterations of the inner mitochondrial membrane composition and defects of mitochondrial dynamics are characteristics of mitochondrial disease. Our recently published proteomic data on comparative proteomic analysis of spermatozoa of infertile men with varicocele identified 22 differentially expressed proteins (DEP) of mitochondrial origin. Proteins involved in mitochondrial organization (LETM1, EFHC1 and MLOS), import receptor TOM22, 3 crucial subunits of electron transport chain (ETC) and the core enzymes of carbohydrate and lipid metabolism were underexpressed in varicocele group. Variocele may result in stagnation in the testicular microcirculation inducing hypo-ischemic degenerative changes in all cell involved in sperm production. During hypoxia, superoxide production at low oxygen concentrations results in oxidative stress. With this background, we hypothesized that hypoxia-mediated oxidative stress (OS) is involved in sperm dysfunction in varicocele due to impaired blood supply to the testis.

Materials and Methods

Mitochondrial disease is characterized by impaired respiratory chain, oxidative phosphorylation, mitochondrial protein import, alterations of the inner mitochondrial membrane composition and defects of mitochondrial dynamics. Decreased sperm motility is associated with defects in sperm mitochondrial ultrastructure. These changes are reported to occur at mitochondrial genome, transcription and proteome levels. Deletions and other changes in the mitochondrial DNA (mtDNA) influence cellular homeostasis which in turn is responsible for sperm dysfunction leading to infertility. Semen parameters such as concentration, vitality and motility of spermatozoa are associated with the activity of sperm mitochondrial enzymes, including ETC complexes. Although poor semen quality, increased oxidative stress and DNA fragmentation are frequently observed, not all men presenting with a varicocele are infertile and men with high grade varicocele can still father children. Considerable difference exists in protein profiles of the spermatozoa in varicocele patients when compared with normozoospermic or fertile donors. Our recently published proteomic data on comparative proteomic analysis of spermatozoa proteins identified 22 differentially expressed proteins (DEP) of mitochondrial origin. Proteins involved in mitochondrial organization (LETM1, EFHC1 and MLOS), import receptor TOM22, 3 crucial subunits of electron transport chain (ETC) and the core enzymes of carbohydrate and lipid metabolism were underexpressed in varicocele group. In varicocele, stagnation in the testicular microcirculation inducing hypo-ischemic degenerative changes may result in oxidative stress.

The goal of this study was to examine if hypoxia-mediated oxidative stress (OS) is involved in sperm dysfunction in varicocele due to impaired blood supply to the testis and results in underexpression of key proteins involved in mitochondrial dysfunction.

Materials and Methods

Following approval of the study by the Institutional Review Board of Cleveland Clinic, all subjects provided written consent to be enrolled in this prospective study. Variocele was diagnosed by clinical analysis, including scrotal palpation and graded. Oxidation-reduction-depletion of protein kinase A regulatory subunit (PKARIA) and DEPs responsible for sperm function were validated by western blotting (WB) and quantified using Image J software. Reduced ORP was associated with underexpression of all studied proteins validated by western blot data and supported by IF findings.

Conclusion

1. cAMP-dependent protein kinase catalytic subunit alpha (PRKACA) and CAMP-dependent protein kinase type I-alpha regulatory subunit (PKAR1A) are regulatory subunits of the cAMP-dependent protein kinases involved in cAMP signalling in cells. Haplo-insufficiency at the protein kinase A RI alpha gene locus leads to fertility defects in men. Therefore, a decline in PKAR1A will lead to a general decline in protein synthesis and abnormal sperm function.

2. Decreased levels of LETM1 (mitochondrial protein import receptor inner), MIC 90 (a member of mitochondrial contact site and cristae organizing system), TOM 22 (translocase of outer membrane receptor complex) and the ETC complexes imply impairment of mitochondrial structure and function leading to decreased ATP level and leakage of ROS leading to altered ORP in varicocele spermatozoa of patients.

3. Low ATP with high ROS in turn are responsible for sperm dysfunction in varicocele men. 4. High ROS may also incur secondary damage to mitochondrial membrane and low ATP level may further decline PKA activity via reduced synthesis of cAMP initiating the vicious cycle.

Therefore, along with conventional management, therapy targeted towards mitochondria may improve the treatment outcome in infertile men with varicocele.