Polymorphism in Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPX) gene in Iranian infertile men

Lakpour N. (M.Sc.)[1], Modaresi M.H. (M.D., Ph.D.)[2], Kharazi H. (Ph.D.)[1], Akhondi M.M. (Ph.D.)[3],Veisi Raygani A. (Ph.D.)[1], Ghasemi J. (B.Sc.)[3], Hodjat M. (M.Sc.)[3], Sadeghi M.R. (Ph.D.)[4]

1- Department of Clinical Biochemistry, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.
2- Nanobiotechnology Research Center, Avesina Research Institute, ACECR, Tehran, Iran.
3- Reproductive Biotechnology Research Center, Avesina Research Institute, ACECR, Tehran, Iran.
4- Monoclonal Antibody Research Center, Avesina Research Institute, ACECR, Tehran, Iran.

Abstract

Introduction: Leukocytes and defective or dead spermatozoa in human semen are a source for the production of reactive oxygen species (ROS) and subsequent injury to intact sperms. Enzymatic and non-enzymatic defensive mechanisms in semen detoxify these compounds. Glutathione peroxidase-4 (GPX-4 or PHGPX) is a major selenoprotein in sperm and it is one of the enzymatic mechanisms that play multiple roles during spermatogenesis. Some of these roles are formation of the mitochondrial capsule, hydro-peroxide detoxification and sperm chromatin condensation. Any decrease in the enzyme activity or content, may create disorders in spermatogenesis and sperm fertilizing ability. Considering defects in the expression of the enzyme gene or presence of mutations which may cause decreases in PHGPX activity or content, this study was carried out to identify a number of important mutations in GPX-4 gene by PCR-RFLP method in Iranian infertile men.

Materials & Methods: This study was performed on 128 Iranian men who had been referred to Avesina Infertility Clinic, including 74 infertile men with defective sperm parameters, 18 normozoospermic and 36 fertile subjects as controls. Mean ± SD for sperm parameters were determined. Genomic DNA was extracted using salting out procedure from peripheral blood leukocytes. PCR-RFLP was done by two sets of primers with 237 bp and 148 bp PCR products that were designed for 1A and 4 exons of GPX-4 gene covering nucleotides of +6 (C→T), +17 (G→A), +1725 (G→A) by MwoI, PshAI and SatI enzymes.

Results: Digestion of a 237 bp intact PCR product by MwoI generates two fragments (151 bp and 86 bp). When a mutation occurs in the restriction site +6 (C→T), the enzyme would not recognize the sequence, therefore 237 bp segment remains undigested. Treatment of 237 bp segment with PshAI generates two fragments (161 bp and 76) in the intact gene but the same enzyme can not digest 237 bp segment when a mutation occurs in the restriction site +17 (G→A). Ultimately, digestion of 148 bp intact segment with SatI generates two fragments (108 bp and 40 bp) but when a mutation occurs in the restriction site +1725 (G→A), the enzyme will not recognize the sequence; therefore 148 bp segment remains undigested. Enzymatic digestion evaluations of 237 bp and 148 bp segments in all participants revealed that neither of the examined mutations existed in GPX-4 gene.

Conclusion: According to the results of this study, it is determined that the prevalence of these mutations in Iranian infertile men is probably low and it may have no association with the etiology of the disorder affecting sperm parameters. Hence, a study with a larger number of patients is suggested to determine the exact prevalence of these and other mutations of the gene in Iranian infertile men.

Key Words: PHGPX, Sperm, ROS, Polymorphism, Male infertility, Selenoproteins.

Corresponding Author: Dr. Mohammad Reza Sadeghi, Avesina Research Institute, ACECR, Tehran, Iran.
E-mail: Sadeghi@avesina.ac.ir