Evaluation of Antioxidant Status of the Seminal Fluid of Pubertal Patients with Beta Thalassemia Major

Thesis

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List of Abbreviations

| ACS | : | Agarose cell support |
|------------------|---|-----------------------------------|
| AD | : | Acid denaturation |
| AI | : | Acrosomal index |
| ALP | : | Alkaline Phosphatase |
| AMP | : | : 2-amino-2-methyl-1-propanol |
| ARE | : | Antioxidant response element |
| ATP | : | Adenosine triphosphate |
| BMI | : | Body mass index |
| Ca ⁺⁺ | : | Serum Calcium |
| CMIA | : | Chemiluminescent Microparticle |
| | | Immunoassay |
| CoQ10 | : | Coenzyme Q10 |
| CPT1 | : | Carnitine Palmitoyl Transferase 1 |
| CUR | : | Curcuminoids |
| DFO | : | Desferrioxamine |
| DFP | : | Deferiprone |
| DFX | : | Desferasirox |
| DNA FI | : | DNA fragmentation index |
| DNA | : | Deoxyribonucleic acid |
| eEF-2 | : | Elongation factor-2 |
| FBG | : | Fasting blood glucose |

🕏 List of Abbreviations 🗷

| FSH | : | Follicle stimulating hormone |
|----------|---|---|
| | • | |
| FTMC | : | Final total motile count |
| GH | : | Growth hormone |
| GI | : | Gastrointestinal |
| GnRH | : | Gonadotropin releasing hormone |
| GPx | : | Glutathione Peroxidase |
| GR | : | Glutathione Reductase |
| GSH | : | Glutathione |
| GSSG | : | Glutathione Disulfide |
| GST | : | Glutathione-S-Transferase |
| H_2O_2 | : | Hydrogen Peroxide |
| HbE | : | Hemoglobin E |
| I.N.T: | : | (4-iodophenyl)-3-(4-nitrophenol)-5- |
| | | phenyltetrazolium chloride |
| ICSI | : | Intra Cytoplasmic Sperm Injection |
| IL | : | Interleukin |
| IQR | : | Interquartile range |
| IVF | : | In Vitro fertilization |
| LH | : | Luteinizing hormone |
| LS | : | Lysis solution |
| MDA | : | Malondialdehyde |
| NAC | : | N-Acetyl Cysteine |
| NADP | : | Nicotinamide adenine dinucleotide phosphate |



| NADPH | : | Nicotinamide adenine dinucleotide phosphate |
|-----------------|---|---|
| | | (reduced) |
| NFκB | : | Nuclear factor κB |
| NHD | : | Number of heads with defects |
| NMPD | : | Number of mid-piece with defects |
| NNF | : | Number of normal forms |
| NP | : | Non-Progressive motility |
| NPPD | : | Number of principle piece with defects |
| NTBI | : | Non-Transferrin-Binding Iron |
| O2 - | : | Superoxide anion |
| OS | : | Oxidative stress |
| PO ₄ | : | Serum phosphorus |
| PR | : | Progressive motility |
| PTH | : | Parathyroid Hormone |
| R.I | : | Reference interval |
| RBC | : | Red blood cell |
| ROS | : | Reactive oxygen species |
| rpm | : | Revolutions per minute |
| SCD | : | Sperm Chromatin dispersion |
| SCS | : | Supper-coated slides |
| SCSA | : | Sperm Chromatin structure assay |
| SDI | : | Sperm deformity index |
| SDS | : | Standard deviation score |

🕏 List of Abbreviations 🗷

| SOD | : | Super Oxide Dismutase |
|-------|---|------------------------------------|
| Sp.C. | : | Spermatogenic cells in millions/ml |
| m/ml | | |
| TAC | : | Total antioxidant capacity |
| TMC m | : | Total motile count in millions |
| TNF: | : | Tumor necrosis factor |
| TZI | : | Teratozoospermicindex |
| WHO | : | World Health Organization |
| XOD: | : | Xanthine oxidase |
| βТМ | : | β-thalassemia major |

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Introduction

Beta thalassemia syndromes are a group of hereditary disorders characterized by a genetic deficiency in the synthesis of beta-globin chains. In the homozygous state, beta thalassemia major causes severe, transfusion-dependent anemia. In the heterozygous state, the beta thalassemia trait (i.e. thalassemia minor) causes mild to moderate microcytic anemia (*Takeshita*, 2013).

Free radicals and other reactive oxidants are generated in biological systems by both endogenous processes (metabolic pathways and enzymes, such as peroxidases, nitric oxide synthases, lipooxygenases and heme protein/enzyme reactions) and exposure to external stimuli such as radiation, nitrogen oxides, mineral fibers and dusts. Reactive oxygen species (ROS) include superoxide anion (O_2^-) , hydrogen peroxide (H_2O_2) and hydroxyl radical (HO) (*Davies, 2012*).

Experimental data confirm the progression of oxidative stress in patients with β -thalassemia major due to activation of free radical processes and lipid peroxidation and decreased antioxidant capacity. The combination of effective iron-chelator agents with natural or synthetic antioxidantscanbe extremely helpful in clinical practice in

the regulation of the antioxidant status of patients with β -thalassemia major (*Pavlova et al.*, 2007).

Spermatozoa are particularly susceptible to damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids, and their cytoplasm contains low concentrations of scavenging antioxidant enzymes. In addition, intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing supplement spermatozoa their limited intrinsic to antioxidant defenses by depending on the protection afforded by the seminal plasma. There is also strong evidence suggesting that DNA fragmentation commonly observed in the spermatozoa of infertile men is mediated by high levels of ROS (Agarwal and Saleh, 2002).

Aim of the Work

- Determining the magnitude of oxidative damage of sperms in thalassemic patients after long period of repeated blood transfusions.
- Assessment of the effect of antioxidant treatment by reassessment of sperm parameters after 6 months of treatment.

Hypothalamic Pituitary Gonadal Axis in β -Thalassemia

Introduction:

β-thalassemia represents a group of recessively inherited hemoglobin disorders characterized by reduced synthesis of β-globin chain. The β-globin gene family is located on human chromosome 11 in a region which is tightly condensed and transcriptionally silent in all non-erythroid cells *(Voon et al., 2008)*. β-thalassemia is subclassified according to whether synthesis of the affected globin chain is totally absent e.g. B^0 or only partially reduced e.g. B^+ *(Adams and Colman, 1990)*.

About 3% of the world's population carries the β-thalassemia genes. The homozygous state results in severe anemia, which needs regular blood transfusion. Treatment with transfusion and chelation therapy has considerably prolonged survival in thalassemic patients. The main adverse effect is iron over load from repeated blood transfusion that leads toiron deposition in various organs. As a result, patients have variable degrees of organ damage and endocrinopathies that can affect their quality of life. Hypogonadotrophic hypogonadism is the commonest

endocrinopathy affecting 80-90% of patients worldwide, for which homozygous β -thalassemia major (β TM) patients have disturbance of growth, sexual maturation and impaired fertility (*Kurtoglu et al.*, *2012*).

The damage to the hypothalamus and pituitary is progressive, even when intensive chelation therapy is given and the occurrence of hypogonadism is often unavoidable. Close follow up and proper management is crucial for every patient with β TM. Early recognition of growth disturbance and prevention of hypogonadism by early and judicious chelation therapy is mandatory for the improvement of their quality of life (*Kyriakou and Skordis*, 2009).

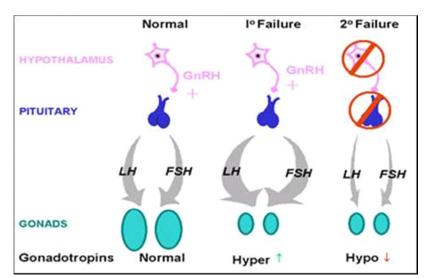


Figure (1): The hypothalamic-pituitary-gonadal Axis in normal individuals, primary gonadal failure and secondary gonadal failure which is either pituitary or hypothalamic failure.

The hypothalamus is the integrative center of the reproductive axis and receives messages from both the central nervous system and the testes to regulate the production and secretion of GnRH. Neurotransmitters and neuropeptides have both inhibitory and stimulatory influences on the hypothalamus. The hypothalamus releases GnRH in a pulsatile nature which appears to be essential for stimulating the production and release of both LH and FSH. LH and FSH are produced in the anterior pituitary and both bind to specific receptors on the Leydig cells and Sertoli cells within the testis. Testosterone, the major secretory product of the testes, is a primary inhibitor of LH secretion in males. Testosterone may be metabolized peripheral tissues androgens in to potent dihydrotestosterone or potent estrogen, estradiol. Both androgens and estrogens act independently to modulate LH secretion. Estradiol produced by both testes and peripheral conversion of androgen precursors is a more potent inhibitor of LH and FSH secretion. The mechanism of feedback control of FSH is regulated by a Sertoli cell product called inhibin. Decreases in spermatogenesis are accompanied by decreased production of inhibin and this reduction in negative feedback is associated with reciprocal elevation of FSH levels (Mcclure, 2005).