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

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

SOD	**:**	Super Oxide Dismutase
Sp.C. m/ml	**:**	Spermatogenic cells in millions/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
βTM	**:**	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 ($^{\bullet}HO$) (***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 the 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, the intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing spermatozoa to supplement their limited intrinsic 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 sub-classified 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 (βTM) 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 βTM. 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).***

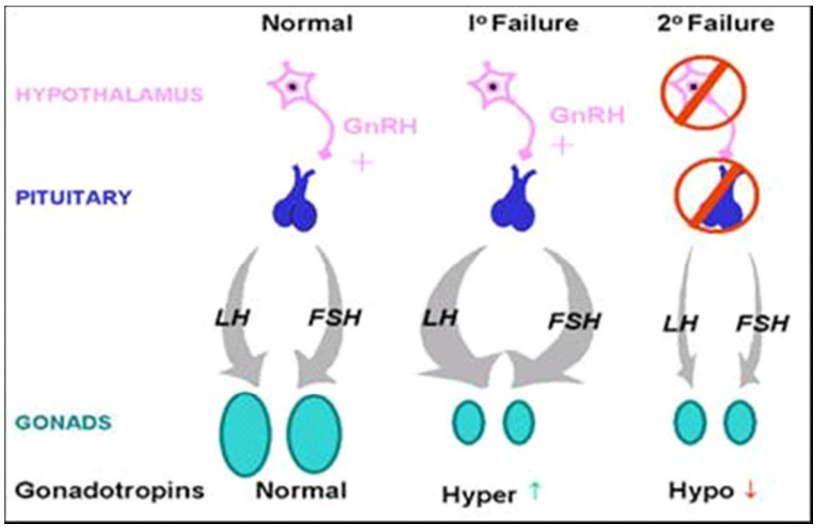

Figure (1): The hypothalamic-pituitary-gonadal Axis in normal individuals, primary gonadal failure and secondary gonadal failure which is either pituitary or hypothalamic failure.

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 in peripheral tissues to potent androgens 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).***