Super-Oxide Dismutase (SOD) Response in Very Low Birth Weight (VLBW) Preterm with Late-Onset Neonatal Septicemia

Thesis

Submitted for Fulfillment of Ph.D. in Childhood Studies

Prepared By

Amr Mohamed Assad Moustfa

M.B., B.Ch., M.Sc. Pediatrics

Supervised by

Dr. Salah El-Din Mostafa

Professor of Preventive Medicine & Epidemiology

Institute of Postgraduate Childhood Studies **Ain Shams University**

Dr. Gamal Samy Aly

Professor of Pediatrics

Institute of Postgraduate Childhood Studies

Ain Shams University

Dr. Hanan Abd-Allah El-Gamal

Professor of Pediatrics

Institute of Postgraduate Childhood Studies

Ain Shams University

2014

قسم الدراسات الطبية

التغيرات في أنزيم السوبر أوكسيد ديسميوتيز في الخدج حديثي الولادة المصابين بالتسمم الدموي المتأخر

رسالة مقدمة للحصول على
درجة دكتوراه الفلسفة فى دراسات الطفولة

مقدمه من

الطبيب/ عمرو محمد أسعد مصطفى

بكالوريوس الطب والجراحة
ماجستير طب الأطفال - جامعة القاهرة

تحـت إشراف

د./ صلاح الدين مصطفى

أستاذ الطب الوقائي والوبائيات
معهد الدراسات العليا للطفولة
جامعة عين شمس

د./ جمـال سامى علي

أستاذ طب الأطفال
معهد الدراسات العليا للطفولة
جامعة عين شمس

د./ حنان عبد الله الجمل

أستاذ طب الأطفال
معهد الدراسات العليا للطفولة – جامعة عين شمس

2014

LIST OF ABBERVIATIONS

ALS	Amyotrophic Lateral Sclerosis
ARDS	Adult respiratory distress syndrome
BSIs	bloodstream infections
CAT	Catalase
CDC	Centers for Disease Control and Prevention
CFUs	Colony forming units
CLABSIs	Central line–associated bloodstream infections
CNS	Central nervous system
CoNS	Coagulase-negative staphylococci
CRP	C-reactive protein
CSF	Cerebrospinal fluid
EC-SOD	Extracellular superoxide dismutase
EOS	Early-onset sepsis
GBS	Group B Streptococcal
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
HSV	Herpes simplex virus
IAP	Intrapartum antibiotic prophylaxis
IVIG	Intravenous immunoglobulin
I/T RATIO	Ratio of immature to total neutrophilic leucocytes
IgG	Immunoglobulin G
LDL	Low density lipoproteins
LOS	Late-onset sepsis
LP	Lumbar puncture
Mn-SOD	Mitochondrial superoxide dismutase

MRSA	Methicillin-resistant Staphylococcus aureus
NI	Nosocomial infection
NICHD	National Institute of Child Health and Human Development
NICU	Neonatal intensive care unit
NS	**Neonatal sepsis**
NRN	Neonatal Research Network
PCT	Procalcitonin
PNR	Patient-nurse ratio
ROS	Reactive oxygen species
SOD	**Superoxide Dismutase**
TNF	Tumor necrosis factor
VLBW	Very low birth weight
WHO	World Health Organization

LIST OF CONTENTS

	Page
List of abbreviations	
List of tables	
List of figures	
Abstract	
Introduction……	1
Aim of the study……	2
Review of literature……..	3
Patients and methods……	60
Results……..	70
Discussion……	85
Summary……..	94
Conclusion……..	98
Recommendations……	99
References……..	100
Arabic summary……	118

LIST OF FIGURES

	Name	Page
1	Timing of bacterial and fungal sepsis in VLBW infants	10
2	An overall picture of the mitochondrial metabolism of ROS and the mechanism of oxidative tissue	45
3	Flow chart outline of the study population	60
4	Ballard score	63
5	Sex distribution of the study group	71
6	Mode of delivery in the study group	72
7	Weight for gestational age in the study group	72
8	SOD in the study groups	80
9	Positive bacterial culture occurrence in different mode of delivery	83

LIST OF TABLES

	Name	Page
1	Risk factors for the development of neonatal sepsis	11
2	Organisms associated with early-onset and late-onset neonatal sepsis	26
3	Risk factors associated with neonatal sepsis in preterm infants	41
4	SOD defense systems	58
5	Hematological scoring system	66
6	Descriptive data of the whole study group	70
7	Infants demographic data in the study groups	73
8	Maternal demographic data in the study groups	74
9	Comparison between the study groups regarding APGAR & BALLARD scores	75
10	Comparison between the study groups at admission to NICU	76
11	WBCs parameters among the study group	77
12	CBC parameters among the study group	78
13	Sepsis score in study groups	79
14	SOD in the study groups	80
15	Sensitivity and specificity of neonatal hematological sepsis score parameters	81
16	Comparing infection occurrence in relation to mode of delivery in the study group	82

INTRODUCTION

Neonatal sepsis is one of the leading causes of morbidity and mortality among the newborns. As many as 2% of fetuses are infected in utero and up to 10% of infants are infected during delivery or the first month of life ***(Gonzalez et al., 2004)***.

Among infected newborns, clinical manifestations develop very early after delivery and most infants will have signs of respiratory distress and cardiovascular instability. Infants with early-onset sepsis are at increased risk for meningitis. Rapid deterioration of the clinical status is expected unless prompt antibiotic management is started. Risk factors for neonatal sepsis include maternal factors, neonatal host factors, and virulence of infecting organism ***(Camacho-Gonzalez et al., 2013)***.

Superoxide Dismutase (SOD) has recently gained notoriety for its connection with amyotrophic lateral sclerosis, more commonly known as Lou Gehrig's disease. This disease is a degenerative disorder that leads to selective death of neurons in the brain and spinal cord, leading to gradually increasing paralysis over a few years ***(Pasinelli and Brown, 2006***

AIM OF THE STUDY

(1) To examine neutrophil counts and indices in preterm very low birth weight (VLBW) newborn infants with culture-proven late-onset sepsis to determine whether the neutrophil responses could predict late-onset sepsis

(2) To evaluate super oxide dismutase (SOD) status as an enzymatic antioxidant in preterm (VLBW) newborn infants with late-onset sepsis.

NEONATAL SEPSIS

❖ Introduction

Sepsis is defined as a systemic inflammatory response syndrome associated with infection on the basis of either microbiologic cultures or strong clinical evidence of the presence of an infection. Severe sepsis is defined as sepsis plus evidence of organ dysfunction defined around pediatric parameters ***(Wynn et al., 2010)***.

Septicemia is a generalized bacterial infection in the bloodstream. Neonatal infections, which may be caused by bacteria, viruses, or fungi, occur as early or late infections and their timing gives care providers' clues for determining causative agents. Transplacental/ intrapartum infections occur in utero and manifest within the first 3 days (72 hours) of life. These early-onset infections (EOI) are associated with high morbidity and mortality. Late-onset infections (LOI) may occur as early as 3 days of age, but more commonly occur after the first week of life ***(Venkatesh et al., 2006)***.

Although most neonatal infections are of maternal or community origin, an increasing proportion are acquired in the nursery. Advances in newborn intensive care have permitted the survival of low-birth-weight and sick infants and have simultaneously created risks for neonatal infections, which are themselves a significant cause of mortality in these infants ***(Zafar et al., 2001)***.

Reported infection rates in the neonatal intensive care unit (NICU) vary from 3.2 to 30 per 100 admissions or discharges, illustrating the wide variability among centers. NICUs that admit surgery patients may have higher rates ***(Moore, 2004)***.

Nosocomial bloodstream infections (BSIs) are increasing in prevalence and result in significant morbidity, mortality, and economic cost. From 1975 to 1996, the proportion of nosocomial infections accounted for by BSIs increased from 5% to 14% ***(Rupp, 2004)*.**

❖ Development of Immune system

The development of the immune system entails a number of changes that occur during the first years of life. Neonates, especially preterm infants, are relatively immunocompromised because of immaturity of the immune

system, as well as decreased placental passage of maternal antibodies. Here we highlight some of the components of the neonatal immune system that are immature and contribute to increased susceptibility to serious bacterial, fungal, and viral infections ***(Camacho-Gonzalez et al., 2013)***.

Innate Immune System

The innate immune system produces an immediate immunologic response and is capable of doing this without previous exposure to a specific pathogen. Recognition of pathogens occurs by identification of conserved biologic regions known as pathogen-associated molecular patterns (PAMPs). Recognition receptors, such as TOLL-like receptors, NOD-like receptors and RIG-like receptors, identify and respond to PAMPs with the production of cytokines and proinflammatory responses that activate the adaptive immune system ***(Kumar et al., 2012)***.

Studies comparing neonatal and adult innate immune functions show that neonatal cells have a decreased ability to produce inflammatory cytokines, especially tumor necrosis factor (TNF) and interleukin (IL)-6 ***(Kollmann et al., 2009)***. In addition, they induce IL-10 production, which in itself is

capable of inhibiting synthesis of proinflammatory cytokines ***(Belderbos etal., 2012)***.

Neutrophil and dendritic cell functions are also reduced; neutrophils show a decreased expression of adhesion molecules, as well as a decreased response to chemotactic factors, and dendritic cells have a decreased capacity of producing IL-12 and interferon (IFN) gamma. The overall reduction in cytokine production in neonates also results in decreased activation of natural killer cells ***(Guilmot et al., 2011)***. Impairment of the innate immune system leads to an increased susceptibility to bacterial and viral infection in this population ***(Kumar et al., 2012)***.

Adaptive Immune System

The adaptive branch of the immune system is designed to eliminate specific pathogens. In newborns, the adaptive immune system slowly increases its function toward an adult like response, minimizing the otherwise overwhelming inflammatory response that would occur when infants transition from a sterile to a colonized environment ***(Schelonka et al., 2011)***.

Decreased cytotoxic function (strong T-helper 2 polarization with decreased IFNgamma production), lack of

isotype switching, and overall immaturity and decreased memory (because of limited pathogen exposure at time of birth), reduce the neonate's ability to respond effectively to infections. For example, the reduction of cell mediated immunity increases the risks of infections caused by intracellular pathogens, such as Listeria, Salmonella, herpes simplex virus (HSV), cytomegalovirus, and enteroviruses ***(Tolar et al., 2009)***.

Transplacental passage of maternal immunoglobulin G (IgG) is inversely related to gestational age and limits the functional ability of the neonate to respond to certain pathogens ***(Palmeira et al., 2012)***.

Minimal IgG is transported to the fetus in the first trimester, whereas fetal IgG rises in the second trimester from approximately 10% at 17 to 22 weeks' gestation to 50% at 28 to 32 weeks' gestation. Thus, preterm infants lack adequate humoral protection against a number of infant pathogens, whereas term infants will often be protected against most vaccine-preventable neonatal infections through transplacental passage from the mother's serum ***(Malek, 2003)***.

Histologic studies have also demonstrated that the marginal zone of the spleen is not fully developed until 2 years

of age, increasing the infant's susceptibility to encapsulated bacterial infections (Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis) ***(Zandvoort & Timens, 2002)***.

Finally, transfer of IgA, IgG, cytokines, and antibacterial peptides present in human milk may be compromised, especially in premature babies. The lack of secretory IgA decreases the ability of the neonate to respond to environmental pathogens ***(Brandtzaeg, 2010)***.

Complement

Complement levels increase with increasing gestational age, but are only about 50% of adult levels at term. Reduced complement levels are associated with deficient opsonization and impaired bacterial killing. Although both pathways seem to be capable of being activated, there may be variations in their activation level. In addition, profound C9 deficiency has been observed in neonates, reducing the ability to form bacteriolytic C5b-9 (m), which will increase the risk of acquiring severe invasive bacterial infections ***(Hogasen,2000)***.