Medical Studies Department

Some Social, Environmental and Laboratory Determinants in Newborns with Necrotizing Enterocolitis

Thesis
Submitted for fulfillment of Ph.D.
in Childhood Studies - Medical Studies Department
(Child Health and Nutrition)

By

Mona Monis Zaki Risk
M.B. B.Ch., M.Sc. (Pediatrics)

Supervised By

Prof. Dr. *Ismail Sadek Ismail*
Prof. of Pediatrics
Faculty of Medicine
Ain Shams University

Prof Dr. *Mostafa Abd-El Aziz El-Hodhod*
Prof. of Pediatrics
Faculty of Medicine
Ain Shams University

Dr. *Karim Yehia Shaheen*
Assist. Professor of Clinical Pathology
Faculty of Medicine
Ain Shams University

Dr. *Rehab Abd-El Kader Mahmoud*
Assist. Professor in Medical Studies Department
Institute of Childhood Studies
Ain Shams University

2005

2005

TABLE OF CONTENTS

contents	*page*
List of Tables	*i*
List of Figures	*v*
List of Plates	*vi*
INTRODUCTION	*1*
AIM OF THE WORK	*5*
REVIEW OF LITERATURE	*7*
Chapter (I): Necrotizing Enterocolitis	*7*
Definition	*7*
Epidemiology	*7*
Risk factors	*9*
Pathogenesis:	*14*
Clinical Presentation	*24*
Diagnosis	*27*
Differential Diagnosis	*31*
Prevention	*32*
Treatment strategies for NEC	*36*
Potential complications	*49*
Mortality & Morbidity	*50*
Chapter (II): Oxygen Free Radicals	*51*
Definition	*51*
Types	*51*
Sources of Oxygen Free Radicals	*59*
Lipid peroxidation	*64*
Mechanisms of Free Radicals in Pathological States	*69*

contents *page*

Chapter (III): Antioxidants *73*

Definition *73*

Mechanism of action of antioxidants *73*

Classification of antioxidants *75*

Antioxidant Enzyme Systems *77*

Superoxide Dismutase *77*

Glutathione Peroxidase *87*

Catalse *95*

Non-enzzymatic Antioxidants *97*

Lipid-soulble antioxidants *98*

Vitamine E *98*

Carotenoids *100*

Quinones *101*

Bilirubin *101*

Water-soluble antioxidants *102*

Vitamin C *102*

Glutathione *104*

Uric acid *105*

Taurine *106*

Metal binding proteins *106*

Binding proteins for heme and heme-proteins *108*

SUBJECTS & METHODS *111*

RESULTS *129*

DISCUSSION *177*

CONCLUSION *201*

RECOMMENDATIONS *203*

SUMMARY *205*

REFERENCES *211*

APPENDIX *275*

ARABIC SUMMARY 1

Acknowledgement

I would like to express my sincere thanks and appreciation to ***Prof. Dr. Ismail Sadek Ismail****, Professor of Pediatrics, Faculty of Medicine, Ain-Shams University, for his magnificent encouragement and useful comments throughout this study.*

I wish to express my deepest gratitude to ***Prof. Dr. Mostafa Abd El-Aziz El-Hodhod****, Professor of Pediatrics, Faculty of Medicine, Ain-Shams University, who kindly offered much of his time for providing me with precious guidance, which is beyond acknowledgment. I wish to thank him for his cardinal support during planning and throughout this study.*

I would like to express my sincere thanks to ***Prof. Dr. Karim Yehia Shaheen****, Assist. Professor of Clinical Pathology, Faculty of Medicine, Ain-Shams University, who offered me much of his time and experience with helpful advice. I record my appreciation to his Nobel character.*

I express my deepest gratitude to ***Prof. Dr. Rehab Abd El-Kader Mahmoud****, Assist. Professor in Medical studies Department, Institute of Childhood Studies, Ain-Shams University, who offered me much of her guidance. She provided me great help, generous and proper support, suggestions throughout this work.*

I should also express my deep gratitude to all the staff members, my colleagues and to every person in the NICU Department, Children's Hospital, Ain-Shams University, for their continuous support, cooperation and help.

Mona Monis

LIST OF ABREVIATIONS

Cl^-	Chloride Ion
OCL^-	Hypochlorous Anion
$O_2^{\bullet}$	Singlet Oxygen
$O_2^{\bullet -}$	Superoxide Radical
$ONOO^-$	Peroxynitrite
C D	Clostridium Defficile
C=C	Carbon-Carbon Double Bond
CCS	Copper Chaperone for Superoxide Dismutase
Cu/Zn SOD	Copper Zinc Superoxide Dismutase
ECMO	Extracorporal Membrane Oxygenation
ecSOD	Extracellular SOD
EGA	Estimated Gestational Age
ELISA	Enzyme-linked Immunosorbant Assay
ET	Endotracheal Tube
ETEC	Enterotoxigenic E. coli
FHM	Fortified Human Milk
G-CSF	Granulocyte Colony-Stimulating Factor
GPX	Glutathione Peroxidase
GSH	Glutathione

GSSG	Oxidized Glutathione
GSSG-R	Glutathione Reductase
H/R	Hypoxia Reoxygenation
HCT%	Hematocrit Percentage
HDP	Hydroperoxide
HNE	Hydroxide Alkenals (4-hydroxy-nonenal)
HOCL	Hypochlouric Acid
I/R	Ischemia/ Reperfusion
IL	Interleukin
$L^{\bullet}$	Carbon Radical
LH	Target PUFA
LOH	Fatty Acid Alcohol
$LOO^{\bullet}$	Lipid Peroxyl Radical
LOOH	Lipid Hydroperoxide
LOOL	Lipid Alkoxyl
MAOIs	Monamine Oxidase Inhibitors
MCH	Mean Corpulscular Hemoglobin
MCHC	Mean Corpulscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
Mn SOD	Manganese Superoxide Dismutase

NEC	Nectrotizing Enterocolitis
$NO^{\bullet}$	Nitric Oxide
NO_2	Nitrogen dioxide
NOS^{-2}	Nitric Oxide Synthase
NPO	Nulla Per Os
NTYR	Nitrotyrosine
O=O	Oxygen – Oxygen Double Bond
OFR	Oxygen Free Radicals
$OH^{\bullet}$	Hydroxyl Radical
ONOOH	Peroxynitrous Acid
PAF	Platelet Activating Factor
PAV	Postero-Anterior View
Plase A_2	Phospholipase A_2
Plase A_2	Phospholipase A_2
PLOOH	Phospholipid Hydroperoxide
PLOOH	Phospholipid Hydroperoxides
PO	Per Os
PPM	Part per million
PROM	Premature Rupture of Membranes
PUFA	Poly-Unsaturated Fatty Acids
R	Initiating Radical

$RO^{\bullet}$	Alkoxyl Radical
$ROO^{\bullet}$	Peroxyl Radical
ROS	Reactive Oxygen Species
SCFAs	Short Chain Fatty Acids
SIADH	Syndrome of Inappropriate ADH
Sig.	Significant
sIgA	Secretory Immunoglobulin A
SOD	Superoxide Dismutase
TGFβ	Transforming Growth Factor Beta
TLC	Total Leukocytic Count
TNFα	Tumor Necrosis Factor alpha
$T\text{-}O^{\bullet}$	Tocopherol radical
T-OH	The Phenolic Hydroxyl Gr. of Tocopherol
TXA2	Thromboxane A2
TXB2	Thromboxane B2
UQ	Ubiguinone
UQH_2	Reduced Ubiguinone
VLBW	Very Low Birth Weight
XO	Xanthine Oxidase

LIST OF TABLES

Comtents	*page*
Table (1): Biochemical mediators in NEC	23
Table (2): Modified Bell Staging Criteria for Necrotizing Enterocolitis	26
Table (3): Medications used in NEC with their dose, contraindications, interactions, and precautions	42
Table (4): The most important antioxidants of human biology	75
Table (5): Antioxidant roles of ascorbic acid	103
Table (6): Comparison of important related points in the history, in both groups under study	129
Table (7a): Frequency distribution of antenatal data among both patients and control groups	130
Table (7b): Comparison of antenatal data among both patients and control groups	130
Table (8a): Frequency distribution of natal data among both patients and control groups	134
Table (8b): Comparison of natal data among both patients and control groups	135
Table (9a): Frequency distribution of post-natal data among both patients and control groups	137
Table (9b): Comparison of post-natal data among both patients and control groups	139
Table (10): Comparison of mean quantitative hematologic parameters, in both groups under study	141

Comtents	*page*
Table (11): Comparison of mean quantitative chemical parameters and arterial blood gases, in both groups under study	143
Table (12): Comparison of mean quantitative enzymatic parameters, in both groups under study	144
Table (13): Correlation between SOD and various clinical & laboratory parameters, among patients with NEC	145
Table (14): Correlation between GPX and various clinical & laboratory parameters, among patients with NEC	147
Table (15): Comparison of mean I/T ratio, and CRP levels, among all patients under study, according to gender	150
Table (16): Comparison among patients regarding mean value of bodyweight according to singlet or multiple delivery	150
Table (17): Comparison among patients regarding mean value og GA according to prolonged second stage of labour	151
Table (18): Comparison of mean RBCs, Hb and HCT levels, among all patients under study, according to polycythemia	152
Table (19): Comparison among patients regarding mean values of pH and TLC according to Apnea	152
Table (20): Comparison of mean levels of I/T Ratio, and CRP, among all patients under study according to bloody stools	153
Table (21): Comparison of mean levels of enzymatic parameters among all patients under study according to bloody stools	154
Table (22): Comparison of mean levels of I/T Ratio and CRP among all patients under study according to bowel sounds	155
Table (23): Comparison of mean levels of enzymatic parameters among all patients under study according to bowel sounds	155
Table (24): Comparison of mean levels of enzymatic parameters, among all patients under study according to Pneumatosis intestinalis (X-ray findings)	156

Comtents	*page*
Table (25): Comparison of important related points in the history in group I (Stage I) and group II (Stage II) under study.	158
Table (26): Comparison of important related points in the history, in group II (Stage II), and group III (Stage III) under study.	159
Table (27): Comparison of important related points in the history, in group I (Stage I), and group III (Stage III) under study.	160
Table (28): Comparison of mean quantitative hematologic parameters, in group I (Stage I), and group II (Stage II) under study.	161
Table (29): Comparison of mean levels of hematologic parameters in group II (Stage II), and group III (Stage III) under study.	162
Table (30): Comparison of mean levels of hematologic parameters in group I (Stage I), and group III (Stage III) under study.	163
Table (31): Comparison of mean values of chemical parameters and arterial blood gases, in group I (Stage I), and group II (Stage II) under study.	167
Table (32): Comparison of mean values of chemical parameters and arterial blood gases, in group II (Stage II), and group III (Stage III) under study.	168
Table (33): Comparison of mean values of chemical parameters and arterial blood gases, in group I (Stage I), and group III (Stage III) under study.	169
Table (34): Comparison of mean levels of enzymatic parameter, in group I (Stage I), and group II (Stage II) under study.	170
Table (35): Comparison of mean levels of enzymatic parameter, in group II (Stage II), and group III (Stage III) under study.	170
Table (36): Comparison of mean levels of enzymatic parameter, in group I (Stage I), and group III (Stage III) under study.	170

LIST OF FIGURES

Contents	*Page*
Fig. (1): Proposed pathways in the pathogenesis of necrotizing enterocolitis	15
Fig. (2): Superoxide and peroxide metabolism	56
Fig. (3): Summary of mechanisms of free radicals in pathological states	70
Fig. (4): Causes and consequences of tissue damage	71
Fig. (5): Glutathione metabolism- GSH Redox cycle	89
Fig. (6): Socio-economic- status in patients and control groups under study	132
Fig. (7): Maternal Problems among patients group	133
Fig. (8): Comparison of mesn quantitive enzymatic parameter, in both groups under study	144
Fig. (9): Correlation between SOD and Band Cell Ratio	146
Fig. (10): Correlation between GPX and TCL	148
Fig. (11): Mean platelets count (x1000/ mm^3) among the groups under study	164
Fig. (12): Mean I/T Ratio (% x0.01) among the groups under study	165
Fig. (13): Mean CRP (ng/ ml) among the groups under Study	166
Fig. (14): Mean SOD (u/ ml x100) among the groups under study	171
Fig. (15): Mean GPX (U/ L) among the groups under study	171

LIST OF PLATES

Contents *Page*

Plate (1): X-ray chest and abdomen PAV of a studied patient with suspected NEC 173

Plate (2): X-ray chest and abdomen PAV of a studied patient with pneumatosis intestinalis 174

Plate (3): X-ray chest and abdomen of a studied patient, upright view showing free air under the diaphragm, portal venous gas and pneumatosis 175

Plate (4): X-ray chest and abdomen of a studied patient showing gasless abdomen 176

Abstract

Necrotizing entero-colitis (NEC), a neonatal disease that primarily affects preterm infants is known to be associated with a high mortality rate and is of unknown etiology. Yet, ischemia-reperfusion appears to be an important contributing factor; also oxygen free-radicals produced during reperfusion most-likely contribute to the injury. So, further studies are needed to elucidate whether disruption of the normally high protective levels of SOD in pre-term infants could lead to NEC. This study included; 30 pre-term neonates and ten healthy controls. Their mean gestational age was 32±2weeks. The 30 pre-term patients were further classified into three groups according to Modified Bell-Staging Criteria for NEC: 8 of them had suspected NEC, 15 had proven NEC, and 7 had advanced NEC. The controls were 10 healthy pre-term neonates free of any major disease. The present study revealed that socio-economic status of the studied patients was below average in 50% in comparison to 20% in the controls. Mean SOD and GPX levels were significantly lower ($P<0.01$) in patients (275Unit/ml, and 4458U/L; respectively), compared to (519Unit/ ml, and 7783U/L; respectively) in the controls. Both anti-oxidant enzymes activity was lower among patients having bad prognostic parameters especially SOD. The natural levels of the antioxidant enzymes (SOD/ GPX), especially SOD were deficient in cases with NEC compared to the controls. This deficiency correlates with the severity of the disease.

Environmental determinants of perinatal insults such as antenatal care, maternal problems and socioeconomic status were also done and 50% of the patients were in the below average, 433% were average and 6.7% were above average. Multiple pregnancies represented one third of the patients compared to 0% among controls. Antenatal care was