Ain Shams University
Institute of Postgraduate Childhood Studies
Department of Medical Studies

MODULATION OF INFLAMMATION AND CACHECTIN ACTIVITY IN RELATION TO TREATMENT AND NUTRITION OF NEONATAL SEPSIS

THESIS

Submitted for Fulfillment of
Ph.D. Degree in Medical Childhood Studies

By

REHAB ABDEL KADER MAHMOUD MOUSTAFA
M.B. B.Ch., M.Sc.

Supervised By

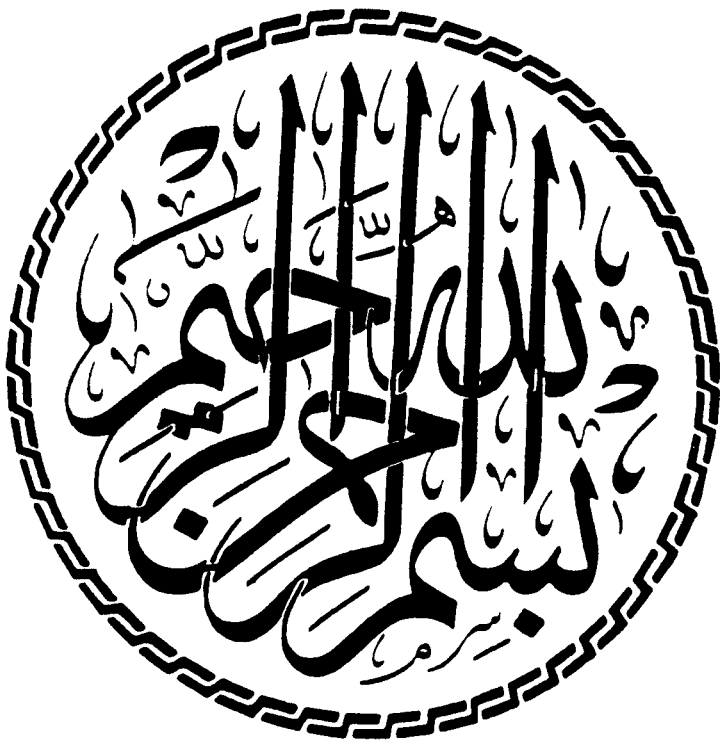
PROF. DR. HAMED MAHMOUD SHATLA
Professor of Pediatrics
Faculty of Medicine - Ain Shams University

PROF. DR. HADIA HUSSEIN BASIEM
Assist. Prof. of Clinical Pathology
Faculty of Medicine - Ain Shams University

PROF. DR. MEDHAT HASSAN SHEHATA
Assist. Prof. of Medical Childhood Studies
Institute of Postgraduate Childhood Studies - Medical Department

DR. MAMDOUH ABDEL MAKSOUD MOHAMED
Lecturer of Pediatrics
Faculty of Medicine - Ain Shams University

1996


بسم الله الرحمن الرحيم

ACKNOWLEDGMENT

Thanks to Allah.

I find no words by which I can express my extreme thankfulness, appreciation and profound gratitude to my great Prof. Dr. Hamed Mahmoud Shatla, *Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for his generous help, guidance and kind encouragement.*

I would like to express my extreme indebtedness and thanks to Prof. Dr. Medhat Hassan Shehata, *Assist. Prof. of Medical Childhood Studies, Institute of Postgraduate Childhood Studies - Medical Department, Ain Shams University, for his great help and valuable advice.*

Also I am greatly indebted to Prof. Dr. Hadia Hussein Basiem, *Assist. Prof. of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her generous guidance and continuous support in the practical part of this work.*

I would like to express my extreme thankfulness and appreciation to Dr. Moustafa Abdel Aziz El-Hodhod, *Lecturer of Pediatrics, Faculty of Medicine Ain Shams University for his great help and continuous encouragement.*

Last, but not least my deep thanks and best wishes to the staff members of N.I.C.U of Gynecology and Obstetrics Hospital of Ain Shams University and to the babies of this study and their parents for their great cooperation.

CONTENTS

LIST OF ABBREVIATIONS

α_1-ACT	Alpha 1-antichemotrypsin.
α_1-AGP	Alpha 1-acid glycoprotein.
α_1-AT	Alpha 1-antitrypsin.
α_1-GP	Alpha 1-glycoprotein.
Ab	Antibody.
ADCC	Antibody-dependent cellular cytotoxicity.
ADH	Antidiuretic hormone.
Ag	Antigen.
AGA	Appropriate for gestational age.
ANC	Absolute neutrophilic count.
ARDS	Adult respiratory distress syndrome.
BM	Bone marrow.
BPD	Broncho-pulmonary displasia.
C_3	The third component of complement.
CFU	Colony-forming unit.
CFU-G	Colony forming unit-granulocyte.
CFU-GM	Colony forming unit-granulocyte monocyte.
CFU-M	Colony forming unit-monocyte.
CRP	C-reactive protein.
CSF	Cerebrospinal fluid.
CT	Computerized tomography.
DIC	Disseminated intravascular coagulation.
E coli	Escherichia coli.
ECMO	Extracorporeal membrane oxygenation.
EDRF	Endothelial derived relaxing factor.
ESR	Erythrocyte sedimentation rate.
F	Female.
G-CSF	Granulocyte-colony stimulating factor.
GAB HS	Group A, beta hemolytic streptococcus.

GBS	Group B-streptococcus.
GE	Gastroenteritis.
GIT	Gastrointestinal tract.
GM-CSF	Granulocyte monocyte-colony stimulating factor.
Hb	Hemoglobin.
HFV	High frequency ventilation.
HI	Hemophilus influenza.
HPF	High power field.
HPG	Haptoglobin.
HSS	Hematological scoring system.
I:T	Immature to total neutrophil ratio.
IFN-γ	Interferon gamma.
Ig	Immunoglobulin.
IL	Interleukin.
IM	Intramuscular.
IPPV	Intermittent positive pressure ventilation.
IV	Intravenous.
IVIg	Intravenous immunoglobulin.
LBW	Low birth weight.
LPL	Lipoprotein lipases.
LPS	Lipopolysaccharide.
LT	Lymphocytes.
M	Male.
M-CSF	Monocyte-colony stimulating factor.
MHC	Major Histocompatibility complex.
MRF	Maternal risk factor.
MTC	Macrophage-mediated tumor cytotoxicity.
MW	Molecular weight.
NEC	Necrotizing enterocolitis.
NICU	Neonatal intensive care unit.

NK	Natural killer.
NS	Non-significant.
NSP	Neutrophil storage pool.
PAF	Platelet activating factor.
PMNs	Polymorphonuclear neutrophils.
PPHN	Persistent pulmonary hypertension of newborn.
PROM	Premature rupture of membrane.
RBC	Red blood cells.
RDS	Respiratory distress syndrome.
Rh	Rhesus factor.
S pneumoniae	Streptococcus pneumoniae.
SD	Standard deviation.
SGA	Small for gestational age.
Sig	Significant.
SS	Sepsis score.
Staph. aureus	Staphylococcus aureus.
TNF	Tumor necrosis factor.
VAECMO	Venoarterial extracorporeal membrane oxygenation.
WBCs	White blood cells.
-ve	Negative.
+ve	Positive.

LIST OF TABLES

Review of Literature Tables

Results Tables

Results Tables

LIST OF FIGURES

Review Figures

Patients and Methods Figures

Results Figures

Results Figures

Introduction and Aim of The Work

INTRODUCTION

Despite steady improvements in antibiotic therapy and intensive care during the past decade, mortality from fulminant sepsis has remained high. This high mortality rate has continued to stimulate interest in pharmacologic agents that might reduce morbidity and mortality (*Kreger et al.* 1980).

Numerous adjunctive therapies that have been proposed, none has created as much controversy as the use of glucocorticoids. Since their use was first proposed three decades ago, many studies in laboratory animals have shown that these drugs decrease morbidity and mortality; however, results in humans have been less convincing (*Sheagren et al.*, 1985).

Even the prospective, randomized study by *Schumer* (1976), which showed improved survival in patients with sepsis treated with glucocorticoids, did not settle the issue. Thus, at the beginning of the present decade the controversy over glucocorticoid administration in fulminant systemic sepsis remained unresolved.

Cytokines are thought to mediate many host responses to bacterial infection. Tumour necrosis factor/cachectin and IL-I play a central role in many responses to infection (*Beutler et al.*, 1988), and antibodies to these cytokines can dramatically improve the course of inflammatory events in bacterial sepsis (*Beutler et al.*, 1987).

Potential benefits of corticosteroid therapy are cell membrane and lysosomal stabilization inhibition of complement-induced granulocyte aggregation, prevention of oxygen radical mediated cell damage, and inhibition of ACTH release (*Stubitz et al.*, 1981).

Potential adverse effects include superinfection, electrolyte disturbances, hyperglycemia, GIT bleeding, and arrhythmias (*Melby*, 1974).

AIM OF THE STUDY

The aim of this study is to determine the clinical value of TNF-α in the follow up of patients with neonatal sepsis treated by dexamethasone.

Review of Literature

NEONATAL SEPSIS

Definition

Neonatal sepsis is a clinical syndrome resulting from the pathophysiologic effects of local or systemic infection in the first month of life (*Gronross*, 1985).

In contrast to bacteremia (bacteria in blood) septicemia usually consists of bacteremia plus a constellation of signs and symptoms caused by microorganisms or their toxic products in the circulation (*Okusawa*, 1988).

Incidence of Neonatal Sepsis

The incidence of neonatal sepsis varies from one nursery to another depending on the presence of conditions that predispose infant to infection (*Guerina*, 1991).

The incidence of neonatal sepsis varies from 1 to 8/1000 live births in developed countries with considerable variability over time and geographic location. Hospital to hospital variability in incidence may be related to rates of prematurity, prenatal care, conduct of labour, and environmental conditions in nurseries (*Gotoff*, 1992).

Among very low birth weight (LBW) infants who are undergoing prolonged hospitalization, the incidence increases dramatically to 300/1000 in very LBW infant (*Cole*, 1991).

As many as 2% of fetuses are infected in utero and up to 10% of infants are infected during delivery or in the first month of life (*McIntosh*, 1992).

In a study performed in Egypt, in 1984, it was found that 54% of infants with risk factors developed septicemia (*El-Maraghi et al.*, 1984).

An increased number of cases of neonatal sepsis have been reported in recent years but no increase in the relative incidence among neonatal infection has been reported (*Bergstrom et al.*, 1992).

Factors Predisposing to Neonatal Sepsis

Although multiple factors have been associated with increased risk of bacterial infections within the first 7 days of life, the most important factors are the degree of prematurity of the infant and maternal medical conditions that may predispose the infant to infection (*Hillier et al.*, 1988).

A. Maternal Risk Factors:

Premature onset of labour, premature rupture of membrane >12 hs, the longest the duration the greater the risk (*Joachin et al.*, 1995).

Pregnant woman with cervical colonization by group B beta hemolytic streptococci, or having tender uterus or a vaginal discharge (*Sperling et al.*, 1988).

Amniotic fluid problems as meconium stained, foul smelling and cloudy amniotic fluid.

Multiple gestation is also associated with an increased incidence of neonatal sepsis (*Guerina et al.*, 1991),

B. Neonatal Risk Factors:

The most important neonatal risk factor is LBW, the rate of sepsis was 8 times greater in infant weighing 1000 to 1500 gms compared with those weighing 2000 gms (*Guerina*, 1991).

Infants who had fetal distress, who were born by traumatic delivery or who were severely depressed at birth and

required invasive procedures, invasive monitoring and respiratory or metabolic support.

TPN, various drains and shunts increase the risk of staph. epidermidis sepsis.

In infants with galactosemia predisposing to E. coli sepsis, G6PD deficiency, immune defect or aspleenia. Other factors such as long term ventilation, problems with swallowing of neurological origin and iron therapy risk may enhance the growth of many organisms (*Roberton*, 1992).

Guerina (1991) classified risk factors into major and minor perinatal risk factors in the following table.

Table (1): Risk factors in neonatal sepsis

Classification	Risk factors
Major risk factors	– PROM>24 hours. – Intrapartum maternal fever (>38° C). – Chorioamnionitis. – Sustained fetal heart rate>160 beats/min.
Minor risk factors	– Intrapartum maternal fever (>37° C). – Twin gestation. – Prematurity (<37 weeks). – Maternal WBC>15x10^9/L. – PROM>12 hours. – Tachypnea<1 hour. – Maternal GBS colonization. – Low Apgar score (<5 at 1 min). – Low birth weight (<1500 gms).

(*Guerina*, 1991)

Causative Organisms

Through the years, there has been a shift in the micro-organisms responsible for neonatal septicemia and meningitis (*Freedman et al.*, 1981).

Prior to available antimicrobial agents, in the first decade of the twentieth century, ***gram positive*** bacteria were apparently the predominant causative agents, in particular, GBS, hemolytic group A, were the most common identical pathogens. In the 1940's and 1950's ***gram negative*** organisms, predominately E. coli were implicated in the vast majority of cases of neonatal sepsis (*Gotoff*, 1992).

In the study done in Egypt at Kasr El-Aini Hospital in 1984 the predominant causative organism of neonatal sepsis were as follows:

Table (2): The predominant causative organisms of neonatal sepsis

Type of organism	Incidence
Klebsiella	51.25%
E. coli	18.15%
Pseudomonas	9.25%
Staphylococci	9.25%
Streptococci	7.40%
Proteus	3.70%

(*El-Maraghi et al.*, 1984)

In another study, done in NICU of Ain Shams University in 1993 by *Ebrahim et al.* it was found that, blood culture was positive in all cases. Klebsiella organisms were isolated in 83.3% of cases Pseudomonas in 10%, mixed (Klebsiella and Pseudomonas) in 3.3% and E. coli in 3.3% of cases.

Vesikari et al., (1985) classified neonatal sepsis into very early onset sepsis occurring in the first 24 hours, early onset sepsis occurring from 1-7 days and late onset sepsis occurring from 8–28 days. From this study the most common organisms are shown in the following table.

Table (3): Aetiology of early and late onset sepsis.

Causative organism	Very early onset <24 hrs	Early onset 1–7 days	Late onset 8-28 days
GBS	52%	14%	27%
E. coli	14%	24%	10%
Staph. aureus	8%	34%	30%
Others	26%	24%	17%

(*Vesikari et al.*, 1985)

Gram-Positive Infections

A. Streptococcal organisms

The group B streptococcus (GBS) is the most common gram positive organism that causes septicemia and meningitis during the first month of life in infants older than 37 weeks gestational age. Vertical transmission of this organism from mother to infant is one route of infection. Nosocomial acquisition of infection has been implicated in some nurseries and may be common than was thought previously (*Taeusch et al.*, 1991).

3 major capsular serotypes of GBS have been identified (I, II, III) these are further sub-classified based on serologically distinct polysaccharide and protein antigens isolated from capsule and cell wall extract (*Guerina*, 1991).

The capsular serotypes causing invasive GBS disease in neonates type III strains have predominated. However 2 new serotypes of GBS, type IV and V, have been recently identified among human isolates. The first report of serotype V GBS as a cause of disseminated neonatal infection was given by (*Rench and Baker,* 1993).

Table (4): Attack rate and mortality of early-onset GBS sepsis by birth weight (*Boyer et al.*, 1983).

Birth weight (g)	No. of live births	No. of cases	Attack rate per 1000 live births	Deaths (%)
501-1000	382	10	26.6	90
1000-2000	1297	11	8.6	27
2001-2500	2102	9	4.3	33
>2500	28603	31	1.1	3

B. Staphylococcal Infection

Coagulase negative ***staphylococci*** is the most common cause of catheter-related sepsis, in the newborn in the United States (*Polin and Ditmar*, 1989). It is a major problem now in neonatal nurseries infection is commonly with slime producing strain (*Patrick*, 1990).

C. Pneumococcal Infection

It is emphasized that pneumococcal septicemia is a rare but highly lethal disease of the newborn. The clinical course strongly resembles early onset GBS disease.

Epidemiologic data suggest that the majority of infants are colonized near birth. Analogous to GBS sepsis, it seems rational to administer penicillin prophylaxis during labour to women in whom S. pneumoniae had been isolated from their genital tract to prevent vertical transmission and neonatal pneumococcal septicemia (*Taeusch et al.*, 1991).

D. Infection with Listeria Monocytogenes

Listeria is small motile, gram-positive rods that grow slowly in the laboratory and that can be mistaken for either corynebacteria or streptococci in gram strains. Infections in human are sometimes seen as a result of contact with domestic animals or contaminated food (*Taeusch et al.*, 1991).

Listeria monocytogenes is a common cause of meningoencephalitis and abortion in ruminants, which also have been linked to disease transmission in human (*Faidas et al.*, 1993).

Gram-Negative Infections

A. Infection with E. coli (K_1)

E. coli is the most common gram negative bacteria that causes septicemia during the neonatal period. Approximately 40% of E. coli strains that cause septicemia posses K_1 capsular antigen, and strains identical with that in blood can usually be identified in the patients nasopharynx or rectal cultures (*Taeusch et al.*, 1991).

B. Haemophilus Influenza Infection (H.I.)

Haemophilus influenza is the cause of about 70% of all cases of neonatal septicemia in American Hospitals.

Both GBS and HI are present at birth or commence within a few hours following birth. The main features are fulminant infection with pneumonia and respiratory distress in neonates of LBW (*Campognone and Singer*, 1986).

C. Pseudomonas Septicemia

Pseudomonas septicemia may present with one or several characteristic violaceous papular lesions, which develop central necrosis after several days. Although this condition is most commonly observed in pseudomonas infection, it may also be associated with other pathogens.

The newborn who receives broad spectrum antibiotics while in an environment that is potentially contaminated by bacteria from respirators is likely to develop disease caused by pseudomonas species or other fastidious organisms (*Taeusch et al.*, 1991).

D. Klebsiella Pneumonia

Klebsiella pneumonia species seem to have increased frequency as causative agents in neonatal septicemia (*Grauel et al.*, 1989).

They are gram-negative, non-sporing, non-motile bacilli (*Sleigh and Duguid*, 1989).

Systemic Candidiasis

The causative organisms are Candida albicans, Candida tropicalis, and Candida parapsilosis. It is most frequent in prematures, very LBW infants, and infants whose mothers had undergone cervical circulage or intrauterine devices.

The site of initial colonization varies with the age at which colonization is detected. At birth colonization is most frequently found in the rectum and oropharynx. At 2 weeks of life, colonization is most frequently in the groin and less likely in the rectum and oropharynx (*Baley et al.*, 1986).

Nosocomial Infections

These are significant problems for the neonate requiring extended care in the special care nursery. This is particularly true for very low birth weight infants.

The overall incidence of nosocomial infections in neonates have been reported to be less than 5%. Beyond the first and the second weeks of life the neonate who has remained in the special care nursery is likely to be colonized with perinatally (endogenous) and nosocomially acquired flora, this places the neonate at risk of infection by coagulase negative staphylococci, enterococci, staphylococcus aureus (including methacillin resistant strains) and gram negative bacteria, including multiple resistant enteric strains. In addition late onset disease caused by GBS and Listeria must be considered.

he most frequently identified factors contributing to nosocomial infections are postnatal age (length of stay in nursery), low birth weight, foreign body (intravenous catheters, chest tubes, endocardial tube etc.,), nursery crowding, surgery, and prolonged treatment with broad spectrum antibiotics (*Jarvis*, 1987).

PATHOPHYSIOLOGY OF NEONATAL SEPSIS

The pathophysiology of neonatal sepsis includes the activation of complement and coagulation cascades, production of B-endorphin and activation of polymorphonuclear neutrophils (PMNs) (*Saez-Liorens et al.*, 1993).

1. The Complement System

The alternative complement pathway can be activated by bacterial endotoxins and some immune complexes. Therefore, it can be considered a first line of defense against invading microorganisms because it functions in the absence of antibodies (Abs). Although the complement system is important in the lysis and phagocytosis, over-stimulation of the system can have deleterious effects.

The increased activation of PMNs induced by complement stimulation can provoke pulmonary leukostasis which is an important factor in the development of pulmonary infection. In addition, patients with deficiency of the terminal components of the complement are less likely to have fulminant disease, although they are more susceptible to meningococcal infection (*Saez-Liorens et al.*, 1993).

2. The Coagulation System

The intrinsic pathway may be activated by a direct interaction between endotoxin and coagulation factor XII (Hageman factor), once it is activated it initiates the clotting sequence that results in the conversion of fibrinogen to fibrin. In addition endotoxins directly or through the participation of cytokines can release tissue factor by monocytes and endothelial cells and thereby activate factor VII and extrinsic pathway (*Schreiber & Austen*, 1973)

3. Production of B-Endorphin

B-endorphin and endogenous opiate, are released from the pituitary gland in response to stress (*Guillermin et al.*, 1977).

It appears to be a fundamental etiological factor in septic shock. It is possible that these peptides function as neurotransmitters to increase vascular permeability and decrease vascular resistance or as endogenous analgesics to control pain and anxiety (*Saez-Liorens et al.*, 1993).

4. Activation of Polymorphonuclear Neutrophils (PMNs)

The increased polymorphonuclear neutrophils (PMNs) is frequently observed in patients with bacterial sepsis, the release of PMNs from bone marrow reserves is induced by endotoxins, cytokines, or complement (*Pizzo*, 1992).

However, an initial transient neutropenia occurs. This transient neutropenia may reflect inadequate bone marrow (BM) function or increased destruction or consumption of circulating PMNs.

A more likely explanation is that bacterial products and cytokines activate leukocyte-endothelium adhesion molecules that promote margination and attachment of PMNs to endothelial cells (*Springer*, 1990).

In addition, trapping of PMNs in the pulmonary vasculature or in capillary vessels may also contribute to initial neutropenia (*Springer*, 1990).

The neonate may have an antibody deficiency owing to absence of maternal antibody (IgG) that can be transferred across to placenta. Alternatively, the very LBW infant may have very low IgG levels (<100 mg/dl) owing to diminished

passive transfer early in gestation. Levels of opsonic C_3, chemotactic, and factor B may further impair opsono-phagocytosis and complement mediated bacterial killing. Neutrophil function may be further impaired by the stress response during sepsis.

The frequent occurrence of granulocytopenia during neonatal sepsis, owing to neutrophil storage pool (NSP) and bone marrow depletion, predispose the infant to a profound quantitative defect in host defenses (*Sameul*, 1992).

In neonates, PMNs have the capacity to phagocytose and kill the microbes, but their ability to develop pseudopods or more in a unidirectional fashion toward a chemotactic stimulus is impaired. This has been shown to be associated with defective signal transduction after chemotactic factor receptor ligand binding occurs (*Gaur et al.*, 1994).

Chemical Mediators of Sepsis

Current evidence indicates that most of the physiologic effects generated by bacterial infections are mediated by a complex interaction of pre-inflammatory cytokines activated in response to the presence of microbial component within the vascular compartment.

Prominent among these cytokines are tumor necrosis factor (TNF), interleukin-1 (IL-I), interleukin-6 (IL-6), interleukin-8 (IL-8), platelet activating factor (PAF), gamma-interferon (γ-INF), macrophage derived proteins and arachidonic acid metabolites. All are participants that amplify the systemic inflammatory response (*Sullivan et al.*, 1992).

The factors that mediated the chain of events occurring at the cellular level in neonatal septic shock are still being actively investigated. They include thromboxane, PAF, TNF,

leukotrienes, prostaglandins, endothelial-derived relaxing factor (EDRF), free radicals, and IL-1.

White blood cells (WBCs) release many of the pre-inflammatory mediators, such as lipo-oxygenase products, free radicals, hydrolytic enzymes, and cytokines which act on the vascular endothelium to cause increased cellular permeability, aggregation of platelets and other blood cells, and release of additional vasoactive substances.

The complex interactions and feedback cycles among these mediators contribute greatly to the hemodynamic instability and vascular leak that so commonly occur with sepsis (*Whitfield*, 1993).

Attention has focused on the inflammatory mediators PAF and TNF as having important roles in a cascade of events that results in the clinical picture of sepsis.

PAF is a phospholipid that is produced by and acts on neutrophils, macrophages, monocytes, platelets, and endothelial cells and is a known inducer of microvascular permeability (*Hosford and Braquer*, 1990).

The extensive review article by *Hosford and Braquet* (1990), discusses the studies that have shown that PAF infusions cause a shock state in animals often paralleling the state seen in neonatal sepsis. This mediator is also produced during shock, as noted in children with sepsis. PAF is not the sole factor responsible for the circulatory changes.

The cytotoxic and immunoregulatory cytokine TNF is considered to be one of the prime mediators of the host response to endotoxin involved in the pathophysiology of sepsis (*Debets et al.*, 1989).

TNF is a powerful vasoactive cytokine produced by activated macrophages including neonatal monocytes, and is

implicated as an important factor in endotoxemia and other shock-like states.

TNF levels are elevated in humans with septic shock, with meningococcal disease after endotoxin administration and in children with gram-negative septic shock. Although TNF is associated with some of the vasomotor responses in sepsis such as pulmonary hypertension and systemic hypotension, it is not linked to other aspects, such as hypoxemia (*Truog et al.*, 1990).

Cytokines are proteins produced by the monocyte-macrophage cell line in response to infectious stimuli, they are important endogenous mediators of the inflammatory response to systemic bacterial disease.

The cardiorespiratory abnormalities and other organ dysfunctions associated with sepsis may be mediated by the monocyte-derived cytokines TNF, IL-I and IL-6 increased circulating levels of TNF and IL-6 were reported during bacterial sepsis (*Catherine et al.*, 1994).

Interleukin-6 (IL-6) is another cytokine that appears to be the most efficient stimulator of the production by the liver of the acute phase proteins.

It is probably a second messenger released by macrophages endothelial cells, or fibroblast and other cells in response to ILI or TNF. High serum levels of IL-6 measured by bioassay have already been found during neonatal sepsis (*Damas et al.*, 1992).

INF-γ was found to enhance the chemotactic response of neonatal PMNs. INF-γ production by mononuclear cells especially by T-helper cells, is deficient in neonates (*Gaur et al.*, 1994).

Additionally, neutrophil-induced digestion of surrounding tissue contributes to separation of high endothelial cell junctions and development of the capillary leak syndrome. Thus, a variety of potential mechanisms exist for PMNs to mediate the vascular injury of septic shock (*Saez-Llorens et al.*, 1993).

CLINICAL PICTURE

The signs and symptoms of sepsis in the newborn are often non-specific and may evolve differently. Sepsis may be fulminant leading to death in several hours or may be more protracted (*McIntosh*, 1992).

The initial signs of infection are often subtle or minimal. The mother or nurse may simply state that the infant "doesn't look well" or "feeds poorly" (*Gotoff*, 1992).

Table (5): Clinical symptoms and signs of neonatal sepsis

Clinical signs	Percent of infants with signs of sepsis
Hyperpyrexia	51%
Hypothermia	15%
Respiratory distress	33%
Apnea	22%
Cyanosis	24%
Jaundice	35%
Hepatomegaly	33%
Refuse feeding	28%
Vomiting	25%
Abdominal distension	11%
Diarrhea	11%
Convulsions	Undetermined

(*Taeusch et al.*, 1991)

Table (6): Clinical signs of early onset sepsis.

Onset	Signs
Early onset	Fetal distress before delivery. Meconium staining of amniotic fluid. Respiratory distress. Persistent tachycardia. Hypothermia. Apneic and bradycardiac spells. Meconium aspiration. Hypotension.

Respiratory Signs

Cyanosis, irregular breathing, dyspnea, and apnea occur frequently in premature infants and infants born after difficult delivery, and may be unrelated to sepsis. If these signs of respiratory distress, however, are accompanied by signs of pulmonary infection, such as pneumonia and empyema, the chances of sepsis being present are increased (*Samuel*, 1992).

Central Nervous System Signs

Meningitis and cerebral vasculitis often result from high-level bacteremia and are found in approximately a third of newborn infants with sepsis. Newborn infants will demonstrate lethargy and/or irritability and convulsions (*Bell and McGuiness*, 1982).

Cardiovascular Signs

Cardiac signs are due to myocarditis, endocarditis pericarditis, or sepsis. Tachycardia ≥160/min, bradycardia, arrhythmias, hypotension and poor peripheral perfusion are

among the most common signs of sepsis (*Baker and Edwards*, 1990).

GIT Signs

Abdominal distension, poor feeding, poor suckling, vomiting, mild diarrhea or may develop an ileus or gastric bleeding (*Guerina*, 1991).

Subcutaneous Tissue and Joints

Sclerema and painful tender bone or joints may be present (*Haque*, 1992).

Skin manifestations occur in about 1/5 of infants with sepsis. The lesions include: cellulitis, impetigo, and subcutaneous abscesses.

Erysipelas, a manifestation of group GABHS infections, is rarely seen today. Impetiginous lesions and subcutaneous abscesses are observed in infections caused by staphylococci, E. coli, and pseudomonas. Sclerema also may be associated with sepsis, especially in premature infants (*Gotoff*, 1992).

Omphalitis, formerly considered an important staring point for neonatal sepsis, occurs in less than 10% of patients. It is characterized by periumbilical erythema and induration are often accompanied by a purulent discharge from the stump (*Samuel*, 1992).

When cellulitis is found, infection with streptococci should be considered. Rapidly developing diffuse erythema of the skin in the absence of discrete eruption, accompanied by sudden development of anemia and leucocytosis in an infant born to mother with PROM should suggest the possibility of clostridium perfringes septicemia (*Feigin et al.*, 1987).

The most dramatic skin lesions are those associated with fulminant meningococcemia in which extensive petechial purpuric, and ecchymotic lesions may involve a large area of the skin. Disseminated intravascular coagulation (DIC) is uniformly found in such patients with sepsis (*McCabe et al.*, 1983).

DIAGNOSIS OF NEONATAL SEPSIS

The diagnosis of systemic bacterial infection must start with a careful look for the infant's signs and symptoms, physical examination, information on longitudinal changes in vital signs and laboratory indicators, and history including relevant recent nursery history (*Cole*, 1991).

There is as yet no single diagnostic test which can reliably diagnose sepsis in the newborn, therefore many diagnostic tests or group of tests are utilized to diagnose or confirm sepsis. The ultimate proof of sepsis rests primarily on discovery of the infecting organism from a body fluid (*Haque*, 1992).

Investigations

I. Hematological Investigations

1. White Blood Cell Count (WBC)

The leucocyte count is believed to be the single most important aid for early detection and screening for the septicemia (*Baley & Stoyk*, 1988).

Total white cell count are often unhelpful in diagnosis of sepsis because the normal range is wide, varies with gestation and postnatal age and can be confused by machines including nucleated RBC (*Roberton*, 1992).

Most, but not all studies have found that an immature to total neutrophil ratio (I:T ratio) >0.2 is a good marker of infection, and all agree that an I:T ratio <0.2 makes infection very unlikely. An abnormal I:T ratio in the presence of a low absolute neutrophil count is very suggestive of infection (*Kite et al.*, 1988).

The maximum normal value is 0.16 during the first 24 hs. 0.14 by 48 hs and 0.13 by 60 hs, where it remains until 5 days of age. There after, the maximum normal I:T ratio is 0.12 until the end of the first month (*Roberton*, 1992).

Both neutrophilia >7.5–8.0 x 10^9/L and neutropenia <2–2.5 x 10^9/L suggest bacterial infection (*Roberton*, 1992).

Both neutrophilia >7.5–8.0 x10^9/L and neutropenia <2–2.5 x10^9/L suggest bacterial infection (*Roberton*, 1992).

Buffy Coat Smear Examination:

Liu et al. (1984) observed higher incidence of degenerative changes of neutrophils including vacuolization and toxic granulation in peripheral blood smears of infant with culture proved bacterial sepsis.

El-Gamal et al. (1989) stated that 70% with sepsis showed organisms in their buffy coat smears. They also had vacuolation and toxic granulations of their neutrophils.

2. Platelet Count

Thrombocytopenia is a common manifestation of bacterial septicemia in neonates and was present in patients without disseminated intravascular coagulation (DIC). A platelet count may be a useful diagnostic tool when the clinician encounters newborn with possible septicemia (*Corrigan*, 1974).

Roberton (1992) reported that about half the babies with proven bacterial infection, the platelet count will fall below 100.000/mm^3 but this may not be until sometime after the baby is obviously clinically septic.

Hematological scoring system (HSS)

From hematologic findings and complete blood cell count criteria, a hematologic scoring system was formulated that assigns a score of 1 for each 7 findings. See table 6.

Table (7): Hematological scoring system

Term	Abnormality	Score
Total WBC count	Increase or decrease $\leq 5,000/mm^3$ or $\geq 25,000$ mm^3 $21,000$ mm^3 at birth 12-24 hrs, and 2 days onward respectively	1
Total PMNs count	Increased or decreased	1
Immature PMNs count	Increased	1
Immature PMNs/total PMNs count ratio	>0.2	1
Immature/mature ratio	≥0.3	1
Degenerative changes in PMNs	≥.3 positive for vacuolization toxic granulation or Dohle bodies	1
Platelet count	$\leq 150,000/mm^3$	1

(*Rodwell*, 1988)

The higher the score the greater was the likelihood of sepsis 96% of septicemic cases had score greater than or equal to 3. With score less or equal to 2 the likelihood that sepsis was absent was 99% (*Rodwell*, 1988).

4. Acute-phase Proteins

A) C-reactive proteins(CRP)

This globulin is produced by the liver during inflammatory process probably as a result of stimulation by IL-1 (*O'Garra*, 1989).

CRP levels take at least 10-12 hours to rise after the onset of infection and so not helpful in the initial diagnosis of early onset disease. However, a normal CRP value 24 hours after sepsis is suspected makes the diagnosis extremely unlikely.

The greatest use of CRP measurement is in following the course of infection falling levels strongly suggest that the infection is responding to treatment (*Roberton*, 1992).

Normal values of CRP are<1.6 mg/dl on day 1 to 2 and <1.0 mg/dl thereafter. Normalization of CRP elevation appears to be a helpful tool in determining response to antimicrobial therapy and duration of treatment. Failure to mount a CRP response may be a poor prognostic sign (*Gerdes*, 1991).

B) Haptoglobin (HPG)

Serum HPG is a glycoprotein present in alpha-2 globulin fraction of plasma proteins (*Salmi*, 1973).

Serum HPG was found to show a highly significant low levels in patients suffering from neonatal bacterial infections, in whom hemolysis was associated. Moreover, serum HPG was significantly higher in infected new-born who survived than those with a lethal outcome (*Ismail et al.*, 1988).

C) Other acute phase proteins

Fibronectin: is an opsonic glycoprotein that is an acute phase reactant but commonly falls during the course of sepsis in neonates. In a prospective study, a low plasma fibronectin

concentration was shown to be a reasonably sensitive indicator of sepsis (*Gerdes*, 1991).

Orosomucoid (α_1-AGP, α_1-AT and α_1-ACT): have all been used in assessing neonatal infection.

Shatla et al. (1988) stated that α_1-GP was significantly increased in serum of infected neonates in comparison to the control group. So it should be considered in the early diagnosis of neonatal infections due to its early synthesis in embryonic life and its rapid estimation.

II. Microbiological Investigations

1. Blood Culture

A specific diagnosis of neonatal septicemia can be confirmed only by the presence of a positive blood culture, when septicemia is suspected, 2 or more blood cultures should be obtained. If the patient is critically ill one culture is adequate (*Feigin, et al.*, 1987).

2. Cerebrospinal Fluid Examination (CSF)

This should be performed in all babies in whom there is a remote possibility of meningitis (*Roberton*, 1992).

Up to 110 WBC/mm^3 have been reported as normal neonatal CSF. However in non-traumatic sample, PMNs count higher than 20/mm^3 should be regarded with suspicion and count above 30/mm^3 are strongly indicative of meningitis. When bloody CSF is obtained, the ratio of red cells to white cells should be calculated. In uninfected CSF this is usually >500 : 1 (*Roberton*, 1992).

The upper normal limit of CSF protein is 1.5–2.0 g/L in the term, and 3.7 g/L in the preterm baby. The levels are usually raised in meningitis.

The CSF glucose should be 50% or more of the blood glucose level, and a low level strongly suggests bacterial meningitis (*Rodriquez, et al.*, 1990).

3. Urine Culture

Urine is difficult to obtain, the best method is supra pubic aspiration. Infection should be considered present if there are more than 10 leucocytes/mm^3 in an uncentrifuged and well shaked specimen or when there are more than 10^5 colony forming units/ml of freshly passed urine (or suprapubic aspiration) on 2 consecutive samples (*Haque*, 1992).

4. Gastric Aspiration

Generally, if there are more than 5 neutrophils per HPF or a large number of bacteria particularly gram-positive cocci in clumps and chains, the test is positive. The low specificity of the test is not surprising since a positive aspirate reflects an infected intrauterine environment, not a fetal inflammatory response (*Guerina*, 1991).

Biochemical Studies

A. Blood Glucose Level

Hypoglycemia has been noted during septicemia with gram negative bacilli with much greater frequency than during infection with gram positive organisms.

Increased peripheral utilization of glucose, perhaps as a result of enhanced insulin sensitivity and lack of glycogen stores, has been suggested as a possible contributing factor for hypoglycemia during neonatal sepsis.

Decreased corticosteroids activity has not been documented even when adrenal hemorrhage is found postmortem (*Feigin et al.*, 1987).

B. Serum Electrolytes Level

Hyponatremia during septicemia probably reflects an appropriate retention of fluids in excess of solute, resulting in dilutional hyponatremia. It may also reflect changes in total body sodium concentration as a result of diarrhea, vomiting, excessive sweating, or insufficient intake.

Measurement of serum electrolytes should be made in infants with possible or proven septicemia, as the result obtained may support the diagnostic impression, have differential diagnostic value and are essential in designing specific regiments of supportive care for each infant (*Feigin et al.*, 1987).

C. Leukocyte Alkaline Phosphates Activity

Donates et al., (1979) found low leukocyte alkaline phosphates activity in all infected neonates they studied. They suggest that this test might be a helpful aid in diagnosing neonatal bacterial infections, and they explained this by a rapid release of functionally, immature neutrophils by the bone marrow.

Again, in a study by *Ismail et al.*, (1988) leukocyte alkaline phosphates activity showed highly significant low scores in patients with neonatal bacterial infections as compared to control group so it can be used as valuable supporting evidence to confirm the presence of neonatal bacterial infection.

D. Kidney and Liver Function Tests

Elevation of blood urea nitrogen (BUN), creatinine, bilirubin and liver enzymes may occur in the course of systemic candidiasis (*Gotoff*, 1992).

E. Blood Gases

Severely infected babies often have a metabolic acidosis. If the pH is below 7.2 and the deficit >10 mmol/L. This must provoke a search for a specific underlying cause such as anemia and hypertension and should be corrected (*Roberton*, 1992).

III. Immunodiagnosis

Isolation of the offending infectious agent in culture remains the mainstay of the diagnosis of infectious disease. However isolation techniques for certain pathogens are sometimes difficult or unsafe. In addition cultures may be negative because of prior antimicrobial therapy or immune complexes.

Under these circumstances, serologic methods can be used to detect specific or non specific host antibody response to the infectious agent and to demonstrate the presence of antigens of the infecting agent in the tissues or body fluids of the host (*Gaur et al.*, 1994).

1. Antigen Detection

Immunologic methods have been developed to detect the presence of antigenic components of various pathogens in patients' serum and other body fluids. This approach has been useful in diagnosis of bacterial infections caused by encapsulated bacteria (H. influenza type B, S. pneumonia, Nisseria meningitides, and GBS).

Counter current immunoelectrophoresis, latex particle agglutination, protein A coagulation, and enzyme immunoassay are the four techniques used for antigens (Ag) detection in CSF. In other body fluids especially urine, false-positive test results may occur due to cross-reactions with Ag from organisms of different species or even material contaminating the urine or perineal area. Serum also may contain non-specific cross-reacting antibodies (*Gaur et al.*, 1994).

2. Antibody Level

An elevated serum IgM concentration (greater than 1720 mg/dl) during the first week of life suggests that infection has occurred in utero or perhaps at birth.

However, infants dying from bacterial infection during the first several days of life may do so with normal IgM levels. Conversely, elevated serum IgM concentrations may occur in newborns who have never been infected, because of contamination with maternal blood (*Pachecoe et al.*, 1994).

3. C_3d Estimation

C_3d is the main metabolite of C_3 the third component of the complement. C_3d appears in the circulation wherever antigen antibody complexes are formed, or whenever bacterial endotoxins activate the alternate pathway.

So C_3d have been tested as a diagnostic marker for neonatal infection. Sensitivity was found 73.7%, specificity 83.6%, positive predictive value 94.8%.

C_3d qualitative test appears to be not only fairly cheap but also reliable in the diagnosis of neonatal bacterial infection (*Guillois et al.*, 1989).

4. Interleukin-6

The human cytokine interleukin-6 (IL-6) is a low molecular weight protein cell regulator produced by various cells including T and B cell, fibroblasts, keratinocytes and endothelial cells, but mainly by macrophages and monocytes.

Both mature and immature neonates were able to produce IL-6 in response to a severe life threatening nosocomial infection compared with a control population and with samples received after the recovery from the infections episode. Measurement of plasma IL-6 concentration might be useful for early diagnosis and monitoring of infection complications in neonate (*Groll*, 1992).

Reviewing the medical literature, IL-6 appears to be predictive of mortality. Patients with IL-6 level of >1000 pg/ml would be likely to be predictive of mortality (*Andrea*, 1995).

Sepsis Screen

Philip and Hewitt (1980), combined the results of five tests into sepsis screen (WBC less than 5000/mm^3, I : T >0.2, ESR greater than or equal 15 mm/hour, CRP and HPG positive).

The screen was considered positive if two or more tests were abnormal, which resulted in improved predictability for early onset of sepsis.

Sepsis Score

It includes both clinical and hematological assessment to identify neonatal sepsis.

Table (8): Sepsis score, examination of clinical and hematological symptoms in neonatal septicemia.

Index	Change	Score
1. Skin colouration	a. Pale, bright pink or pink. b. Little or inconstant discolouration. c. Pronounced and constant change towards dirty green grey skin colouration.	0 2 4
2. Microcirculation	a. Rapid filling of capillaries following pressure on the skin. b. Marbling of the skin after pressure on the skin, the pressure mark noticeable for one second. c. Sustained, seriously disturbed blood circulation of the skin, pressure marks on the skin remain noticeable for many seconds.	0 2 3
3. Metabolic acidosis	a. No, pH = 7.35-7.45 b. Slight acidosis, pH = 7.20-7.30 c. Pronounced acidosis, pH below 7.20	0 1 2
4. Heart rate	a. No abnormal change in the heart rate by monitor control b. Dropping of heart rate below 80/min with deterioration of general condition	0 1
5. Muscle tone and activity	a. Normal, corresponding to gestational age b. Hypotonic; tone reduced perhaps impaired spontaneous movement. c. Flappy, no muscle tone no spontaneous movement.	0 1 2
6. Apneic spells	a. Uninterrupted regular breathing b. Apneic spells of more than 20 second duration.	0 2

Table (8): Continued.

Index	Change	Score
7. Liver enlargement measured in the midclavicular line	a. 0-2 cm: normal size. b. 2-4 cm: moderate liver enlargement. c. More than 4 cm considerable liver enlargement.	0 0.5 1
8. Gastro-intestinal (GI) symptoms	a. No: GIT symptoms. b. Yes: one or more symptoms such as rising of stomach contents before next feeding, vomiting, abdominal distention and diarrhea.	0 1
9. White blood cell count (WBC)	a. Within the normal range for healthy newborn infants. b. Leucocytosis, above one standard deviation for healthy newborn infants. c. Leucocytopenia, below one standard deviation for healthy newborn infant.	0 1 2
10. Shift to the left in differential leucocytic count	a. No increase in proportion of band. b. Moderate up to 25% of band. c. Considerable more than 25% of band or appearance of immature forms of myelo- or erythropoiesis.	0 2 3
11. Platelet count	a. Thrombocytes more than 100,000/mm^3. b. Thrombocytopenia, thrombocytes less than 100,000/mm^3	0 2

(*Tollner*, 1982)

The interpretation of sepsis score can be done as follows:

1. 0-4.5 = No sepsis.
2. 5-10 = Observation range of sepsis.
3. More than 10 = Suspicion of sepsis (*Tollner*, 1982).

The sepsis score is helpful for early detection of septicemia and differential diagnosis in newborn infants (*Tollner*, 1982).

Radiological Investigations

Chest X-ray is a procedure which should be a part of the investigation of all babies with suspected sepsis, whether respiratory symptoms are present or not.

Abdominal X-ray if there is clinical evidence of intra-abdominal disease should be taken to exclude necrotizing enterocolitis (NEC).

Abdominal ultrasound may be valuable in NEC with intra-abdominal sepsis and in evaluating renal sepsis and intra-abdominal masses and fluid (*Roberton*, 1992).

Ultrasound of catheter-tip sites (to rule out infected thrombi), kidney and heart should be performed (*Samuel*, 1992).

Plain-film radiographs on the involved bone or joint in case of neonatal sepsis are valuable in establishing the diagnosis of osteomyelitis or arthritis (*Guerina*, 1991).

CT scan provides information about the degree of cerebral oedema occurrence and site of obstruction of CSF fluid, and the presence of major infarction (*Klein and Marcy*, 1995).

COMPLICATIONS OF NEONATAL SEPSIS

There are certain complications that may occur during the course of septicemia which require special attention because of the nature of the management problems which may present. These include meningitis, septic shock, adrenal insufficiency, DIC and hyponatremia (*Samuel*, 1992).

I. Focal infections

1. Meningitis

Meningitis is more common in the neonatal period than any other period of life, the exact incidence is not known but lies somewhere between 0.2–1.36/1000 live births (*Haque*, 1992).

There are no reliable symptoms to differentiate sepsis from meningitis in the neonate, such as a full fontanelle or nuchal rigidity. Both seizures (frequently focal and present in up to 75% of patients) and focal findings such as hemiparesis and horizontal eye deviation, are often associated with neonatal meningitis (*Holbrook*, 1993).

The neonates surviving after neonatal meningitis may show communicating or non-communicating hydrocephalus, subdural effusions, ventriculitis, deafness, and blindness (*Cole*, 1991).

2. Otitis Media

The incidence of otitis media is appreciable (0.6-2.4%) due to the shorter, widely patent and horizontally placed Eustachian tube. Predisposing factors are chorioamnionitis, asphyxia, prematurity, ventilatory support and infants with cleft palate or Down's syndrome (*Haque*, 1992).

3. Osteomyelitis and Septic Arthritis

During the first year of life, capillaries perforating the epiphyseal plate of the long bone provide communication between the joint space and the metaphysis.

Thus, septic arthritis and osteomyelitis often occur together. The source of infections is usually hematogenous but direct trauma during arterial/venous puncture has also been implicated as a cause. Organisms vary but the most predominant are Staph aureus followed by GBS and gram-negative organisms.

In the extremely preterm, LBW babies who are cared for in intensive care units, candida albicans is emerging as an important agent (*Haque*, 1992).

4. Necrotizing Enterocolitis

Bacteria play a major part in the pathogenesis and complication of necrotizing enterocolitis. Evidence to support this concept includes clustering of cases that my be interrupted by enforcement of infection control measures, positive blood cultures in 30 percent of affected infants at the onset of symptoms and identification of bacteria in the involved intestinal submucosal and muscularis layers (*Koslaske et al.*, 1978).

Seigel et al., (1981) found increased frequency of isolation of gram negative aerobic organisms (especially klebsiella pneumonia) from gastric and fecal material obtained from infants with necrotizing enterocolitis as compared with material from cohort neonates, and prevention of disease by administration of oral aminoglycosides to high risk infants. Formula feeding, intestinal ischemia, and low birth weight are

other factors that have been implicated in the pathogens of this syndrome.

II. Disseminated Intravascular Coagulation (DIC)

Abnormalities of the coagulation system may accompany bacteremia and other serious infections and may produce thrombocytopenia, purpura fulminans, symmetrical peripheral gangrene, and DIC (*Kreger et al.*, 1980).

III. Septic Shock

Shock is a syndrome which is produced by failure of the circulation of blood at the capillary blood vessels leading to tissue hypoxia, metabolic disorders and cellular death. When all these appear together with local or general infection, it is called septic shock (*Ellner*, 1983).

Changes observed early in the course of shock in gram negative bacteremia consist of:

1. Striking diminution in systemic vascular resistance.
2. Increased or occasionally normal to slightly decreased cardiac output.
3. Increased stroke volume.
4. Decreased central venous pressure.
5. Hyperventilation.
6. Lactate accumulation.

These findings are characteristic of what is sometimes termed ***warm shock*** (*McCabe and Olans*, 1981).

As shock progresses in gram-negative bacteremia, systemic vascular resistance increases, cardia output diminishes, and hyperventilation and further lactate accumulation develops, the classic findings of ***cold shock*** (*McCabe et al.*, 1983).

Hyponatraemia (Electrolyte Disturbances)

It may occur as a complication of severe septicemia and or meningitis. The altered electrolyte concentration is often symptomatic and served to normal without therapy once acute phase of the disease is over. The clinical signs of symptomatic hyponatremia, such as lethargy, irritability, tremors and convulsions are often indistinguishable from those of septicemia in the newborn infant.

Therefore serum electrolytes determination should be obtained in severe neonatal septicemia. The stress of infection may result in excessive release of antidiuretic hormone which leads to water retention and dilutional hyponatremia. It may also reflect changes in total body sodium concentration as a result of diarrhea, vomiting, excessive sweating or insufficient intake (*Feigin et al.,* 1987).

IV. Adrenal Insufficiency

Adrenal hemorrhage in severely ill infants with septicemia has been documented. In most cases of neonatal septicemia the adrenal glands will respond adequately and replacement therapy is not indicated. Nevertheless, adrenal insufficiency should be considered in patient with neonatal septicemia who has not responded to appropriate antibiotic therapy and has developed a shock like syndrome.

Serum sodium, potassium and glucose should be obtained to support this diagnosis and after the initial treatment for shock, adrenal corticoids should be continued for the presumptive diagnosis of adrenal failure (*Samuel,* 1992).

TREATMENT OF NEONATAL SEPSIS

Treatment of neonatal sepsis is more likely to be successful the earlier in the septic process that therapy is initiated (*Holbrook,* 1993).

The treatment of septicemia includes:

I. Standard therapy
   1. Antimicrobial therapy
   2. Supportive therapy

II. Adjunctive therapy
   1. Exchange transfusion
   2. Granulocyte transfusion
   3. Human IV immunoglobulins (IV Ig)
   4. Purified human fibronectin transfusion

III. Newer therapies for neonatal sepsis and septic shock
   1. Cytokines
   2. Venoarterial extracorporeal membrane oxygenation (VAECMO).
   3. High frequency ventilation techniques (HFV) (Jet or oscillator HFV)
   4. Surfactant therapy
   5. Heparin therapy

I. Standard Therapy

1. Antimicrobial Therapy

Initial treatment of suspected neonatal sepsis is determined by the pattern of disease and the organisms that are common for the age of the infant and flora of the nursery (*Gotoff,* 1992). Some of the antimicrobials commonly used in neonates are listed in table (9).

Table (9): Antibiotics Commonly Used in the Newborn

	First week of life or preterm			Term infant or more than 7 days old			
Drug	Dosage	Route	Frequency	Dosage	Route	Frequency	Comment
Amikacin	10–15 (mg/kg/day)	i.v./i.m.	8–12	22.5 (mg/kg/day)	i.v./i.m.	8	Infuse over 30 min.
Ampicillin	100 (mg/kg/day)	i.v./i.m.	8–12	150–300 (mg/kg/day)	i.v./i.m.	6–8	Use higher dose for meningitis
Cefotaxinic	100–150 (mg/kg/day)	i.v./i.m.	12	150–200 (mg/kg/day)	i.v.	6–8	Use higher dose for meningitis
Ceftazidime	50–100 (mg/kg/day)	i.v.	12	50–100 (mg/kg/day)	i.v.	12	
Ceftriaxone	50–100 (mg/kg/day)	i.v.	12–24	50–100 (mg/kg/day)	i.v.	12–24	
Erythromycin	40–60 (mg/kg/day)	i.v.	6	40–60 (mg/kg/day)	i.v./p.o.	6–8	Infuse over 60 min.
Gentamicin*	5 (mg/kg/day)	i.v./i.m.	12–18	7.5 (mg/kg/day)	i.v./i.m.	8–12	Infuse over 60 min.
Kanamycin*	15 (mg/kg/day)	i.v./i.m.	12–18	25 (mg/kg/day)	i.v./i.m.	8–12	
Methicillin	100 (mg/kg/day)	i.v./i.m.	8	200 (mg/kg/day)	i.v./i.m.	6–12	
Metronidazole	20 (mg/kg/day)	i.v.	8	20 (mg/kg/day)	i.v.	8	
Moxalactam	100 (mg/kg/day)	i.v./i.m.	12	100–200 (mg/kg/day)	i.v./i.m.	8–12	Do not use as a single drug in neonatal sepsis
Netilmicin*	5 (mg/kg/day)	i.v./i.m.	12	5 (mg/kg/day)	i.v./i.m.	12	
Nystatin	20,000–400,000 U	p.o. or local	6	200,000–400,000 U	p.o. or local	12	
Penicillin	30,000–50,000 U	i.v./i.m.	12	40,000–60,000 U	i.v./i.m.	8	
Tobramycin	4.5 (mg/kg/day)	i.v./i.m.	12	5.75 (mg/kg/day)	i.v./i.m.	6–8	Infuse over 30 min.
Vancomycin	20–40 (mg/kg/day)	i.v.	12	30–60 (mg/kg/day)	i.v.	6–8	Infuse over 60 min.

* Monitor concentration levels (From *McIntosh,* 1992)

The possibility that the cause of septic syndrome is due to herpes simplex dictates the addition of acyclovir, often empirically. If fungal infection is considered, then amphotericin is indicated (*Whitflied et al.,* 1993).

If neonatal infection is suspected, treatment should be initiated as soon as possible after specimens for diagnostic studied have been obtained. The choice of antibiotics must be based on the historical experience of the nursery, the antimicrobial susceptibility of bacteria recently isolated from both sick and healthy newborns, and the maternal history (*Guerina,* 1991).

The choice of antibiotics must be based on:

1. Consideration of the type of micro-organisms that may be encountered.
2. Sensitivity of the organism to the antibiotic employed
3. The likelihood of achieving bactericidal concentrations of antibiotics at the site of infection.
4. Consideration of possible adverse effects of therapy with specific antibiotic agents (*Feigin et al.,* 1987).

Roberton, (1992) started a cocktail used by most neonatologists which is a penicillin plus an aminoglycoside. In the UK, penicillin G and gentamycin are widely used for infants treated within 48-72 hours of birth, though where listeria is a problem ampicillin and gentamycin is preferred. Tobramycin, amikacin and netilmicin are acceptable alternatives to gentamycin.

After 48 hours of age when streptococcal infections became less common they usually give flucloxacillin instead of penicillin to cover staphylococci, especially staph

epidermis. If the staph epidermis in the unit is usually resistant to flucloxacillin, then vancomycin should be used.

If pseudomonas infections are present the add of antipseudomonas antibiotic to the cocktail usually ceftazidime if there is any suspicion of intra-abdominal disease, in particular NEC, metronidazole is added.

All these drugs should be given intravenously. There is no place for oral antibiotic therapy in serious neonatal infections and intramuscular injections can be hazardous in babies because of their small muscle bulk and the chance of nerve damage.

Feigin et al., (1987) said that when septicemia is suspected after 3 days of age, a penicillinase resistant penicillin should be added to the regimen. Methicillin is preferred because it is bound to protein to a much lesser extent than nafcillin or oxacillin, thus the risk of displacing bilirubin from albumin binding sites is reduced.

Ampicillin and gentamicin are generally recommended for empiric treatment of suspected serious neonatal bacterial infections but there are three exception: when one suspects a staphylococcal infection, when one suspects a resistant enteric bacillary infections during a nursery outbreak and when one has to retreat an infant who has already received prolonged treatment with ampicillin and gentamycin. For the latter infants, it would be prudent to initiate treatment with amikacin, to which many gentamicin resistant enteric bacilli are susceptible, and/or an extended spectrum penicillin or newer cephalosporin (*Jacobs*, 1990).

Third generation cephalosporins such as cefatoxine are valuable addition for treating documented neonatal sepsis and meningitis because:

1. The minimal inhibitory combinations for Gram negative enteric bacilli are much lower than for the aminoglycosides.
2. There is excellent penetration into C.S.F. in the presence of inflamed meninges and
3. Much higher doses than those of aminoglycoside can be given since toxicity is quite limited. The end result is much higher bactericidal titer in serum and C.S.F. than are achievable with ampicillin/aminoglycoside combination (*Gotoff*, 1992).

Most anaerobic bacteria, although resistant to the aminoglycosides are susceptible to penicillin and ampicillin. The notable exceptions are strains of bacilli fragilis which are usually susceptible to metronidazole, clindamycin and some of the newer B lactams especially imipenem and sulbactam.

The antibiotic therapy must be reviewed once the cultures and sensitivities are back from the laboratory 48–72 hours after the baby first become ill (*Pursley*, 1994).

Duration of Therapy

Once antibiotics are given they should be continued for at least 48 hours, when the results of the cultures and clinical course of the patients can be reviewed.

If cultures are negative the baby is well and on review it appears that he was not infected, then the antibiotics should be stopped.

If cultures are negative, the baby has improved, yet as other diagnosis has been established, and on review infection is still possible despite negative cultures, then therapy should probably be continued for a minimum of 5–7 days.

For infections such as pneumonia, urinary tract infections or NEC, intravenous antibiotics should be maintained for at least 10 days, increasing to 14 days if the blood culture is positive.

Meningitis should also be treated with parenteral antibiotics for at least 14 days in the case of GBS and listeria, rising to at least 21 days for most other organisms, including coliforms (*Roberton,* 1992).

Monitoring Antibiotic Therapy

Whenever potentially toxic antibiotics such as aminoglycosides or chloramphenicol are used, the plasma levels of antibiotics must be measured two or three times per week to check whether adequate therapeutic levels are being achieved.

However, with third generation cephalosporins because the difference between the toxic and therapeutic level is so wide, therapeutic drug monitoring is not necessary (*Delovois and Harvey,* 1988).

Steroid Therapy

The use of steroid in management of neonatal sepsis remains controversial. Only future studies with sufficient adherence to sound principle of clinical trail design can resolve the controversy surrounding the use of corticosteroids as an adjunctive therapy in bacterial infections (*Kreger et al.,* 1980).

Corticosteroid Administration

Many corticosteroid regimens were described in the 32 paper reviewed in table (10). The different studies will not be compared here. However numerous studies lacked consistency in details of corticosteroid administration.

Table (10): Details of corticosteroid administration*.

Study	Drug	Dosage (mg/day)	Route	Duration (days)	Initiation†
Hahn et al.	C	VAR	im	5	1–2
Jahn et al.	VAR	‡	VAR	VAR	VAR
Magil et al.	C	300/100 or100/50§	NS	3–7 21	VAR
Wisseman et al.	C	300/100 or 500	po	4 1	VAR
Kinsell and Jahn	VAR	‡	VAR	‡	NS
Hall and Gold	VAR	NS	NS	NS	NS
Henegar et al.	VAR	NS	VAR	NS	NS
Wagner et al.	HC	80/10	po	5	NS
Lepper and Spies	VAR	VAR	VAR	NS	VAR
Cassidy	HC,C	NS	VAR	NS	NS
Lepper and Spies	HC A	250 100	iv im	5 2	VAR
Peerman and McGanity	C	300/100	NS	VAR	NS
Weil and Spink	VAR	NS	NS	NS	NS
Altemeier and Cole	VAR	NS	NS	VAR	VAR
Ribble and Braude	VAR	VAR	VAR	VAR	VAR
Lepper and Spies	HC A	250¶ 100¶	iv im	5 2	•• ••
Spink	VAR	VAR	VAR	VAR	VAR
McCabe and Jackson	NS	VAR	NS	NS	VAR
Bennett et al.	HC	300/0	iv po	2 4	NS
Weil et al.	HC	<300 or 300>	iv	2+	NS
Bodey et al.	P	NS	po	NS	NS
Breen	NS	NS	NS	NS	NS

Table (10): Continued

Study	Drug	Dosage (mg/day)	Route	Duration (days)	Initiation†
Melnick and Litvak	HC	100–400 or 400–1000	NS	NS	NS
Weiss et al.	VAR	VAR	NS	VAR	VAR
Cavanagh et al.	D	VAR	iv	VAR	NS
Blair et al.	NS	NS	NS	NS	NS
deLemos and Haggerty	M	160	iv	3	NS
Jensen et al.	HC P	100 40	im po	7+	•• ••
Kanski	VAR	VAR	VAR	VAR	NS
Motsay et al.	VAR	NS	NS	NS	NS
Klastersky et al.	B	1/kg	iv	3	NS
Christy	NS	VAR	NS	NS	NS

* Abbreviations: C, cortisone; HC, hydrocortisone; B, betamethasone; A, ACTH; P, prednisone; M, methylprednisolone; D, dexamethasone; VAR, variable; NS, not stated or not stated in all instances; iv, intravenously; po, by mouth; im, intramuscularly.

† Denotes day of illness on which therapy was initiated.

‡ Sample regimen given in introduction to study.

§ Slash denotes progression of dosage regimen.

¶ Adult dosage.

••Tabulated, but not specifically controlled for.

(*Weitzman and Berger*, 1989)

Corticosteroid preparations vary with respect to actions, toxicity, metabolism, distribution and particularly, effect on inflammation and host response to infection (*Weitzman and Berger*, 1985).

Routine administration of dexamethasone is recommended by some authorities although its efficacy requires further clarification. If dexamethasone is to be used it should be administered as soon as the first dose of antibiotics administered the regimen of dexamethasone suggested is 0.15 mg/kg/dose every 6 hours for the first 4 days (16 doses).

One must be certain to discontinue therapy at this time or as soon as bacterial infections is no longer a consideration. It is common for fever to recur once dexamethasone therapy is discontinued (*Sheagren*, 1985).

Mechanism of Action

Cytokines are thought to mediate many host responses to bacterial infection. Tumor necrosis factor and IL-1 play a central role in many responses to infections.

Corticosteroids therapy can dramatically improve the course of inflammatory events in bacterial sepsis (*Beulter and Cerami*, 1987).

Dexamethasone significantly reduced both TNFα concentration and indexes of inflammations and concentration of interleukin-1 and prostaglandin E_2. It was chosen as adjunctive therapy in infant and children with sepsis in attempt to reduce the inflammatory response and to improve long term outcome (*Baker*, 1988).

The indexes of inflammation and concentration of cytokines in blood improved rapidly within 12 hrs as

compared with the same measures in patient given only antibiotics (*Beulter et al.*, 1988).

Potential benefit of corticosteroids therapy are cell membrane and lysosomal stabilization inhibition of complement induced granulocyte aggregation, prevention of O_2 radical mediated cell damage and inhibition of ACTH release (*Stubitz and Metal*, 1981).

Side Effects

Potential adverse effects include superinfection, electrolyte disturbances, hyperglycemia, arrhythmia and gastrointestinal bleeding.

Upper GIT bleeding in any patient was due to the stress resulting from sepsis rather than due to administration of steroid. However if bleeding occurred within 12 hrs of steroid administration, the hemorrhage was considered to be due to steroid (*Melby*, 1974).

Supportive Therapy

Maintenance of fluid and electrolyte balance as well as acid-base balance, is important in the management of neonatal sepsis. Hypoglycemia should be treated promptly. Hyperbilirubinemia should be monitored and treated with exchange transfusion since the risk of kernicterus increases in presence of sepsis. Parenteral nutrition should be considered for infants who do not sustain feeding (*Gotoff*, 1992).

a. Monitoring

The septicemic neonate requires all the neonatal intensive care monitoring: blood pressure, ECG, respiration and temperature continuously and his fluid input and urinary output must be accurately monitored because of the high risk

of renal failure. He should have his blood count, electrolytes, calcium, glucose and albumin checked daily, coagulation studies, cultures and X-rays should be repeated as necessary. Several hematocrit and blood gas estimation per day may be required (*Roberton,* 1992).

b. Temperature Control

The use of servo-controlled incubators is probably contraindicated in septic neonates, because pyrexia will turn off the heater exposing the body to thermal stress. Conversely, if despite pyrexia, the infant is peripherally vasoconstricted with a cool skin temperature, this may increase the heat power considerably and result in hyperthermia.

If the baby is hypothermic he should be maintained in an environmental temperature at the top end of or just above, his thermoneutral range (*Roberton,* 1992).

c. Blood Pressure (Hypotension)

Shock may be prominent feature particularly in Gram negative septicemia, and should be treated with whole blood or plasma as appropriate, if this doesn't increase the blood pressure, dopamine should be given, starting at 5 μg/kg/min., as myocardial sensitivity to isotopes is maintained or even increased in sepsis (*McDonough,* 1988).

d. Fluid Therapy

Urine output must be monitored carefully as acute renal failure and in appropriate secretion of anti-diuretic hormone (ADH) are not uncommon in septic neonates, particularly those with meningitis and pneumonia.

Hyponatremia should be treated by fluid restriction if sodium losses are excessive, sufficient sodium should be given to relieve symptoms of hyponatremia (*Feigin et al.*, 1987).

Sodium and potassium may be lost in large amounts in NEC, gastroenteritis and the diuretic phase of acute tubular necrosis should be replaced. Hypoglycemia is a common finding in septicemia, the blood glucose should, therefore, be checked two or three times per day at kept ≥1.2–2 mmol/L.

In septicemia with peripheral vasoconstriction, capillary samples must always be taken from an artery. Metabolic acidemia (pH <7.20, base excess >-10 mmol/L) is common in severe early onset sepsis and should be corrected (*Roberton,* 1992).

e. Respiratory Support

Many babies, in particular those with GBS sepsis, will become apnoeic when infected. In babies, in particular those with NEC, the abdominal distention may splint the diaphragm and compromise respiration and PPHN may complicate any severe early onset septicemia in all these situation IPPV will be required (*Roberton,* 1992).

f. Haemorrhagic Diathesis

Treating the underlying infection and metabolic problems will often result in improved homeostasis but if it doesn't, platelet and fresh frozen plasma (FFP) transfusion will be necessary. Exchange transfusion corrects the bleeding by simultaneously washing out coagulation inhibitors including fibrin degradation products, as well as by supplying missing coagulation factors (*Roberton,* 1992).

g. Nutrition

Infected babies are hypercatabolic but rarely tolerate enteral feeds because of an ileus, NEC or gastroenteritis. Intravenous feeding, in particular the use of intralipid is also poorly tolerated in early sepsis.

For this reason I.V. dextrose 10%, which gives some calories, should be used for the first 24–48 hours, after which a low concentration of intravenous Vamin can usually be given safely. More complete parenteral or even enteral nutrition should be started as soon as there is improvement in the baby's condition (*Roberton*, 1992).

II. Adjunctive Therapy

1. Exchange Transfusion

The use of exchange transfusion as an adjunctive therapy for several neonatal septicemia was widely used, it helped to remove bacterial endotoxins, improved peripheral and pulmonary perfusion and enhanced humoral and cellular immunity in the infected newborn infant (*Baley*, 1988).

The following are the main indications of exchange transfusion in neonatal sepsis:

- Septicemia with DIC.
- Septicemia with sclerema.
- Septicemia with significant neutropenia when granulocyte or buffy coat transfusion are lacking (*Feigin et al.*, 1987).

2. Granulocyte Transfusion

The rationale for WBC transfusion as therapy for neonatal sepsis included the common occurrence of neutropenia in septic neonates.

The ability of transfused WBCs to migrate to the site of inflammation and phagocytose invading organisms, the low probability of survival of neutropenic septic neonates and the disturbed PMNs function in newborns. It is given as a transfusion of 20 ml/kg of a preparation obtained by continuous flow filtration leucopheresis of ABO/Rh compatible blood and containing WBC (*Yoder and Polin,* 1986).

3. Human I.V. Immunoglobulins (IV Ig)

Recently pooled human Igs have been used IV both for prevention and treatment of neonatal sepsis. (IVIg) are known to stimulate PMNs release into the tissues, so increasing the number of Ag presenting cells and potentiating the action of complement (*McIntosh,* 1992).

IVIgs have reduced the mortality from neonatal sepsis when used in conjunction with antibiotics. Currently, the prophylactic dose is 100-200 mg/kg infused over 2–3 hours within the first hours of birth to infants weighing 1500 gms or less. For the treatment of sepsis, it is infused over 2–3 hours in a dosage of 200 mg/kg/day given once every 24 hours for 4 days (*Berger,* 1991).

4. Purified Human Fibronectin Transfusion

Fibronectin, a glycoprotein found in plasma and tissues, contributes to adhesion of neutrophils and monocytes and binds to certain bacteria such as GBS with or without Ab. It is diminished in premature newborns and newborns with sepsis, birth asphyxia and RDS.

Recently it is proposed to administrate purified human fibronectin as an additional immunotherapy for infected neonates (*Mills*, 1995).

III. Newer Therapies for Neonatal Sepsis and Septic Shock

1. Cytokines

The recent use of cytokines to enhance host defense mechanisms in the adult has suggested a role for this new form of immunotherapy in the newborn. Several studies over the past years have indicated that a number of these cytokines may enhance neonatal myeloid progenitor proliferation, modulate neonatal B.M. neutrophil storage and proliferation pools induce peripheral neutrophilia and protect against the high mortality associated with experimental bacterial sepsis to enhance neonatal host defense against overwhelming bacterial infection (*Mitchell*, 1991).

These cytokines include the following:

a. Granulocyte-monocyte colony stimulating factor (GM-CSF)

It is a small glycoprotein acts at an early B.M. progenitor stage and influences the growth and proliferation of CFU-GM and CFU-eosinophil resulting in an increase in granulocytes, macrophage and eosinophils in the peripheral blood (*Mitchell*, 1991, *Wheeler and Givner*, 1992).

Recombinant human GM-CSF, administered in animals with bacteremia, improved the outcome (*Givner and Nagaraj*, 1993).

b. Granulocyte-colony stimulating factor (G-CSF)

Human G-CSF is a low molecular weight glycoprotein (*Gabrilove and Jakubowski,* 1989).

G-CSF like GM-CSF promotes and enhances mature effector neutrophil function (*Mitchell,* 1991).

Administration of G-CSF (5 μg/kg) in newborn with GBS, induced a significant peripheral neutrophilia and increased the size of the neutrophil storage and proliferative pool G-CSF therapy significantly reduces the mortality rate during GBS sepsis in newborn, and this therapeutic effect appears to be synergistic with antibiotic therapy (*Cairo,* 1991).

Animal study suggested that G-CSF treatment prevent not only bacterial infections, but also endotoxic organ failure. We extended these studies on healthy human volunteers treated with G-CSF. Whole blood from these subjects was stimulated in vivo with Gram negative or positive initiations of cytokine release.

The result indicate that in blood from G-CSF treated donors a shift from the release of proinflammatory to the release of anti inflammatory mediators had occurred and this encourage use of G-CSF as a prophylaxis patients prone to sepsis (*Wendel,* 1995).

c. Monocyte-colony stimulating factor (M-CSF)

Is a large glycoprotein that stimulates CFU-M resulting in increased levels of circulating monocytes and an enhancement of mature effector monocyte function. M-CSF enhances Ab dependent cellular toxicity, monocyte oxidative metabolism, and other physiologic functions (*Mitchell,* 1991).

d. Monoclonal Antibodies Against TNFα

New studies reveals that patient with IL-6 levels of >1000 pg/ml would be likely benefit from treatment with monoclonal antibodies against TNFα (*Andrea,* 1995).

e. Other Cytokines

- IL-6: appears to induce differentiation of B-cell, lymphocytes, enhances Ig secretion by B-cell stimulates hepatocytes to produce fibrinogen, stimulate natural killer cell activity and enhances differentiation of cytotoxic T-cell (*Mitchell,* 1991).
- IL-3: stimulates the growth and proliferation of relatively early B.M. progenitor cells and appears to affect almost all of the hematopoietic lineages. It also stimulates monocyte Ab-dependent cellular cytotoxicity (*Cannistra et al.,* 1988).
- γ-INF: it is an antiviral glycoprotein produced by T lymphocytes with the aid of monocytes has been demonstrated to enhance neutrophil superoxide anion production, phagocytic activity, fungicidal activity, Ab dependent cytotoxicity and expression of chemotactic receptors. It is a major stimulus of G-CSF production. γ-INF enhance neonatal host defense against listeria monocytogenes (*Andrea,* 1992).

2. Veno-Arterial Extra Corporeal Membrane Oxygenation (VAECMO)

VAECMO can be a very useful form of rescue therapy for the neonate with fulminant septic shock unresponsive to the mentioned discussed conventional treatment modalities (*Whitfield,* 1993).

3. High-Frequency Ventilations (HFV) Techniques (Jet or Oscillator HFV)

These techniques have not been studied systematically in the septic shock. The experience with HFV suggests that in some neonates, when hypocapnic alkalosis is not attainable with conventional time cycled, pressure-limited ventilation techniques, hypocapnia can be achieved at lower mean airway pressure with HFV and is often associated with improved oxygenation (*Carter et al.*, 1990).

Others have reported a decreased need to VAECMO rescue therapy in patients who have received HFV trials, even when they meet accepted ECMO criteria (*Whitfield*, 1993).

4. Surfactant Therapy

The evidence that prophylactic or rescue administration of various surfactant is beneficial in the survival of infants with primary surfactant deficiency is excellent (*Kending et al.*, 1991).

The use of this therapy in the neonate with secondary surfactant deficiency states, as can occur in septic shock remains to be studied (*Dohyns*, 1993).

5. Heparin

The use of heparin by continuous infusion (10–20 units/kg/hrs) has been recommended as a prophylactic anticoagulant in the seriously ill neonate with sepsis and associated disseminated intravascular coagulation.

Some evidence exist that heparin interferes with bacterial opsonization by complement (*Cairo*, 1992).

PREVENTION OF NEONATAL SEPSIS

This can be achieved through:

I. Prevention and Control of Predisposing Risk Factors

A. Maternal prophylaxis

Aggressive obstetrical management of suspected maternal chorioamnionitis with antibiotics and rapid delivery of the newborn infants appear to have decreased the morbidity and mortality of early onset neonatal sepsis (*Gibbs et al.*, 1988).

Another approach to maternal prophylaxis is to immunize women to GBS so that high titre Ab will reach the fetus (*Roberton*, 1992).

B. Neonatal prophylaxis

Prophylactic penicillin given to all babies or just those less than 2 kg in weight was found to cause significant reduction of the incidence of GBS sepsis (*Ralph et al.*, 1987 and *Roberton*, 1992).

II. Encouraging Breast Feeding

III. Prevention of Nosocomial Neonatal Sepsis

The frequent indiscriminate use of systemic antibiotics in NICU and nursery which lead to the selection of resistant gram-negative organisms should be avoided (*Bennet et al.*, 1982).

Prevention of nosocomial infection is based on appropriate design of nurseries and following the policies of neonatal care published by the American Academy of Pediatrics. Careful hand washing, the use of gloves, bathing

infants with hexachlorophene containing soap to decrease skin colonization with staphylococci and umbilical disinfection (*Gotoff*, 1992).

Immunoprophylaxis

The prophylactic use of IVIg for high risk newborns has been shown to decrease the incidence of neonatal sepsis (*Christensen et al.*, 1984 *and Haque et al.*, 1986).

Morbidity and Mortality

Babies with neonatal septicemia often have multi addition problems, they may develop BPD secondary to hyper-ventilation, or they may have an intracranial hemorrhage or periventricular leucomalacia.

However, unless they develop one of these serious complication, sequelae are rare, particularly in term babies the overall figure for the mortality of blood culture proven neonatal sepsis was quoted at 25–50% in late 1970 (*Hill and Christensen*, 1988).

Mortality from neonatal septicemia in Ain Shams University Hospital both pediatric and obstetric rate was 42.9% in 1993 (*Amal et al.*, 1994).

TUMOR NECROSIS FACTOR-α (TNFα)

Cytokines

The term cytokines was initially given to a group of potent polypeptides that were later subdivided into two broad functional groups. Polypeptides with a primary regulatory action on mature cells were called cytokines, with the additional distinction that lymphokines were made by lymphocytes (for example interleukin 2) and monokines by monocytes (for example interleukin 1).

The other group were characterized by their ability to support the proliferation and differentiation of immature precursor cells and were labelled growth factors (for example, fibroblast growth factor).

More than 30 peptides are currently recognized as cytokines, including tumor necrosis factor-α (*Windebank*, 1990).

They form a network divided into five broad groups:

1. Interferons (IFNα, β and δ) recognized by their ability to protect cells from viral infections.
2. Interleukins (IL: from 1 to 10) they have diverse immunoregulatory functions.
3. Hemopoietic colony stimulating factors : they are peptides who are structurally dissimilar, but functionally related in controlling hemopoiesis.
4. Tumor necrosis factors (TNFα and β): these are structurally related proteins with cytotoxic activity in vivo.
5. Assorted growth factors: for example, platelet derived growth factor and colony stimulating growth factor, named according to the cell system in which their activity was discovered (*Windebank*, 1990).

Table (11): Characteristic Properties of Cytokines

Cytokine	MW	Principal Cell Sources	Primary Type of Activity	Preeminent Effects
IL-1	17,500	Macrophages & others	Immuno-augmentation	Inflammatory & hematopoietic
IL-2	15,500	T lymphocytes & LGL	T & B cell growth factor	Activates T & NK cells
IL-3	14,000-28,000	T lymphocytes	Hematopoietic growth factor	Promotes growth of early myeloid progenitor cells
IL-4	20,000	TH cells	T & B cell growth factor promotes IgE reactions	Promotes IgE switch & mast cell growth
IL-5	18,000	TH cells	Stimulates B cells & eosinophils	Promotes IgA switch & eosinophilia
IL-6	22,000-30,000	Fibroblasts	Hybridoma growth factor, augments inflammation	Growth factor for B cells & polyclonal immuno-globulin production
IL-7		Stromal cells	Lymphopoietin	Generates pre B & pre T cells & is lymphocyte growth factor
IL-8	8,800	Macrophages & others	Chemoattractants neutrophils & T lymphocytes	Regulates lymphocyte homing & neutrophil infiltration
G-CSF	18,000-22,000	Monocytes & others	Myeloid growth factor	Generates neutrophages
M-CSF	18,000-26,000	T cell & others	Macrophage growth factor	Generates macrophages
GM-CSF	14,000-38,000	T cell & others	Monomyelocytic growth factor	Myelopoietic
IFN-α	18,000-20,000	Leukocytes	Antiviral, anti-proliferative, & immunomodulating	Stimulates macrophages & NK cell
IFN-β	25,000	Fibroblasts		
IFN-γ	20,000-25,000	T lymphocytes & NK cells		Induce cell membrane antigens (e.g. MHC)
TNF-α	17,000	Macrophages & others	Inflammatory Immunoenhancing & tumoricidal	Vascular thromboses & tumor necrosis
LT=TNF-β	18,000	T lymphocytes		
TGF-β	25,000	Platelets, bone, & others	Fibroplasia & immunosuppression	Wound healing & bone remodeling

(*Oppenheim*, 1991)

Functions of Cytokines

Cytokines control both specific and non-specific aspects of normal growth, inflammatory repair, and immune responses throughout the body with up and down regulation (*Windebank*, 1990).

They function as intercellular signals that regulate local, and at times, systemic inflammatory responses. They are produced by a number of cells rather than by specialized glands. They are not usually present in serum and act in paracrine (i.e. locally near the producing cells) or autocrine (i.e. directly on the producing cells) rather than in an endocrine manner on distant target cells (*Oppenheim et al.*, 1991).

A single purified cytokine can have multiple effects on the growth and differentiation of many cell types. Consequently, cytokines may exhibit considerable overlap in their biologic effects on lymphoid, myeloid, and connective tissue target cells (*Oppenheim et al.*, 1991).

Cytokines, in turn, regulate each other by competition, interaction, and mutual induction in a series of lymphokine cascades and circuits with positive or negative feedback effects. For example, cytokines such as IL-1 and IL-2 induce the production of other cytokines such as TNF and interferon. Furthermore, IL-1 and IL-2 induce each other reciprocally.

Recombinant DNA technology has provided virtually unlimited supplies of highly purified cytokines, permitting the clinical evaluation of these substances in the absence of biologically active contaminants.

Most cytokines have been studied to determine their safety, their immunomodulatory activity in specific clinical situations and to define treatment regimens (*Quesenberry*, 1992).

Cachectin/TNFα

Tumor necrosis factor (TNF) is name given to a factor that appears in the serum of affected animals during the acute phase of an inflammatory response (*Kriegler et al.*, 1988).

TNF was first identified in the serum of mice challenged with endotoxin after BCG inoculation. Infusions of serum containing TNF into untreated mice induced hemorrhagic necrosis of transplanted subcutaneous tumours.

The purified TNF protein was also found to be cytotoxic or sytostatic for a number of human and murine tumor cell lines in vitro and in vivo (*Spriggs et al.*, 1988).

The availability of such in vitro assays for TNF lead to the isolation, purification and subsequent molecular cloning of the gene encoding TNF (*Kriegler et al.*, 1988).

Around the same time, another factor was discovered and named cachectin recognized as a circulating mediator of wasting during parasitic diseases (*Oppenheim et al.*, 1991).

TNF consists of 2 distinct peptides (α and β) with multiple immunologic and local as well as systemic inflammatory activities (*Oppenheim et al.*, 1991).

TNFα is produced by activated macrophages and other cells as monocytes, lymphocytes and natural killing cells. It has a broad spectrum of biologic actions on many immune and non-immune target cells.

Lymphotoxin (LT) which is primarily a product of T-lymphocytes, has been called TNFβ. Both TNFα and TNFβ bind to the same receptors on target cells and consequently have the same biologic activities (*Boride*, 1995).

Early in 1985a, *Beutler et al.*, noted that the amino terminal sequence of mouse cachectin was strongly homologous to that reported for human TNFα.

It was also observed that cachectin and TNFα possessed an identical spectrum of bioactivities and were immunologically indistinguishable, suggesting that they were in fact the same molecule. This assumption was soon confirmed by genetic sequence analysis (*Caput et al.*, 1986).

Biochemistry of TNFα

Two forms of TNFα are produced : a 17-KD secreted form and a 26-KD membrane associated form. Although it has been suggested that the membrane - bound form may act primarily as a mediator of local (paracrine) tissue effects, and is processed to the 17-KD circulating form, its mechanism of action is uncertain (*Tracey and Cerami,* 1992).

In man, TNFα and lymphotoxin are encoded by separate genes on the short arm of chromosome 6 (*Tracey et al.*, 1989).

The degree of homology between TNFα and TNFβ is 46% at the nucleotide level and 28% at the amino-acid level. The precursor form of TNFα consists of 236 amino-acids. Prior to or during secretion 79 N-terminal amino-acids are enzymatically removed from the pro-form to yield the soluble mature TNFα of molecular weight 17,400 (*Oppenheim et al.*, 1991).

The biological functions of TNFα also overlap with those of another 17-KD cytokine, Interleukin-1 (IL-1), but the molecules are structurally different and do not compete for a common receptor (*Tracey et al.*, 1988).

High affinity membrane receptors for TNF are present in a variety of tissues (most notably in the liver, kidney, spleen, lung, muscles, endothelium and intestine) and mediate maximal cellular responses even with low receptor occupancy.

Two different TNF receptors have recently been identified and sequenced and their genes cloned (*Tracey and Cerami*, 1992).

Table (12): Properties of Human TNF.

Property	TNF	
	TNF-α	TNF-β
Chromosome	6	6
Pro-form	236 amino acids	204 amino acids
Mature form	157 amino acids	171 amino acids
Cell sources	Macrophages, T and B lymphocytes, keratinocytes, fibroblasts, endothelial	T lymphocytes (TH1 subset), EBV B cell lines
Receptor	80-KDa glycoprotein KD = 10^{-10} mol/L 1000-10,000 sites/cell	The same for TNF-α
In vivo effects	Local neutrophilic infiltration Schwartzman reaction and necrosis of tumors Endogenous pyrogen Acute-phase reactants Cachexia, neutrophilia Radioprotection Adjuvant Angiogenesis	The same for TNF-α

(*Oppenheim et al.*, 1991)

Biosynthesis of TNFα

TNFα is synthesized by various activated phagocytic and non phagocytic cells, including macrophages/monocytes, lymphocytes, natural killer cells, astrocytes and microglial cells of the brain, and Kupffer cells of the liver (*Tracey et al.*, 1989).

A pivotal role in inflammation is suggested by the wide variety of infections or inflammatory stimuli capable of triggering TNFα biosynthesis including bacterial endotoxins/ lipopolysaccharide (LPS), enterotoxin, toxic shock syndrome toxin-1, mycobacterial cord factor, viruses, complement 5a, fungal or parasitic antigens, IL-1, and in an autocrine manner TNFα itself (*Hesse et al.*, 1988).

In response to LPS, both transcription and translation of the TNFα precursor are increased and large amounts of mature protein are released within minutes (*Tracey et al.*, 1989).

A number of endogenous mediators are also active inducers of TNFα such as IL-3, GM-CSF (granulocyte macrophage-colony stimulating factor), leukotriene β_4, platelet activating factor (PAF) and interferon-δ (IFNδ) in conjunction with Lipopolysaccharide (LPS) for macrophages (*Oppenheim et al.*, 1991).

By contrast, dexamethasone inhibits TNFα biosynthesis, but this effect is not observed if the steroid is given after the cells have been exposed to LPS. Thus, the up or down regulation of TNFα biosynthesis by IFNδ and dexamethasone, respectively, probably contributes to the pro-inflammatory or anti-inflammatory effects of these mediators (*Beutler et al.*, 1986).

Biological Effects of TNF-α

The polypeptide hormone tumour necrosis factor-α (TNF-α) is a primary mediator in the pathogenesis of infection, injury and inflammation, and in the beneficial processes of host defence and tissue homeostasis (*Tracey et al.*, 1988).

Depending on its concentration, duration of cell exposure, and presence of other mediators in the cellular environment, the net biological effects of this peptide regulatory factor may ultimately benefit or injure the host.

Thus, acute systemic release of TNF-α causes septic shock and tissue injury while persisting TNF-α production provokes cachexia, and these sequelae are synergistically influenced by other mediators including interleukin-1 (IL-1) and interferon-δ (IFN-δ).

When lesser amounts are released in tissues, the beneficial effects may predominate to mediate enhanced host defence against pathogens and to coordinate normal tissue remodeling (*Tracey et al.*, 1989).

Thus, the various biological activities of TNF-α may be classified into (*Beutler and Cerami*, 1987) :

1. Anti-tumoral and growth regulatory activities : TNF-α displays a selective toxicity for tumoral and virus-infected cells. Conversely, it is angiogenic and stimulates the growth of cultured fibroblasts.
2. Immunomodulatory and pro-inflammatory activities : TNF-α activates macrophages, neutrophils and eosinophils, as well as endothelial cells (which display procoagulant activity). It regulates the production of antibodies by B-cells and stimulates cytotoxic T-cells. It induces the production of several other inflammatory mediators such as

IL-1, IL-6, colony stimulating factors, prostaglandins, platelet activating factor (PAF) and collagenase.

3. Metabolic activities : TNF-α strongly inhibits lipoprotein lipase and adipocyte gene expression.

TNF-α as an Inflammatory Mediator

TNF-α has emerged as a mediator of general inflammation, and a variety of observations suggest that the molecule may play an important part in diverse human disease processes.

TNF-α is an endogenous pyrogen, capable of inducing fever both through a direct effect on hypothalamic neurones and through the peripheral induction of IL-1, which in turn elicit fever.

Hence, administration of LPS-free preparations of TNF-α to rabbits evokes a biphasic febrile response. The initial rise in temperature is attributable to the direct effect of the hormone, whereas the second rise results from IL-1 release (*Dinarello et al.*, 1986).

Table (13): Mediators Stimulated by Cachectin/TNF-α

Peptide regulatory factors	• IL-1 • IL-6 • Cachectin/TNF-α
Eicosanoids	• Prostaglandins • Leukotrienes • Platelet activating factor
Hormones	• Corticotropin/Cortisol • Adrenaline • Noradrenaline • Glucagon

(*Tracey et al.*, 1989)

Table (14): Biological Effects of Tumor Necrosis Factor-α.

Acute, high dose
Shock and death
Tissue injury with multiple organ system failure
Fever
Respiratory arrest
Pulmonary edema and ARDS
Hemorrhagic necrosis and capillary thrombosis
Capillary leak syndrome
Lactic acidosis
Hyperglycemia followed by hypoglycemia
Catabolic hormone release
Platelet activating factor and eicosanoid release
Chronic, low dose
Anorexia
Weight loss
Anemia
Depletion of body protein and lipid
Hypertriglyceridemia and hyperaminoacidemia
Increased whole-body energy expenditure
Acute-phase protein biosynthesis
Neutrophilia
Catabolic hormone release
Euthyroid-sick syndrome
Local tissue production
Fibroblast growth factor
Angiogenesis factor
Stimulation of growth factors (PDGF, GM-CSF, TGF)
Induces collagenases and elastases
Chemoattractant
Superoxide radical release
Localization of infection
Increases macrophage cytotoxicity against fungus and parasites

(*Tracey and Cerami*, 1992)

In addition, it enhances chemotaxis of macrophages and neutrophils, increase their phagocytic and cytotoxic activity, and promotes leucostasis by inducing increased expression of intercellular-leucocyte adhesion molecules and endothelial-leucocyte adhesion molecules at sites of inflammation. Moreover, it may function in both walling off of early infections and as anti-viral mediator (*Mestan et al.*, 1988).

TNF-α also exhibits osteoclast-activating factor activity and like IL-1, TNF-α is capable of stimulating synovial cell production of prostaglandin E_2 and collagenase (*Dayer et al.*, 1985).

Numerous inflammatory disorders of diverse origins may depend on the production of TNF-α, with all its attendant consequences. For example, excessive production of collagenase and prostaglandin E2 production may lead to the loss of bone and cartilage in rheumatoid arthritis, this entire process may depend in part on the production of TNF-α at a local level.

Similarly, inflammatory diseases of the central nervous system, gastrointestinal tract, lungs, kidneys, and other tissues may depend on TNF-α release (*Beutler and Cerami,* 1987).

Beneficial Functions of TNF-α

It has been shown that sublethal quantities of TNFα are capable of protecting mice from challenge with an otherwise lethal inoculum of Plasmodium berghei.

It has also been shown that the hormone exerts an anti-viral effect in vitro. In addition, C3H/HeJ mice, which can not produce TNFα because they have a genetic lesion, are far more susceptible to gram-negative infections than normal mice are (*Wong and Goeddel,* 1986).

While physiological studies have generally focused on the role of TNFα as a potent mediator in the progressive development of inflammation, other studies provide a new understanding of its physiological role.

For example, *Otsuka et al.* (1990) have shown that TNFα inhibits neutrophil migration to local inflammatory sites, a result that might indicate an inhibitory role for TNFα.

Also in support of a beneficial role, infection of mice with Mycobacterium bovis leads to the growth of monocytic cells, organized in granulomas, which harbor the mycobacteria.

TNFα mRNA and protein accumulate in the granulomas as they expand and levels decline as they repress. Injection of anti-TNFα antibodies suppress the formation of the granulomas and markedly increase the number of mycobacteria (*Kindler et al.*, 1989).

This result can be interpreted as the requirement for TNFα in granuloma formation to allow bacterial elimination and to prevent further bacterial spread (*Jacob*, 1992).

The role of TNFα in silica-induced pulmonary fibrosis may be explained in a similar fashion. Although the presence of non degradable particles in the alveoli of humans or rodents leads to fibrotic reaction, the role of TNFα in causing the fibrosis can be viewed as preventing further dissemination of free-floating silica particles (*Piguet et al.*, 1990).

Thus, the presence of TNFα at the site of inflammatory reactions does not necessarily imply that it propagates the inflammatory reaction; depending on timing, target cells and magnitude of inflammatory reaction, TNFα can display beneficial properties for the host (*Jacob*, 1992).

Actions of TNFα

Effect on Neoplasm

TNFα share in the several modes of tumor cell control by macrophages that have been reported:

a. Inhibition of Tumor Cell Division

Inhibition of tumor cell division may occur by mediators secreted by macrophages which act on all proliferating cells present. These mediators largely uncharacterized, but include prostaglandins, IL-1 and TNFα. This inhibition is not thought to require cell contact and occur rapidly (*Lewis*, 1992).

b. Macrophage Mediated Tumor Cytotoxicity

Macrophage-mediated tumor cytotoxicity (MTC) is a contact dependent, non phagocytic process which occurs very slowly over 1–3 days. It is selective for neoplastic cells and is independent of antibody production after recognition of the neoplastic cells, binding to macrophages occurs followed by the secretion of toxic substances, which result in the eventual lysis of the bound tumor cells.

TNFα and a novel serine protease are the major candidates for toxic mediators TNFα (*Lewis et al.*, 1992).

c. Antibody-Dependent Cellular Cytotoxicity (ADCC)

Antibody-dependent cellular cytotoxicity (ADCC) is a process whereby macrophages are able to lyse antibody coated tumour cells. The classical form is rapid and mediated by polyclonal antisera.

Reactive oxygen intermediates specially hydrogen peroxide, play a major role in cytolysis, but other mediators

may also be important such as complement components, neutral protease and TNFα.

Though TNFα appears to be an important mediator of the cytotoxicity of human mononuclear phagocytes in vitro and IFN-γ can increase this cytotoxicity by sensitizing tumour cells of the lytic action of TNFα.

Phase 1 trial in patients with various types of cancer given recombinant TNFα systematically have shown response rates of less than 5%. However direct injection into tumor has more encouraging results (*Lewis et al.*, 1992).

However, the evidence that TNF is therapeutic in vivo is not particularly convincing in that it is not based on the ability of TNFα to cause tumour regression, but on its ability to cause hemorrhagic necrosis of the centers of established tumors (*Havell et al.*, 1988).

A study by *Vreugdenhil et al.* (1992) reported that B-cells from patients with chronic lymphocytic leukemia not only express receptor sites for TNFα but also proliferate in a dose dependent manner when exposed to increasing concentrations to TNFα in vitro.

It was found that the primary pathogenic event in juvenile chronic myeloid leukemia is the autocrine production and release of TNFα which directly induces proliferation of malignant monocyte macrophage elements (*Freedman et al.*, 1991).

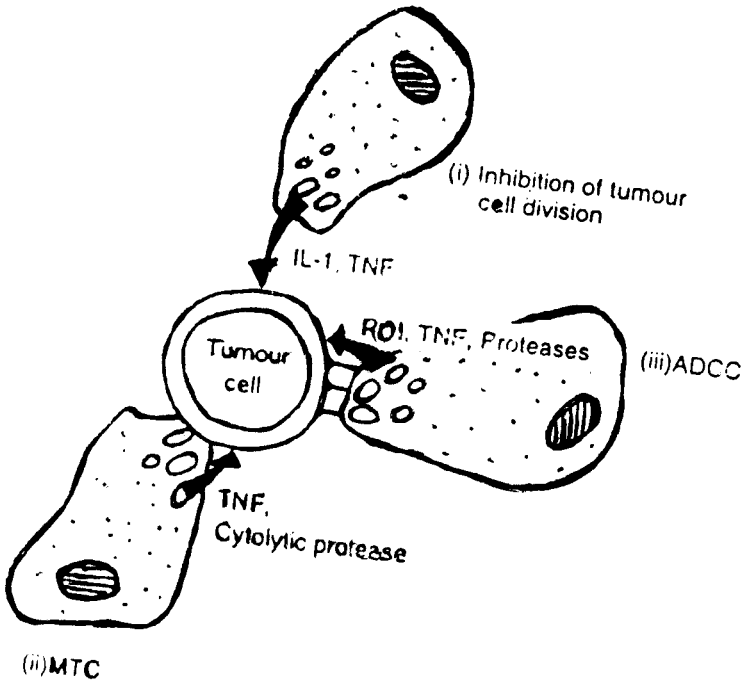


Fig. (1): Mechanisms of tumor cell control by macrophages (*Lewis et al.*, 1992).

TNFα in Cachexia

The investigations done on the effects of TNFα on tissue metabolism provided evidence that TNF causes a state of net catabolism in adipocytes and a depletion of stored cellular lipid in what had been termed an "in vitro model of cellular Cachexia" (*Tracey and Cerami*, 1992).

After binding to its receptors, TNF induces transcriptional changes that lead to reduction of the mRNAs for several lipogenic enzyme and lipoprotein lipases (LPL) without altering the biosynthesis of the normal house-keeping genes. The metabolic result of these cellular responses is lipolysis, with net losses of free fatty acids and depletion of the triglyceride storage pools.

Muscle cells also become catabolic when inoculated with TNF, they are rapidly depleted of glycogen stores, release lactate and develop a defect in the ability to maintain a normal resting membrane potential (*Tracey and Cerami*, 1992).

In contrast to the monocyte and adipocyte effects, hepatocytes display relatively anabolic responses to TNF and manifest increased rate of lipogenesis and glycogen-mediated amino-acid uptake, accelerated biosynthesis of acute phase protein and decreased albumin biosynthesis.

Considered together, these finding suggest that the net effects of TNF are mobilization of peripheral tissue energy stores from adipose and muscle tissue that can be used by the liver in order to meet the increased energy and synthetic demands associated with infection or cancer (*Tracey and Cerami*, 1992).

The complex responses that are characteristic of cachexia (protein and lipid loss, anorexia and anemia) were

demonstrated in studies using rates given recombinant TNF twice daily for 7–10 days.

Hypophagia, weight losses, neutrophilia, hepatic inflammation and diminished blood cell mass were induced by prolonged exposure to TNF. TNF treated animals became depleted of both whole body proteins and lipids, indicating that the net catabolic effect of TNF differed markedly from normal adaptive response to food restriction (*Tracey and Cerami*, 1992).

It should be stressed, however that the net effects of this potent cytokine are dependent upon its site of production in tissues, the duration of its biosynthesis and the quantity produced during the response to illness and inflammation.

For example, acute production of large quantities of TNF induces a catastrophic state of shock and tissue injury while chronically produced lesser quantities of TNF trigger a state of net catabolic and cachexia (*Tracey and Cerami*, 1992).

New Described Effect of TNF

a. Cutaneous Response to Injury and Infection with Cutaneous Sensitization

In response to skin sensitization keratinocytes are induced to synthesis TNFα and the local availability of this cytokine serve to provide one signal for Langerhans cell migration from the skin to the draining lymph nodes also cutaneous trauma other than that resulting from skin sensitization will also result in production of TNFα by keratinocytes.

There is evidence that both ultra-violet B irradiation and topical exposure to Non-sensitizing skin irritants will induce keratinocytes to produce this cytokine and both such treatment

cause accumulation of dendritic cells in the draining lymph nodes. The synthesis and secretion of TNFα and the consequent stimulation of Langerhans cell migration may represent an important early step in cutaneous response to injury and infection (*Cumberbatch et al.*, 1994).

b. In Response to Hemorrhage

TNF plays a critical role in initiation and regulation of Kupffer cells to present antigen and express major histocompatibility complex class II antigen and also cytokine production following hemorrhage.

Hemorrhage increase TNF level by 215% at 2 hours and by 76% at 24 hours also following hemorrhage the significant elevation of TNF plasma levels correlated with the severe suppression of Kupffer cell antigen presentation and expression MHC class II antigen, it has been found that the significant reduction of circulating TNF in plasma by pretreatment with anti TNF antibodies resulted in restoration of these function of Kupffer cells at 2 and 24 hours.

It is more likely that secondary mediators (prostaglandin E_2 or IL-6) which are up regulated by TNF, are involved in the regulation of Kupfer cell cytokine release in this regard, several studies have demonstrated that TNF not only induces the synthesis of IL-1, but also up regulates the synthesis of prostaglandin E_2 Prostaglandin E_2 on the other hand inhibits the synthesis of IL-1 and TNF through negative feedback mechanism.

Moreover studies have shown that following hemorrhage, a significant elevation of PGE_2 in serum occurs. Neutralization of circulating TNF with monoclonal antibodies result in reduced PGE_2 production.

TNF also appears to up regulate IL-6 production following hemorrhage. In summary, TNF play a central, pivotal role in hemorrhage induced suppression of Kupffer cell antigen presentation and MHC class II antigen expression and it enhances the release of IL-6 following hemorrhage (*Ertel et al.*, 1991).

c. Its Role in Inflammatory Bowel Disease

TNF regulates the intestinal IgG Fc binding site. This binding site is involved in reaction between mucus and bacteria on mucosal surface and thereby help to protect the mucosa. This binding site is under the effect of TNFα as TNFα may suppress the expression of this site by differentiation of the cells towards mucin producing cells.

The implication of TNFα in regulation of the binding site and perhaps goblet cell differentiation warrants special attention to the pathogenesis of chronic inflammatory bowel disease since increased production of TNFα, diminished amounts of goblet cells mucins intestinal IgG binding site are all features of chronic ulcerative colitis (*Hamade et al.*, 1991).

d. In Purpura Fulminans

TNFα can be used as a marker of morbidity and mortality in children with purpura fulminans and antibodies to TNFα are used in the treatment of these children (*Girardin et al.*, 1988).

e. Adult Respiratory Distress Syndrome (ARDs)

Cytokines are believed to mediate pulmonary injury as high circulating levels of TNFα, IL-1, IL-8 are found in ARDs patients. Thus, inhibition of lung TNFα production by monoclonal antibodies to TNFα markedly reduces lung oedema and inflammation in ARDs (*Fleverstein*, 1995).

Therapeutic Efficiencies and Future Possible Application of TNFα and Its Antibodies

As a single agent in tumor therapy, r-TNFα would be of limited value if judged by its ability to cause complete tumor regression. Natural TNFα seems more therapeutic, but the doses needed to cause tumor regression are also extremely toxic.

Also, TNFα-induced tumor regression requires an immunocompetent host and the therapeutic effects do not occur in the presence of T cell deficiency (*Havell et al.*, 1988).

Efforts aimed at modifying the TNFα molecule to produce a second generation mediator capable of lysing tumors without eliciting fever, hypotension and coagulopathy are in progress.

It is hoped that certain tumors will prove uniquely sensitive to the effects of TNFα and may be destroyed by concentrations of the hormone that are tolerated by the affected individual (*Tracey et al.*, 1989).

Data from studies in animal models suggest that treatment or pretreatment with polyclonal or monoclonal antibodies against TNFα decrease the mortality associated with septic shock (*Girardine et al.*, 1988 *and Tracey et al.*, 1987).

It remains to be seen whether immunization (either active or passive) might similarly protect against other deleterious effects of the hormone and whether this procedure might be used to clinical advantage (*Beutler and Cerami*, 1986).

Pateints and Methods

PATIENTS AND METHODS

This study was conducted on 44 neonates with sepsis admitted to Neonatal Intensive Care Unit (NICU) of Obstetrics and Gynecology Hospital of Ain Shams University as well as 16 healthy neonates from the same hospital.

Group of Neonates

Patients with sepsis were randomly classified into 2 groups:

- **Group I**: They were 22 septic newborns, 12 males and 10 females, their gestational ages ranged between (30–40 weeks) with mean age of (34.68 weeks). Preterm patients below 37 weeks were 12, while fullterm patients more or equal to 37 weeks were 10. Their birth weight ranged between (1.050–3.800 kg) with a mean of (2.550 kg).
  This group of septic patients received dexamethasone + antibiotics in treatment of neonatal sepsis in addition to the other line of treatment (e.g. fluid, Na, K, etc.). Dexamethasone was given in a dose of 0.15 mg/kg I.V. every 6 hours (total dose 0.6 mg/kg/24 hours) for 4 days.
- **Group II**: They were 22 septic newborns, 8 males and 14 females. Their gestational age ranged between (30–40 weeks) with mean age of (35.42 weeks). Preterm patients, less than 37 weeks, were 12 while fullterm patients, more or equal to 37 weeks were 10. Their birth weight ranged between (1.110–4.400 kg) with a mean of (2.74 kg). This group of patients received antibiotics only in treatment of neonatal sepsis beside the other lines of treatment.
  In all cases treatment was started within 2 hours from the clinical diagnosis of presumed sepsis.

- **Control group**: They were 16 healthy neonates serving as a control group. They were 9 males and 7 females. Their gestational age ranged between (35–40 weeks) with mean age of (37.31 weeks). Preterm neonates, below 37 weeks were (5), their birth weight ranged between (1.7–4 kg) with a mean of (3.04 kg).

All studied neonates were subjected to the following:

I. Prenatal, natal and postnatal medical history taking including:
   - Premature rupture of membranes.
   - Instrumental delivery.
   - Resuscitation.
   - Presence of chorioamnionitis.
   - Other complications e.g. RDS, NEC.
   - Symptoms of neonatal septicemia as poor feeding, vomiting, jaundice, irritability, convulsion, cyanosis and skin changes as mottling.

II. Neonatal physical examination including:
   - Determination of birth weight.
   - Assessment of gestational age by the duration of pregnancy, and combined physical and neuromuscular evaluation using Dubowitz Score (*Dubowitz*, 1977).
   - Assessment of Apgar score at 1 and 5 minutes.
   - Manifestations of neonatal sepsis as thermal instability, respiratory distress, lethargy, abdominal distention, increase gastric residue, petechiae, purpura, bleeding from puncture sites, hepatomegaly, splenomegaly, seizures and sclerema (*Tollner*, 1982).

III. Laboratory investigations:
   1. Complete blood picture (using Coulter counter and differential count of Lishman stained blood film):
      - Total leucocytic count and differential count.
      - RBCs count.
      - Immature to total neutrophil ratio I/T ratio.
      - Hb gm%.
      - Platelet count.
   2. Detection of C-reactive protein in the serum (using Latex agglutination test).
   3. Blood culture was obtained on admission, and was repeated 24–48 hours after start of therapy (using nutrient fluid media then subculture on suitable agar media).

IV. Determination of the activity of tumour necrosis factor-α (TNF-α) in blood at the time of diagnosis and after 4 days from the start of treatment (using ELISA technique).

V. Patient monitoring: The following were accurately recorded throughout the period of study which was continued till patient discharge or death:
   1. Physical examination.
   2. Neurologic examination.
   3. Vital signs.
   4. The volume of all fluid intake and output as well as the type of fluids.
   5. Medications administered (type, dose, time of administration and possible adverse occurrences).

Methodology

Assessment of TNF-α in the Serum using ELISA Technique

Sample Collection

For assessment of TNF-α, blood samples were taken from septic patients (2 blood samples) and from control (one blood sample). Blood samples of about 1–1 1/2 ml from a peripheral vein or venous catheter inserted in the umbilical vein collected in sterile, clean dry tubes and rapidly separated by centrifugation. Samples were kept frozen at –20° C till time of assessment.

Procedure

1. Sufficient strips were selected to accommodate standards, controls and all test samples and to fit into the holding frame.
2. 200 µl of each standard (15, 50, 150, 500, 1500 pg/ml in human serum and merthiolate), control (1, 2 in human serum with merthiolate) or sample were dispensed into the appropriate wells.
3. 50 µl of incubation buffer (tris-maleate buffer, with BSA and preservatives) was dispensed into the wells and incubated for 2 hours at room temperature on an horizontal plane shaker 700 ±100 RPM.
4. Washing of the plate was done by:
   a. Aspirating the liquid from each well.
   b. 0.4 ml of washing solution (Tween 20, 20%) after dilution in 400 ml distilled water is added into each well.
   c. Aspirating the content was done. Steps b and c were repeated twice.

5. 100 µl of standard 0 (0 pg/ml in human serum and preservatives) was dispensed into the wells.
6. 50 µl of anti-TNF-α HRP conjugate was dispensed into all wells.
7. Incubation for 2 hours was done again.
8. Washing the plates again.
9. 200 µl of freshly prepared revelation solution (0.2 ml of chromogen TMB was pipetted into one of the vials of substrate buffer (H_2O_2 in acetate/citrate buffer) was dispensed into each well within 15 minutes following washing step.
10. Incubation for 30 minutes.
11. 50 µl of stopping reagent (H_2S0_4 1.8 N) was dispensed into each well.
12. Reading the microtiter plate at 450 nm (reference filter 630 nm) was done.
13. Construction of a standard curve using all standard points was done by plotting the optical density on the ordinate against the standard concentrations on the abscissa using linear paper and drawing the curve by connecting the plotted points with straight lines.

Expected Values

Cut off values of 70 pg/ml were used to differentiate normal from septic newborn.

- Level <70 pg/ml means normal.
- Level ≥70 pg/ml means sepsis.

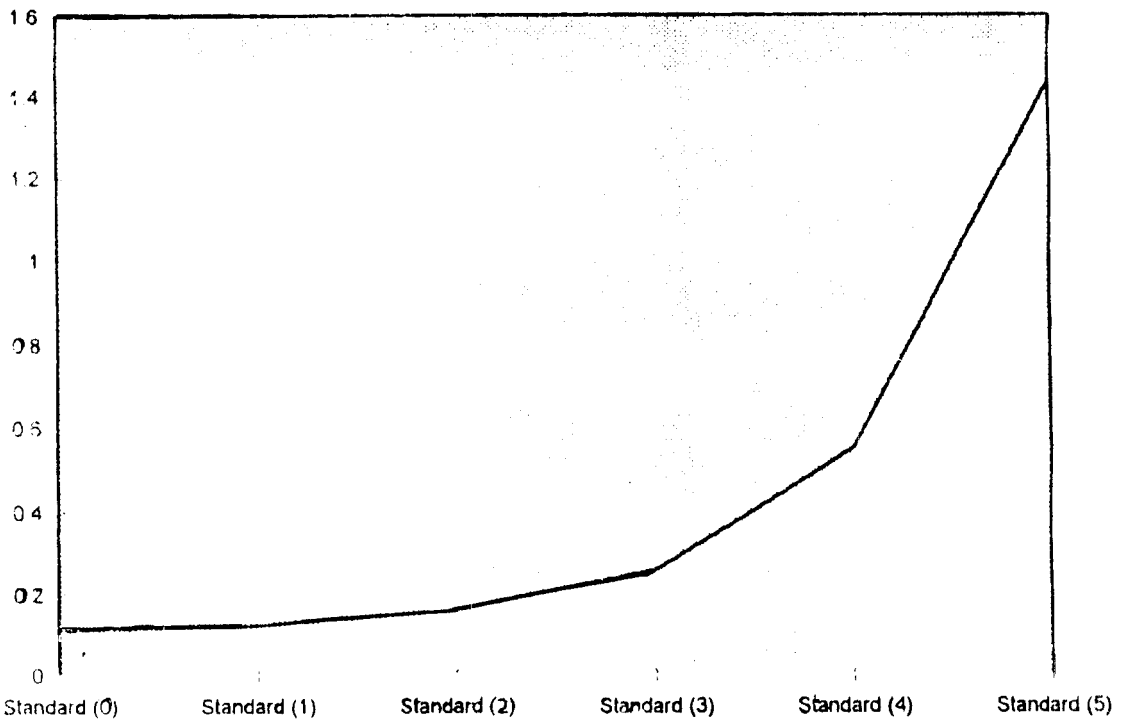


Fig. (1): Curve for calculation of serum TNFα level

Results

RESULTS

The results of this study are presented in the following tables and figures.

Table (1): Descriptive data of control group (healthy newborn)

No	Sex	GA (wks)	Birth wt. (kg)	Mode of delivery	TNF level	CRP
1	F	36	2.8	CS	45	-ve
2	F	38	3.3	CS	50	-ve
3	M	39	3.5	CS	60	-ve
4	F	37	3	NVD	60	-ve
5	M	36	2.7	NVD	50	-ve
6	F	40	4	CS	65	+ve
7	M	36	2.7	NVD	52	-ve
8	F	37	3	NVD	50	-ve
9	M	37	2.5	NVD	50	-ve
10	M	38	3.35	CS	60	-ve
11	M	38	3	NVD	50	-ve
12	M	40	4	NVD	65	+ve
13	M	37	3	CS	60	-ve
14	M	35	1.7	NVD	65	-ve
15	F	37	3.4	CS	50	-ve
16	F	36	2.75	NVD	55	-ve
Range		35–40	1.7–4		45–65	
Mean		37.31	3.04		55.44	
±SD		1.45	0.57		6.61	

As shown in table (1): control group includes 16 healthy neonates (7) females and (9) males.

- 44% of cases delivered by C.S while 56% delivered vaginally.
- Gestational age ranged between 35–40 weeks with a mean of 37.3 weeks.
- Birth weight ranged between 1.7–4 kg with a mean of 3.04 kg.
- Preterm cases below 37 weeks were 4 while fullterm were 12.
- CRP was positive in 12.5% of cases.
- TNFα serum level ranged between 45–65 pg/ml with a mean of 55.4 pg/ml (normal serum TNFα is ≤70 pg/ml).

Table (2): Descriptive data of group I (septic group steroid treated)

No	Sex	Mode of delivery	GA (wks)	Birth wt. (kg)	Day of sepsis	Sepsis score Pre	Sepsis score Post	Hemat Score Pre	Hemat Score Post	CRP
1	F	CS	39	3.8	1	12	10	3	2	+ve
2	F	CS	30	1.6	1	16	16	4	5	+ve
3	M	VD	35	2.6	3	12	12	3	2	+ve
4	M	CS	37	2.9	2	18	16	4	4	+ve
5	F	VD	30	1.7	2	14	12	3	2	+ve
6	M	VD	36	3.12	2	12	10	3	2	+ve
7	M	VD	38	3.5	4	14	12	3	2	+ve
8	M	CS	32	2.37	12 hrs	12	10	3	2	+ve
9	M	CS	37	3	6	16	18	4	4	+ve
10	F	CS	38	3.5	2	12	10	3	2	+ve
11	F	CS	30	1.05	2	18	18	5	4	+ve
12	F	VD	30	1.1	2	18	18	4	4	+ve
13	F	VD	40	3.4	6	14	12	4	2	+ve
14	F	CS	37	2.9	1	18	18	5	3	+ve
15	M	VD	39	3.3	4	16	12	3	2	+ve
16	M	VD	38	3.1	2	14	12	3	2	+ve
17	F	CS	30	1.6	16 hrs	18	16	5	6	+ve
18	M	CS	32	2.2	1	14	12	3	3	+ve
19	M	CS	30	1.7	2	16	16	4	6	+ve
20	F	VD	32	2	3	14	12	3	2	+ve
21	M	CS	39	3.5	3	12	10	3	2	+ve
22	M	CS	34	2.1	1	12	10	4	3	+ve
Range			30–40	1.05–3.8	0.5–6	12–18	10–18	3–5	2–6	
Mean			34.68	2.55	2.33	14.64	13.27	3.59	3.00	
±SD			3.71	0.84	1.54	2.34	3.06	0.73	1.35	

Table (2): Continued.

No	TNF Pre	TNF Post	TLC (x1,000) Pre	TLC (x1,000) Post	I/T ratio	Platelets (x1,000) Pre	Platelets (x1,000) Post	Culture	Fate
1	515	135	28	21	0.21	70	90	Kleb	R
2	365	520	4.5	11	0.52	40	50	Kleb	D
3	210	105	22	20	0.22	60	80	Kleb	R
4	310	170	4	6	0.61	40	30	Staph	D
5	315	110	22	20	0.12	80	100	Kleb	R
6	250	120	25	21	0.24	70	70	Pseud	R
7	255	110	27	20	0.31	60	70	None	R
8	260	125	26	25	0.34	50	100	Pseud	R
9	260	270	11	12	1.00	80	80	Kleb	D
10	275	120	25	20	0.82	120	130	Staph	R
11	850	650	4	6	0.71	40	40	Kleb	D
12	365	520	4.5	16	0.82	80	80	Kleb	D
13	210	90	13	15	0.31	130	150	Kleb	R
14	750	600	5	10	0.71	90	100	Kleb	D
15	415	135	15	15	0.33	80	80	Kleb	R
16	320	120	20	15	0.41	70	60	Staph	R
17	750	760	10	13	0.91	40	40	Pseud	D
18	310	110	22	20	0.15	70	50	Kleb	R
19	550	560	20	25	0.81	50	60	E. coli	D
20	270	100	12	10	0.24	80	80	Kleb	R
21	310	100	22	20	0.31	70	60	Kleb	R
22	400	130	23	25	0.22	60	80	E. coli	R
Range	210–850	90–760	4–28	6–25	0.12–1	40–130	30–150		
Mean	387.05	257.27	16.59	16.64	0.47	69.55	76.36		
±SD	183.56	223.37	8.47	5.79	0.28	23.60	28.71		

Table (2) shows that group I included 22 septic neonates, 10 females and 12 males with male to female ratio 1.2.

- 60% of cases delivered by C.S while 40% delivered vaginally.
- Gestational age ranged between 30–40 weeks with a mean of 34.68 weeks.
- Birth weight ranged between 1.05–3.8 kg with a mean of 2.55 kg.
- Preterm patients below 37 weeks were 12 while fullterm were 10.
- CRP was positive in 100% of cases.
- Blood culture was positive in 95.4% of cases.
- Mean value of sepsis score was 14.64 while mean value of hematological score was 3.59.
- Mean TNFα serum level was 387.05 pg/ml at the time of diagnosis.

Table (3): Descriptive data of group II (septic non-steroid treated)

No	Sex	Mode of delivery	GA (wks)	Birth wt. (kg)	Day of sepsis	Sepsis score Pre	Sepsis score Post	Hemat Score Pre	Hemat Score Post	CRP
1	F	CS	38	3.38	2	16	16	5	6	+ve
2	M	VD	38	3.7	0.5	12	10	4	3	+ve
3	M	CS	37	3.14	2	20	20	5	6	+ve
4	F	CS	38	3.6	1	12	12	3	2	+ve
5	M	VD	34	2	2	12	12	3	2	+ve
6	F	VD	32	1.6	1	18	16	5	6	+ve
7	F	CS	40	4.4	1	14	12	3	2	+ve
8	F	CS	36	2.5	1	14	12	3	3	+ve
9	M	CS	30	1.45	2	18	16	5	5	+ve
10	F	VD	37	3.3	3	12	12	3	3	+ve
11	F	VD	32	1.33	3	16	16	4	5	+ve
12	F	VD	30	1.11	3	18	16	4	6	+ve
13	F	CS	36	3.2	3	12	10	3	2	+ve
14	M	CS	30	1.56	2	18	18	5	5	+ve
15	F	VD	34	2.4	0.5	14	12	3	2	+ve
16	F	VD	40	4.2	1	12	12	3	2	+ve
17	F	VD	30	1.7	1	18	16	4	6	+ve
18	M	CS	32	2.1	2	12	12	4	6	+ve
19	F	CS	37	3.5	1	12	10	4	2	+ve
20	M	VD	36	3.2	6	20	20	4	5	+ve
21	M	VD	38	3.6	1	12	12	3	2	+ve
22	F	VD	39	3.4	2	16	16	4	6	+ve
Range			30–40	1.11–4.4	0.5–6	12–20	10–20	3–5	2–6	
Mean			35.18	2.74	1.86	14.91	14.00	3.82	3.95	
±SD			3.42	1.00	1.24	2.94	3.09	0.80	1.79	

Table (3): Continued

No	TNF Pre	TNF Post	TLC (x1,000) Pre	TLC (x1,000) Post	I/T ratio	Platelets (x1,000) Pre	Platelets (x1,000) Post	Culture	Fate
1	575	580	4	5	0.51	80	40	Kleb	D
2	200	210	18	20	0.34	100	90	Kleb	R
3	1060	1070	5	8	0.22	40	30	Pseud	D
4	100	50	19	20	0.21	60	70	Pseud	R
5	175	100	22	20	0.11	80	80	Kleb	R
6	610	620	4	10	0.34	40	30	Staph	D
7	580	100	25	15	0.21	90	90	Kleb	R
8	310	100	28	30	0.22	110	100	Kleb	R
9	410	650	30	20	0.63	40	40	Staph	D
10	180	125	22	15	0.12	70	60	E. coli	R
11	650	720	21	20	0.65	30	20	Kleb	D
12	700	740	28	25	0.92	30	30	Kleb	D
13	185	120	27	20	0.31	80	50	E. coli	R
14	420	520	25	20	0.51	20	60	Strep	D
15	330	220	22	21	0.21	50	60	Strep	R
16	580	220	21	20	0.23	60	50	Pseud	R
17	620	630	4.2	6	1.00	40	30	None	D
18	170	200	20	25	0.47	50	70	None	D
19	110	60	5	6	0.24	60	80	Pseud	R
20	1020	1030	4.8	8	0.84	40	30	Kleb	D
21	200	210	11	15	0.31	70	60	Staph	R
22	560	600	14.9	20	0.58	40	50	Kleb	D
Range	100–1060	50–1070	4–30	5–30	0.11–1	20–110	20–100		
Mean	442.95	403.41	17.31	16.77	0.42	58.18	55.45		
±SD	276.99	320.21	9.09	6.91	0.26	24.23	23.04		

As shown in table (3) group II included 22 septic neonates, 8 males and 14 females with male to female ratio 0.57.

- 45.4% of cases delivered by C.S while 54.6% delivered vaginally.
- Gestational age ranged between 30–40 weeks with a mean of 35.18 weeks.
- Birth weight ranged between 1.11–4.4 kg with a mean of 2.74 kg.
- Preterm patients below 37 weeks were 12 while fullterm were 10.
- CRP was positive in 100% of cases.
- Blood culture was positive in 90.9% of cases.
- Mean value of sepsis score was 14.91 while mean value of hematological score was 3.82.
- Mean TNFα serum level was 442.95 pg/ml at the time of diagnosis.

Table (4): The incidence of clinical symptoms and signs among studied septic groups.

	Group I		Group II		
	No	%	No	%	P-value
Prematurity	12	54.5%	12	54.5%	P >0.05 (NS)
Risk factors	14	63.6%	14	63.6%	P >0.05 (NS)
Difficult resuscitation	14	63.6%	8	36.4%	P >0.05 (NS)
Meconium aspiration	2	9.1%	3	13.6%	P >0.05 (NS)
Diminshed activity	22	100%	22	100%	P >0.05 (NS)
Lethargy	8	36.4%	6	27.3%	P >0.05 (NS)
Apnea	3	13.6%	2	9.1%	P >0.05 (NS)
Respiratory distress	11	50.0%	12	54.5%	P >0.05 (NS)
GIT symptoms	16	72.7%	20	90.9%	P >0.05 (NS)
Bleeding tendency	14	63.6%	16	72.7%	P >0.05 (NS)
Hepatomegaly	14	63.6%	8	36.4%	P >0.05 (NS)
Splenomegaly	2	9.1%	2	9.1%	P >0.05 (NS)
Convulsions	4	18.2%	2	9.1%	P >0.05 (NS)
Hypotonia	12	54.5%	6	27.3%	P >0.05 (NS)
Poor periph circulation	5	22.7%	4	18.2%	P >0.05 (NS)
Mottling	3	13.6%	2	9.1%	P >0.05 (NS)
Skin changes	7	31.8%	8	36.4%	P >0.05 (NS)
Oedema LL	12	54.5%	12	54.5%	P >0.05 (NS)
PROM	10	45.5%	10	45.5%	P >0.05 (NS)
M:F ratio	1.2		0.57		P >0.05 (NS)

Table (4) shows that in both septic groups:

- Risk factors either maternal or neonatal risk factors occurred in 63.6% of cases of neonatal sepsis.
- Prematurity was the most important neonatal risk factor which occurred in 54.5% of cases of neonatal sepsis.
- PROM considered as the most important maternal risk factor which occurred in 45.5% of cases of neonatal sepsis.
- Regarding clinical symptoms and signs in the studied cases there was no significant difference between group I and group II ($P > 0.05$).

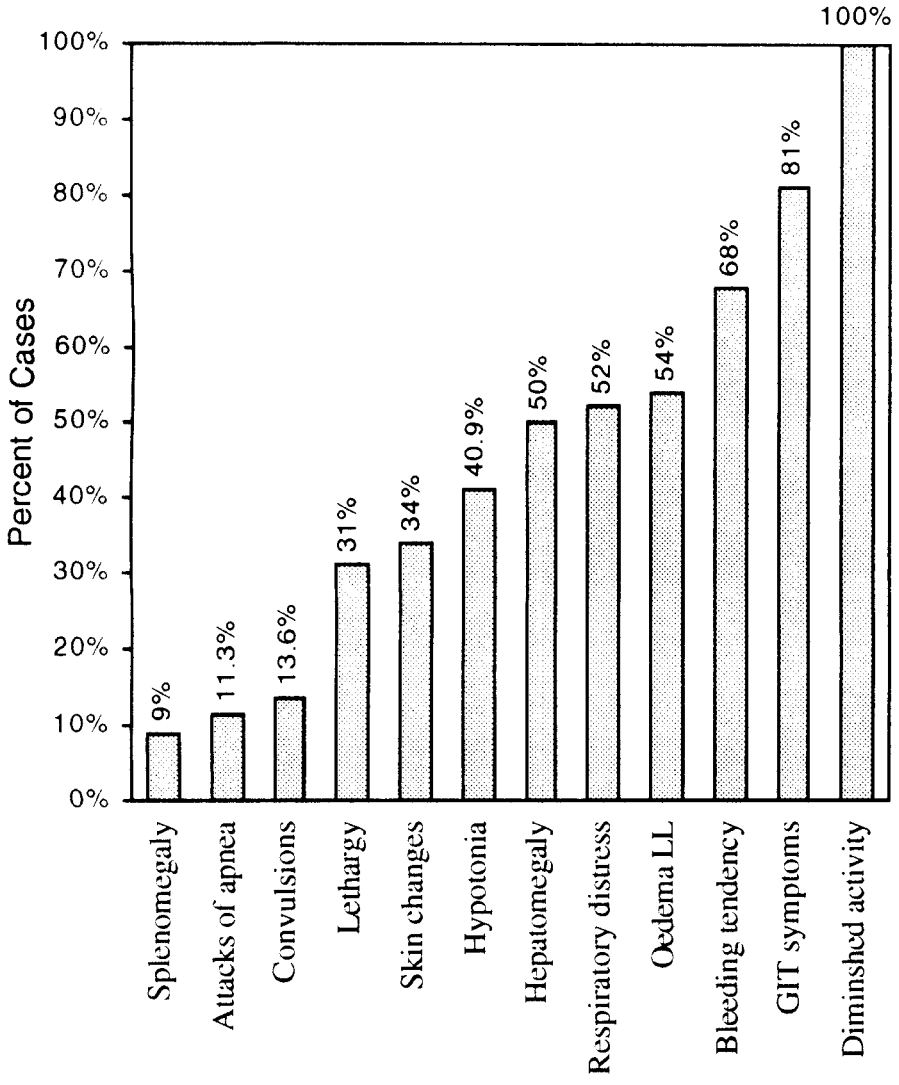


Fig. (1): The incidence of clinical symptoms and signs among the studied septic groups (n = 44 cases)

Figure (1) shows that splenomegaly occurred in 9% of cases, attacks of apnea in 11.3%, convulsions in 13.6%, lethargy in 31%, sking changes in 34%, hypotonia in 40.9%, hepatomegaly in 50%, respiratory distress in 52%, oedema of LL in 54%, bleeding tendency in 68%, GIT symptoms in 81% and diminished activity in 100% of septic cases.

Table (5): Incidence of laboratory findings among studied septic groups

	Group I		Group II		
	No	%	No	%	P-value
Leucopenia	5	22.7%	6	27.3%	P >0.05 (NS)
Leucocytosis	11	50.0%	10	45.5%	P >0.05 (NS)
I/T ratio	20	90.9%	20	90.9%	P >0.05 (NS)
Thrombocytopenia	20	90.9%	20	90.9%	P >0.05 (NS)
CRP	22	100%	22	100%	P >0.05 (NS)
+ve Blood culture	21	95.5%	20	90.9%	P >0.05 (NS)

Table 5: Regarding laboratory investigations of the studied cases:

- CRP was positive in 100% of cases in group I and II while in control group it was positive in 12.5% of cases.
- In group I:
  - Mean WBCs was 16.59 ±8.47 $x10^3/mm^3$.
    Leucocytosis occurred in 50% and leucopenia in 22.7%.
  - I:T ratio ≥0.2 was presented in 90.9% of cases with a mean of 0.47 ±0.28.
  - Mean platelet count was 69.55 ±23.6 $x10^3/mm^3$.
    Thrombocytopenia occurred in 90.9% of cases (below 100,000/mm^3).

- In group II:
  - Mean WBCs was 17.31 ±9.09 x10^3/mm^3. Leucocytosis occurred in 45.5% while leucopenia was in 27.3% of cases.
  - I:T ratio ≥0.2 was presented in 90.9% of cases with a mean of 0.42 ±0.26.
  - Mean platelet count was 58.18 ±24.2 x10^3/mm^3. Thrombocytopenia occurred in 90.9% of cases (below 100,000/mm^3).
- There was no significant difference between group I and II (P >0.05) as regarding laboratory findings.

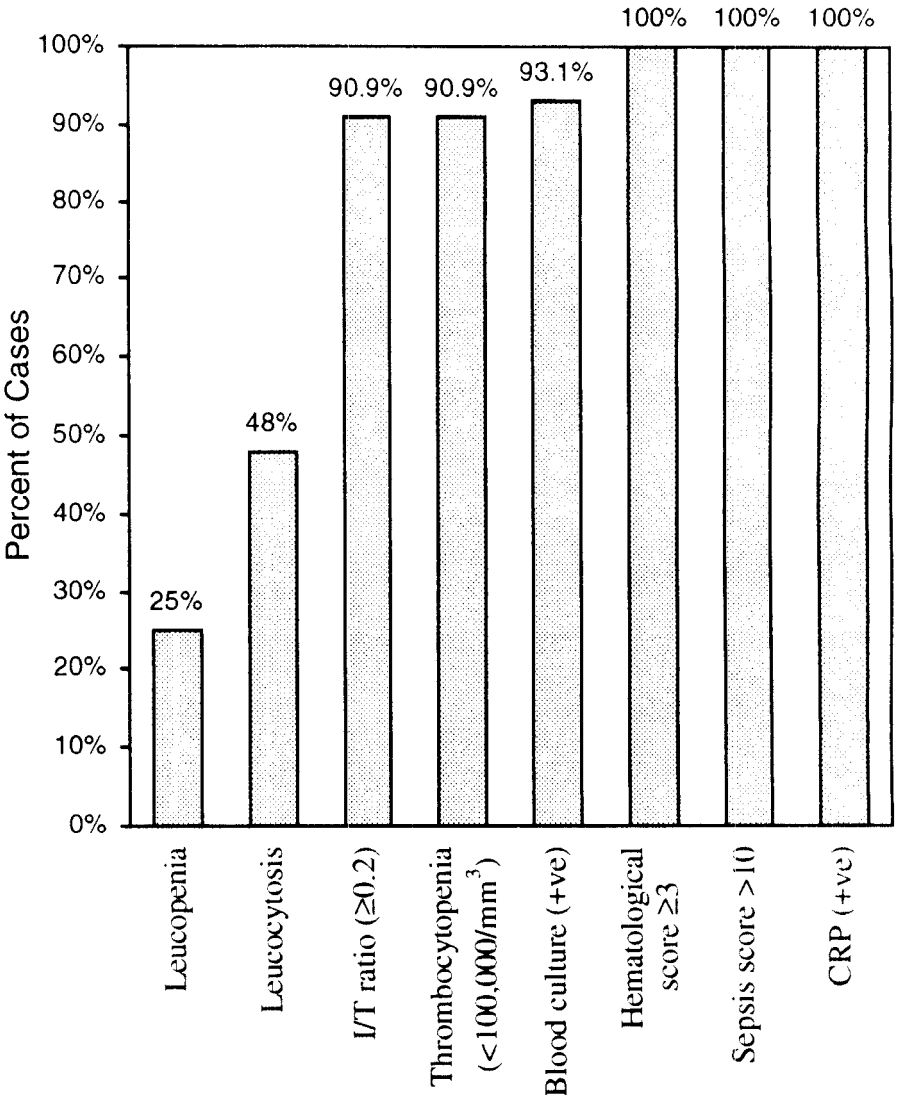


Fig. (2): The incidence of laboratory findings among the studied septic neonates (n = 44 cases).

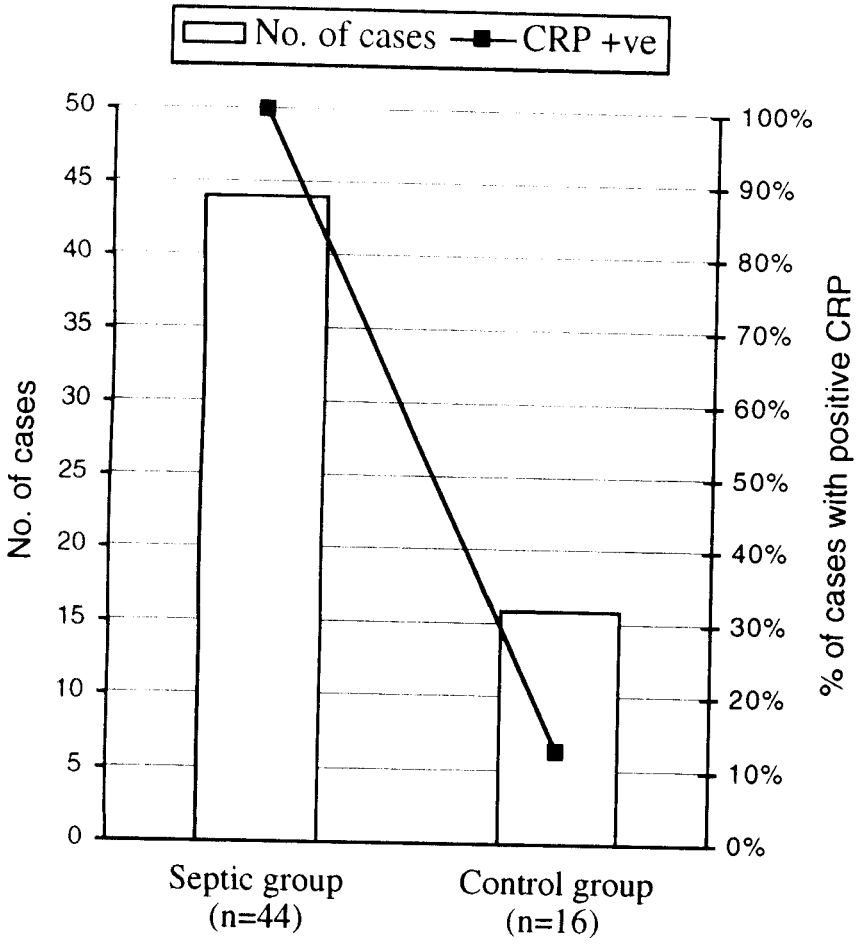


Fig. (3): Incidence of positive CRP test in septic groups (I, II) and control group.

Table (6): Mean serum level of TNFα in pg/ml in the 3 studied groups.

	Control	Group I	Group II	P-value		
				Group I vs. Control	Group II vs. Control	Group I vs. Group II
n	16	22	22			
Range	45–65	210–850	100–1060	<0.001	<0.001	>0.05
Mean	55.44	387.05	442.95	(Sig)	(Sig)	(NS)
SD	6.61	183.56	276.99			

Comparison of mean TNFα serum level in the 3 studied groups revealed the following (table 6):

- Mean TNFα serum level in septic patients in group I (387.05 ±183.56 pg/ml) was not significantly different from that of group II (442.95 ±276.99 pg/ml) (P >0.05).
- Mean TNFα serum level in septic patients in group I (387.05 ±183.56 pg/ml) was significantly higher than that of the control group (55.44 ±6.61 pg/ml) (P <0.001).
- Mean TNFα serum level in septic patients in group II (442.95 ±276.99 pg/ml) was significantly higher than that of the control group (55.44 ±6.61 pg/ml) (P <0.001).

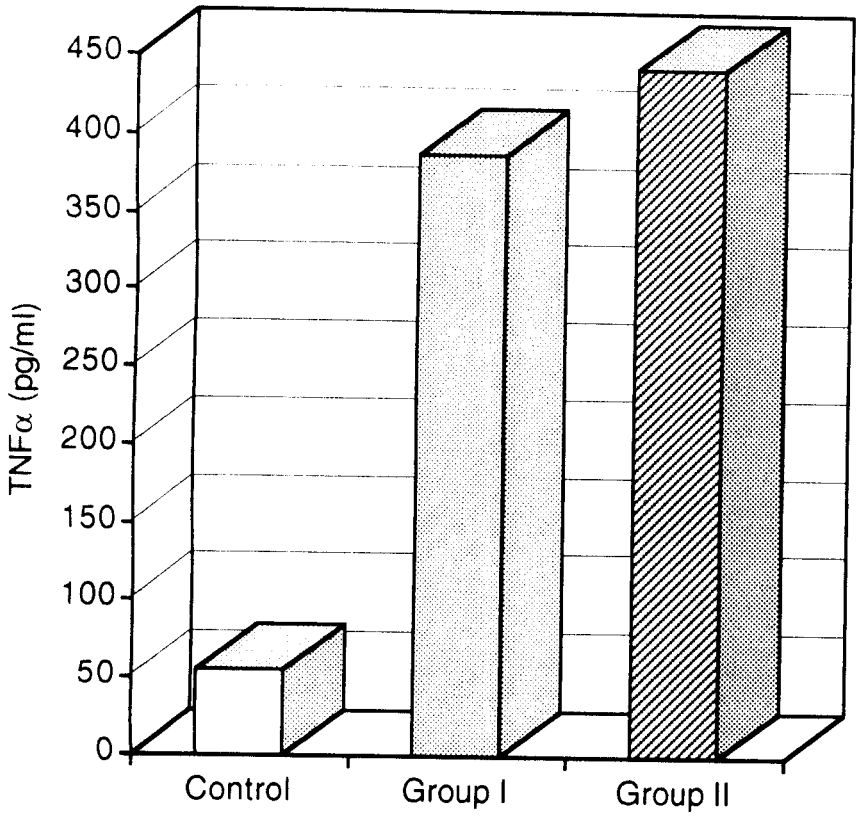


Fig. (4): Mean serum level of TNFα in pg/ml in the 3 studied groups.

Table (7): Mean age, weight, sepsis score and hematological score in group I and II.

		Group I (n=22)	Group II (n=22)	t/z value	P-value
Gestational Age (wks)	Range	30–40	30–40	0.47	>0.05 (NS)
	Mean	34.68	35.18		
	SD	3.71	3.42		
Birth weight (kg)	Range	1.05–3.8	1.11–4.4	0.71	>0.05 (NS)
	Mean	2.55	2.74		
	SD	0.84	1.00		
Hematological score	Range	3–5	3–5	0.90	>0.05 (NS)
	Mean	3.59	3.82		
	SD	0.73	0.80		
Sepsis score	Range	12–18	12–20	0.14	>0.05 (NS)
	Mean	14.64	14.91		
	SD	2.34	2.94		

As shown in table (7): there was no significant difference between group I and II as regard gestational age, birth weight, sepsis score and hematological score with P >0.05.

Table (8): TNFα level in pg/ml among males and females in septic groups

	Males	Females
n	20	24
Range	170–1060	100–850
Mean	375.25	448.13
SD	247.52	221.77
Z-value	1.48	
P	>0.05 (NS)	

Table (9): Mean TNFα level, age, day of sepsis and weight in different types of causative organisms.

	Klebsiella		Other G -ve		G +ve		P
	Mean	±SD	Mean	±SD	Mean	±SD	
Age	35.14	3.75	34.79	3.40	34.63	3.58	>0.05 (NS)
Day of Sepsis	2.52	1.68	1.73	1.04	1.56	0.62	>0.05 (NS)
Weight	2.60	1.00	2.80	0.84	2.51	0.89	>0.05 (NS)
TNF	450.68	233.07	390.71	283.51	359.38	123.30	>0.05 (NS)

Table (10): Mean serum TNFα level in vaginal delivery and C.S in both septic groups.

	VD	CS
n	21	23
Range	175–1020	100–1060
Mean	401.67	427.17
SD	225.72	245.60
Z-value	0.32	
P	>0.05 (NS)	

Table (11): Mean serum TNFα level in premature and mature neonates in both septic groups.

	Premature	Mature
n	24	20
Range	170–1020	100–1060
Mean	437.29	388.25
SD	230.28	241.41
Z-value	0.95	
P	>0.05 (NS)	

From tables (8–11) it was found that:

- Mean serum TNFα level in males septic neonates (375.25 ±247.52 pg/ml) was not significantly different from that of females septic neonates (448.13 ±221.77 pg/ml) (P >0.05).

- Mean serum TNFα level in septic neonates delivered by C.S (427.17 ±245.6 pg/ml) was higher than those who delivered vaginally in both groups (401.67 ±225.72 pg/ml) but with no statistical significant difference (P >0.05).

- Mean serum TNFα level in preterm septic neonates in both groups (437.29 ±230.28 pg/ml) was not significantly different from that of fullterm patients (388.25 ±241.41 pg/ml) (P >0.05).

- There was no significant difference between TNFα serum level and type of causative organisms (Gram +ve or Gram -ve) (P >0.05).

Table (12): Mean serum TNFα level in group I and II post-treatment.

	Group I	Group II
n	22	22
Range	90–760	50–1070
Mean	257.27	403.41
SD	223.37	320.21
Z-value	1.96	
P	<0.05 (Sig)	

As shown in table (12): Mean TNFα serum level in group I (257.27 ±223.37 pg/ml) (after 4 days of treatment) was significantly lower than that of group II (403.41 ±320.21 pg//ml) ($P < 0.05$).

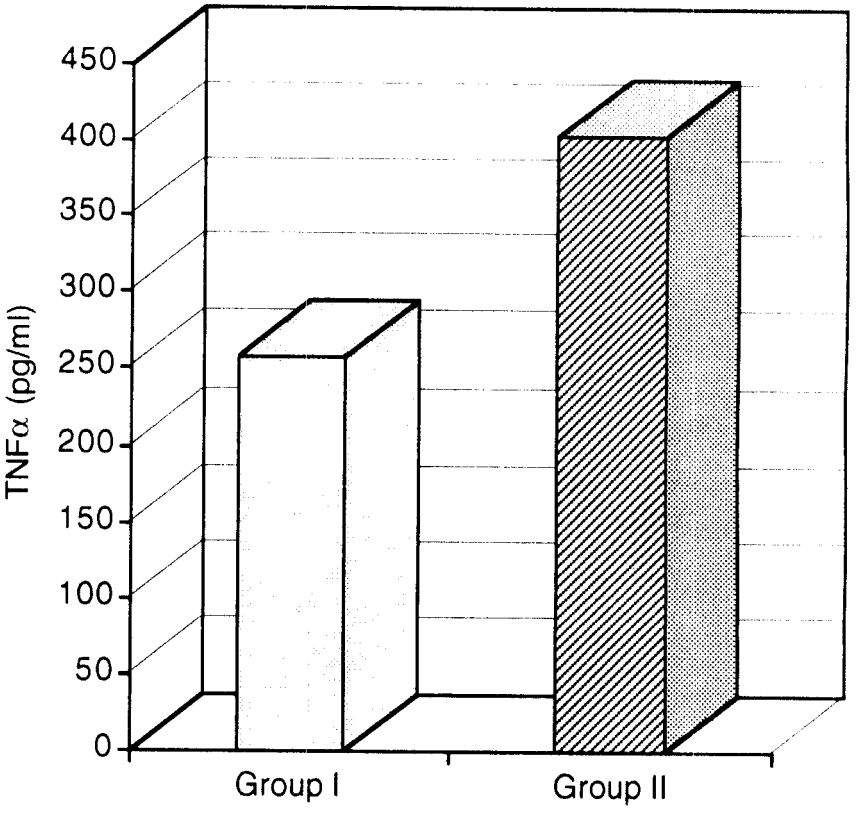


Fig. (5): Mean serum level of TNFα in pg/ml in group I and II post-treatment.

Table (13): TNFα level in patients who recovered and who died in both groups and in each group alone (I & II)

		Recovery	Death	Z-value	P
Groups I and II	n	25	19		
	Range	50–220	170–1070		
	Mean	125.00	600.53	5.44	<0.001 (Sig)
	SD	44.77	229.33		
Group I	n	14	8		
	Range	90–135	170–760		
	Mean	115.00	506.25	3.82	<0.001 (Sig)
	SD	13.73	194.93		
Group II	n	11	11		
	Range	50–220	200–1070		
	Mean	198.64	669.09	3.71	<0.001 (Sig)
	SD	68.81	236.20		

Comparison in table (13) revealed the following:

- Mean TNFα serum level in septic neonates who died in group I (506.25 ±194.93 pg/ml) was significantly higher than those who recovered (115 ±13.73 pg/ml) (P <0.001).

- Mean TNFα serum level in septic neonates who died in group II (669.09 ±236.2 pg/ml) was significantly higher than those who recovered (198.64 ±68.81 pg/ml) (P <0.001).

- Mean TNFα serum level in septic neonates who died in both groups (600.53 ±229.33 pg/ml) was significantly higher than those who recovered (125 ±44.77 pg/ml) (P <0.001).

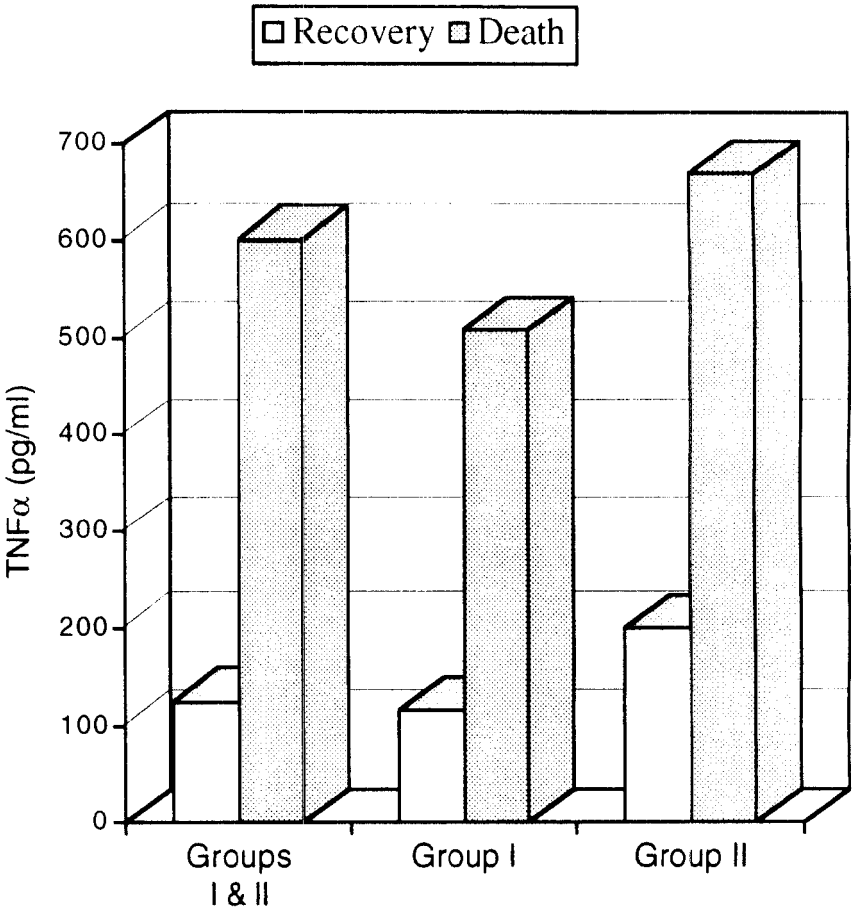


Fig. (6): Mean serum level of TNFα in pg/ml in patients who recovered and who died in both groups and in each group alone (I & II).

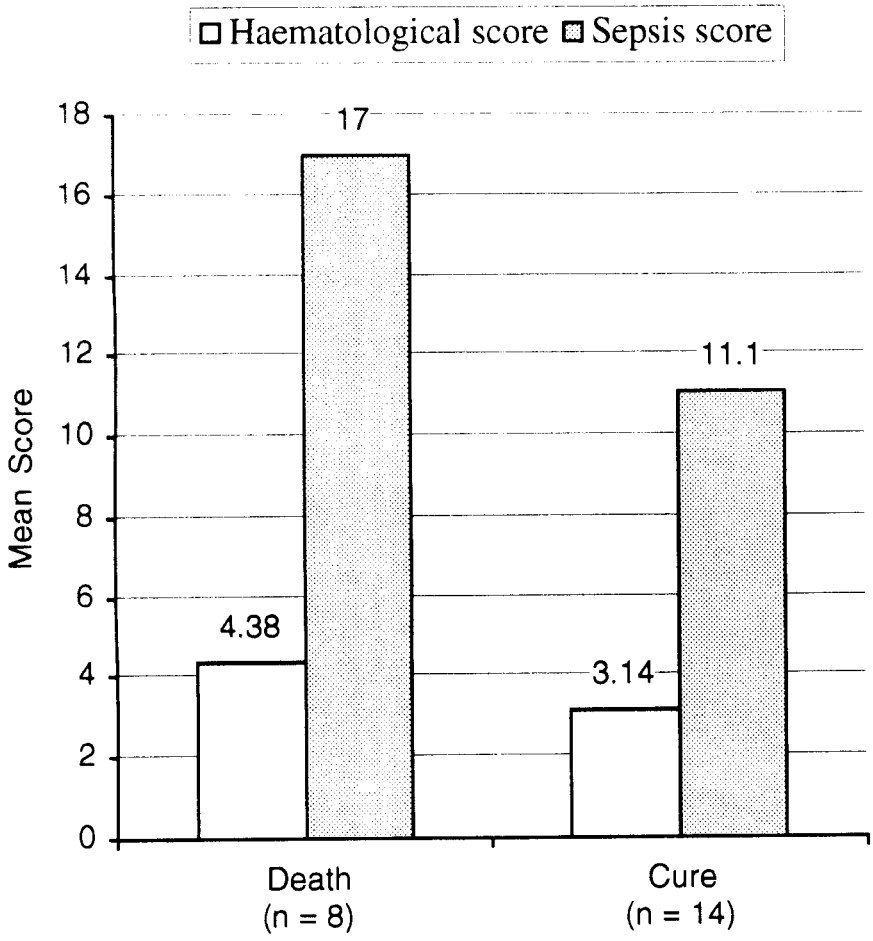


Fig. (7): Relation of hematological and sepsis score to prognosis in group I.

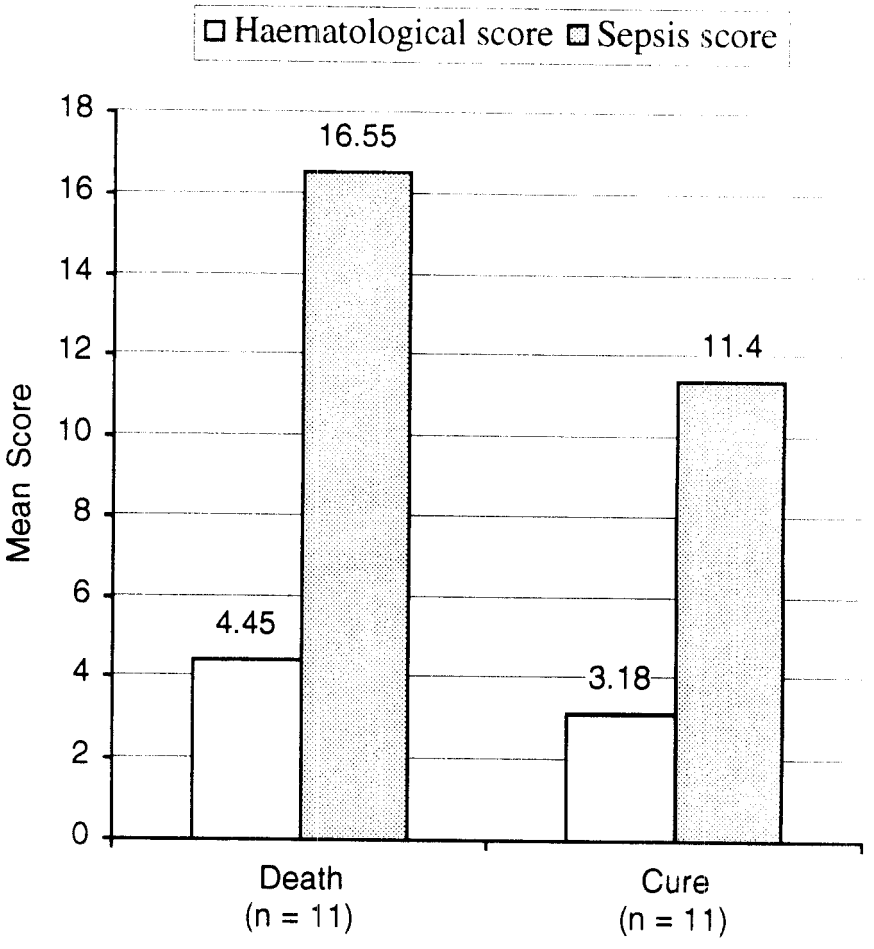


Fig. (8): Relation of hematological and sepsis score to prognosis in group II.

Table (14): Comparing TNFα serum level in recovered patients in group I and II.

	Group I	Group II
n	14	11
Range	90–135	50–220
Mean	115.00	198.64
SD	13.73	68.81
Z-value	2.83	
P	<0.01 (Sig)	

Table (14) shows that mean TNFα serum level in septic patients who recovered in group I (115 ±13.73 pg/ml) was significantly lower than that of group II (198.64 ±68.81 pg/ml) (P <0.01).

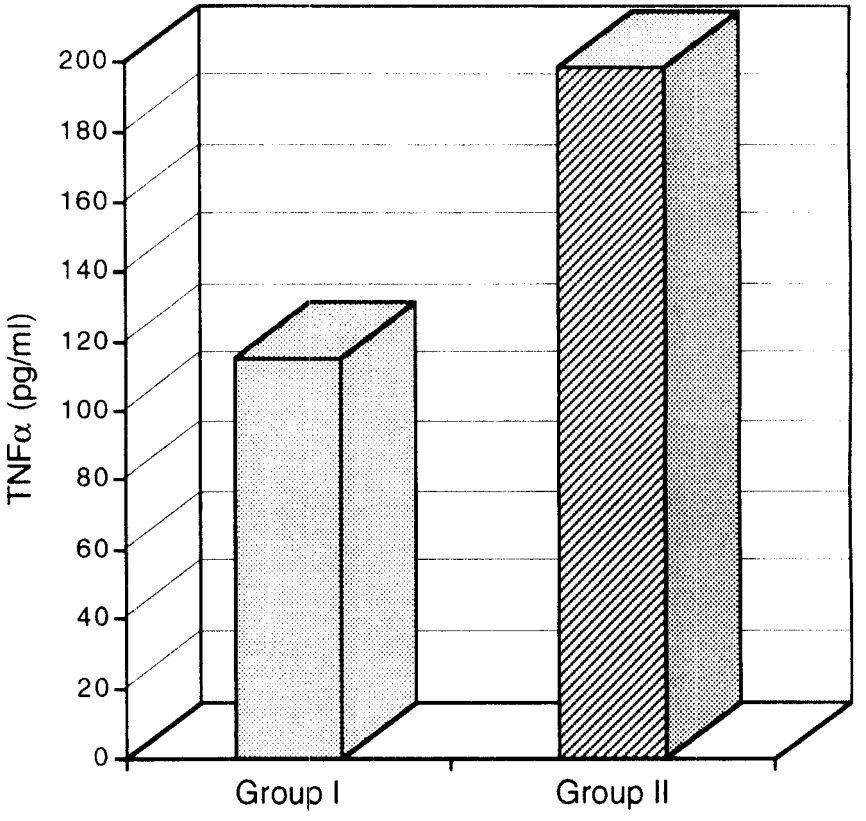


Fig. (9): Mean TNFα serum level in recovered patients in group I and II.

Table (15): Comparison between pre and post treatment values of TNFα, sepsis score and hematological score in group I and II.

	Pre (mean ±SD)	Post (mean ±SD)	Mean % change	Paired-t	P
Group I					
TNF	387.05 ±183.56	257.27 ±223.37	-38.8%	4.67	<0.001 (Sig)
Sepsis score	14.64 ±2.34	13.27 ±3.06	-10.0%	4.95	<0.001 (Sig)
Hematological score	3.59 ±0.73	3.00 ±1.35	-18.5%	2.89	<0.01 (Sig)
Group II					
TNF	442.95 ±276.99	403.41 ±320.21	-14.1%	1.23	>0.05 (NS)
Sepsis score	14.91 ±2.94	14.00 ±3.09	-6.2%	4.18	<0.001 (Sig)
Hematological score	3.82 ±0.80	3.95 ±1.79	+0.1%	0.51	>0.05 (NS)

Table (15) shows that:

In group I

- Mean TNFα serum level post-treatment (257.27 ±223.37 pg/ml) was significantly lower than mean serum TNFα serum level pre-treatment (387.05 ±183.56 pg/ml) (P <0.001).
- Mean value of sepsis score post-treatment (13.2 ±3.06) was significantly lower than that pre-treatment (14.64 ±2.34) (P <0.001).

- Also mean value of hematological score post-treatment (3.0 ±1.3) was significantly lower than that pre-treatment (3.59 ±0.73) ($P < 0.001$).

In group II

- Mean TNFα serum level post-treatment (403.41 ±320.21 pg/ml) was lower than that pre-treatment (442.95 ±276.99 pg/ml) but with no statistical significant difference ($P > 0.05$).
- Mean value of sepsis score post-treatment (14 ±3.09) was significantly lower than that pre-treatment (14.91 ±2.9) ($P < 0.001$).
- Also there was no significant difference between hematological score pre and post-treatment ($P > 0.05$).

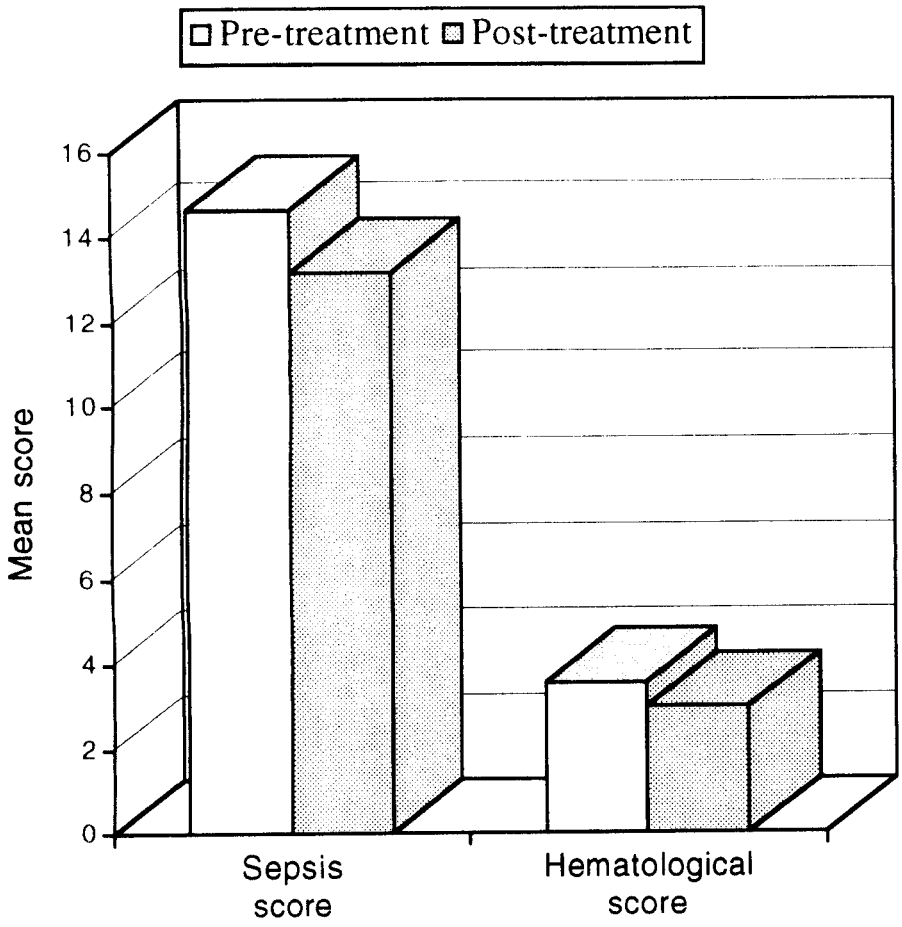


Fig. (10): Comparison between pre and post treatment values of sepsis score and hematological score in group I.

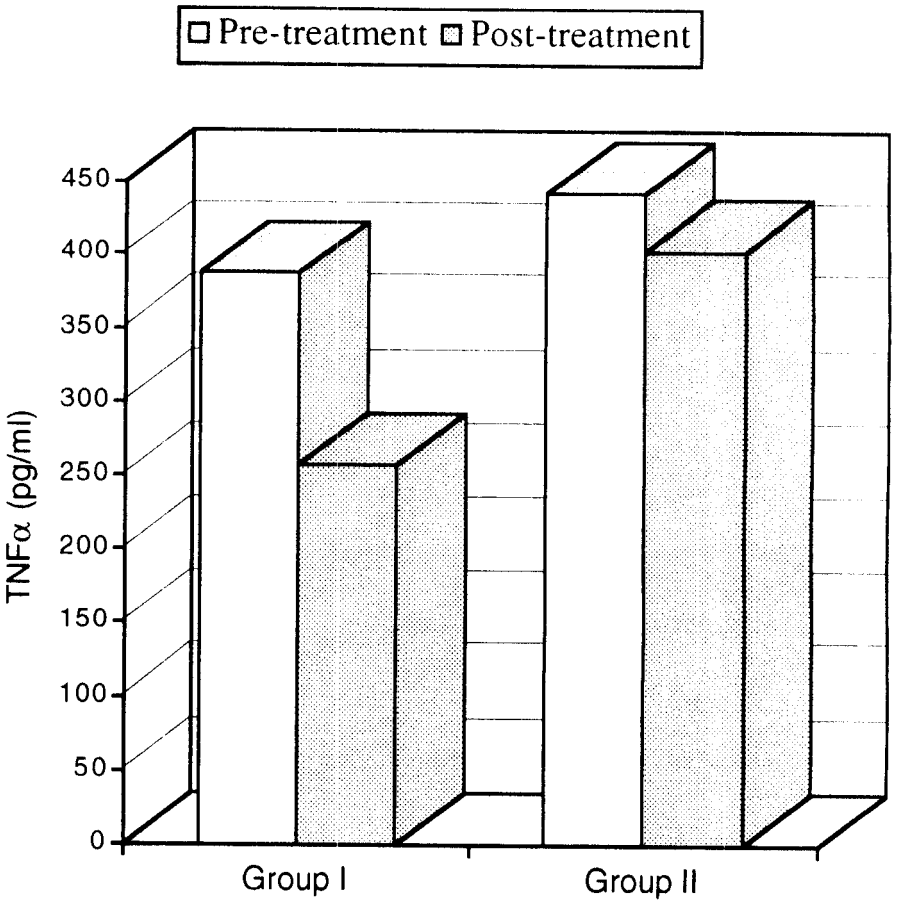


Fig. (11): Comparison between pre and post treatment values of TNFα levels in group I and II.

Table (16): Correlation matrix of TNFα to different studied parameters.

Parameter	Group I		Group II	
	r	P	r	P
Pre treatment				
Gestational age	-0.33	>0.05 (NS)	-0.09	>0.05 (NS)
Birth weight	-0.4	>0.05 (NS)	-0.13	>0.05 (NS)
Day of sepsis	-0.42	>0.05 (NS)	0.45	<0.05 (Sig)
Hematological score	0.75	<0.001 (Sig)	0.48	<0.05 (Sig)
Sepsis score	0.6	<0.01 (Sig)	0.84	<0.001 (Sig)
TLC	-0.42	>0.05 (NS)	-0.37	>0.05 (NS)
Platelets	-0.35	>0.05 (NS)	-0.47	<0.05 (Sig)
Post treatment				
Hematological score	0.83	<0.001 (Sig)	0.8	<0.001 (Sig)
Sepsis score	0.81	<0.001 (Sig)	0.94	<0.001 (Sig)
TLC	-0.41	>0.05 (NS)	-0.37	>0.05 (NS)
Platelets	-0.38	>0.05 (NS)	-0.81	<0.001 (Sig)

Table 16 shows that:

In group I pre-treatment:

- TNFα serum level was significantly correlated positively with hematological score ($r = 0.75$; $P < 0.001$) and sepsis score ($r = 0.6$; $P < 0.01$).
- No significant correlation existed between TNFα serum level and TLC ($r = -0.42$; $P > 0.05$).
- There was no significant correlation between TNFα serum level and gestational age or birth weight ($r = -0.33$ and -0.4, respectively) ($P > 0.05$).

In group I post-treatment:

- TNFα serum level was still significantly correlated positively with hematological score and sepsis score (r = 0.83 and 0.81, respectively) (P <0.001).
- No significant correlation existed between TNFα serum level and TLC (r = –0.41; P >0.05).

In group II pre-treatment:

- TNFα serum level was significantly correlated positively with hematological score (r = 0.48; P <0.05) and sepsis score (r = 0.84; P <0.001).
- No significant correlation between TNFα serum level and TLC, gestational age or birth weight (r = –0.37, –0.09 and –0.13, respectively) (P >0.05).
- There was a significant negative correlation between TNFα serum level and platelet count (r = –0.47; P <0.05).

In group II post-treatment:

- TNFα serum level was still significantly correlated with hematological score, sepsis score and platelet count (r = 0.8, 0.94 and –0.81, respectively) (P <0.001).
- There was no significant correlation between TNFα serum level and TLC (r = –0.37; P >0.05).

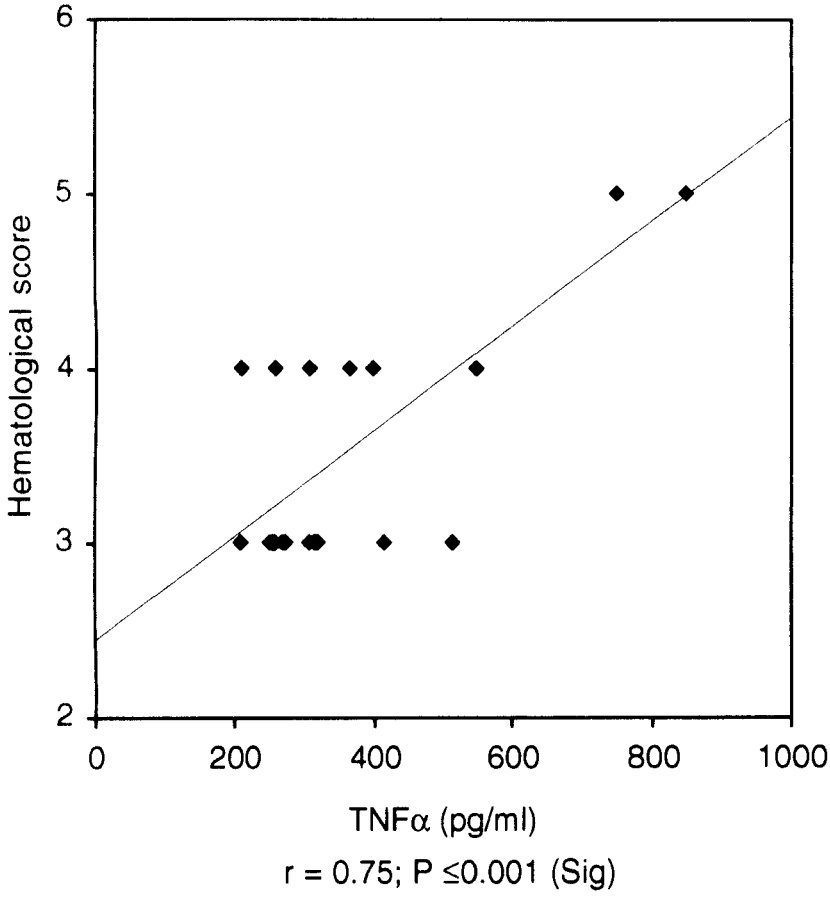


Fig. (12): Correlation between TNFα serum level and hematological score in group I (pre-treatment).

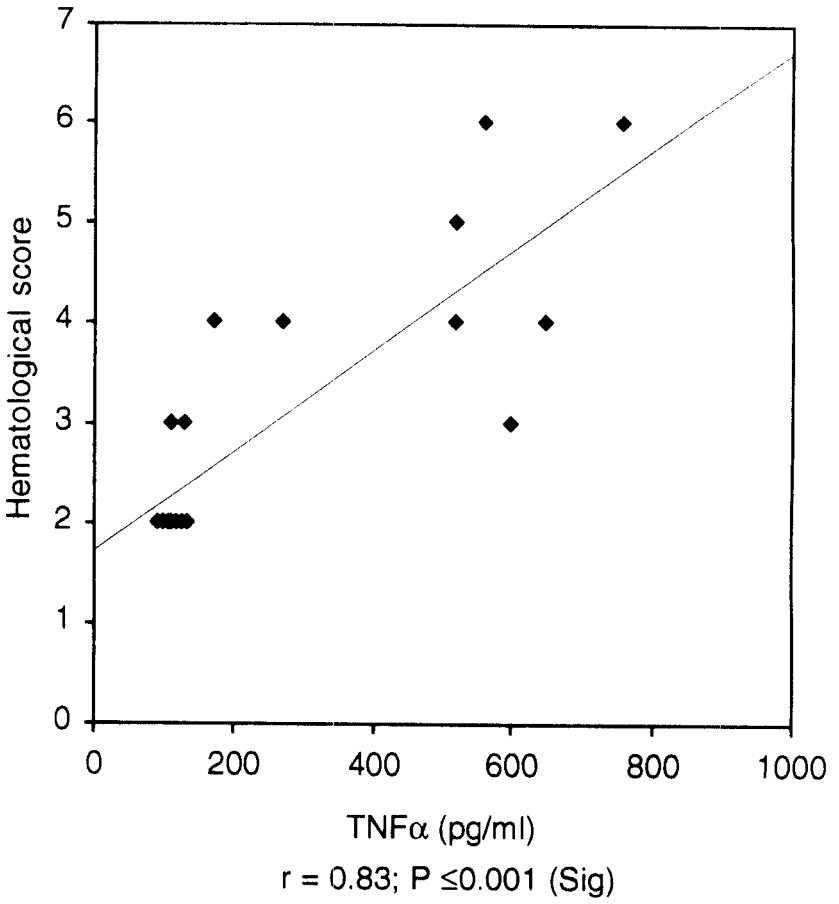


Fig. (13): Correlation between TNFα serum level and hematological score in group I (post-treatment).

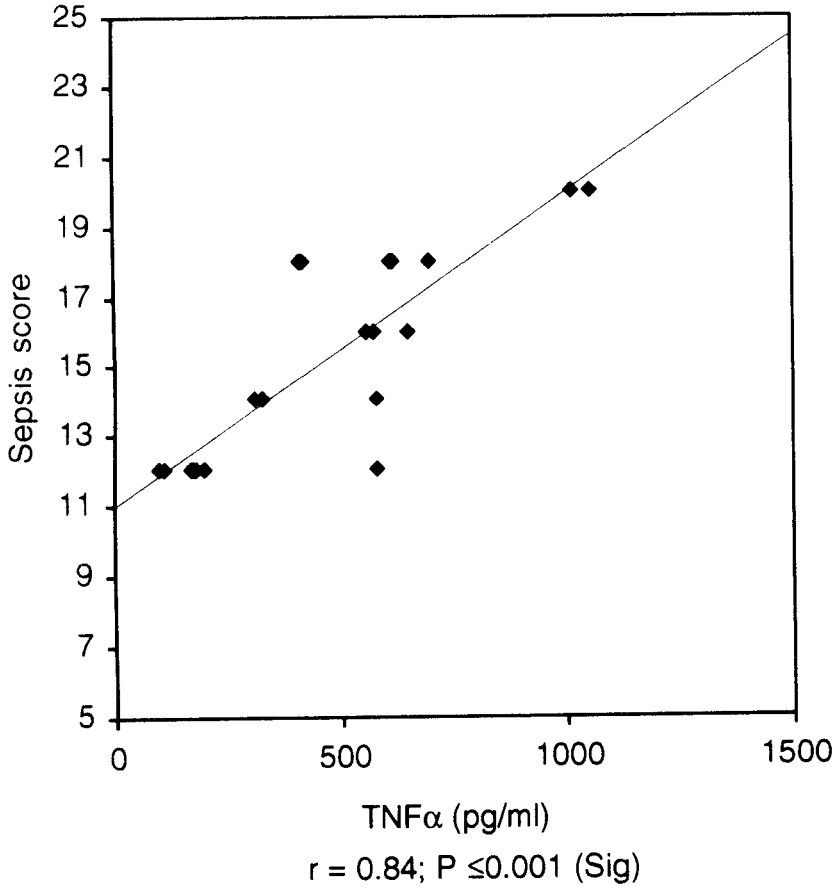


Fig. (14): Correlation between TNFα serum level and sepsis score in group II (pre-treatment).

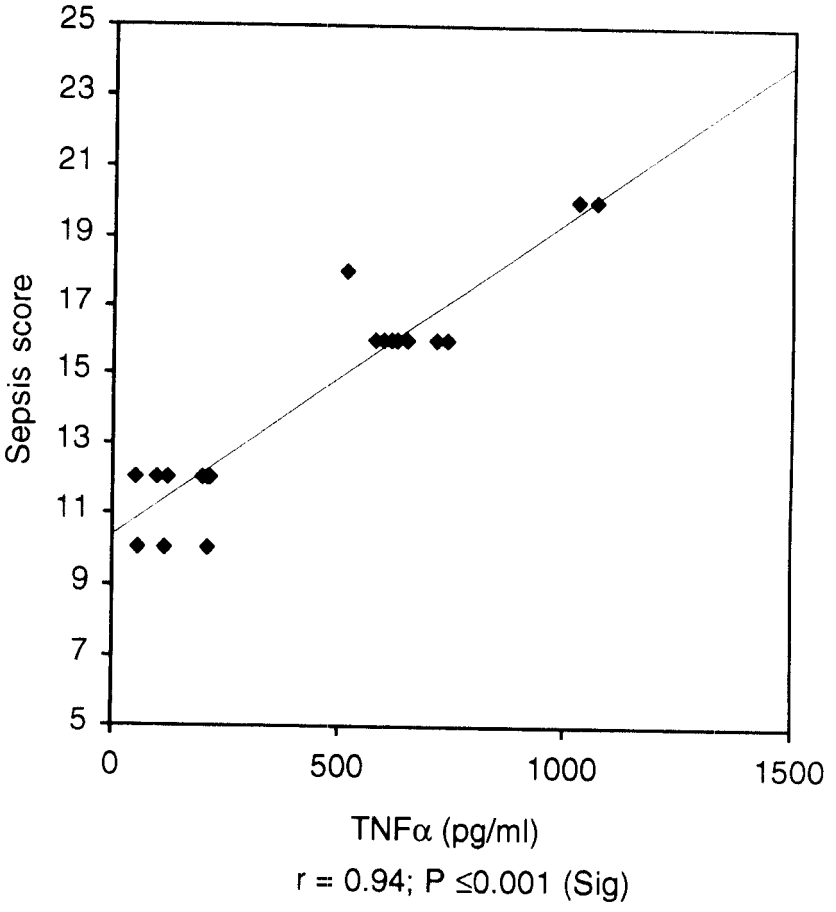


Fig. (15): Correlation between TNFα serum level and sepsis score in group II (post-treatment).

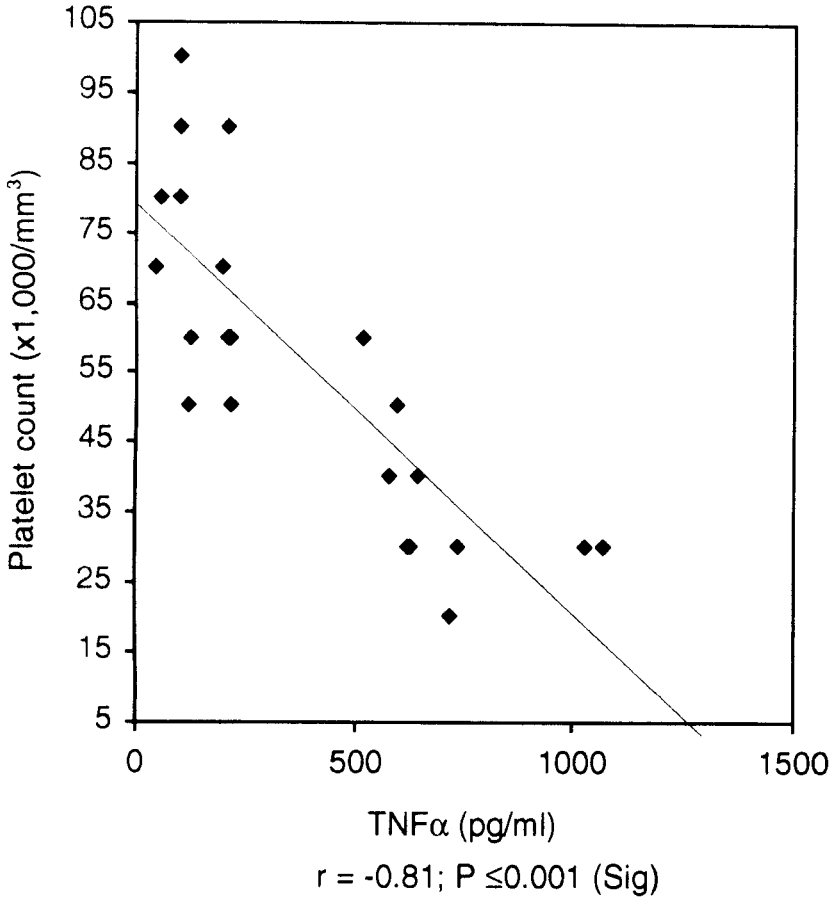


Fig. (16): Correlation between TNFα serum level and platelet count in group II (post-treatment).

Table (17): Relation between prognosis and TNFα level.

	TNFα Decrease		TNFα Increase		Totals	
	No.	%	No.	%	No.	%
Recovery	23	88%	2	11%	25	57%
Death	3	12%	16	89%	19	43%
Totals	26	100%	18	100%	44	100%

Chi Square =25.94; P <0.001 (Sig).

TNFα serum level decrease as a predictor of recovery:

Sensitivity	92%
Specificity	84%
Predictive value of positive test	88%
Predictive value of negative test	89%
Efficiency	89%

Table (17) shows that:

- There was a significant relation between prognosis and TNFα level (Chi-square = 25.94; P <0.001).
- As TNFα serum level decreased in 88% of recovered septic patients and increased in 89% of died septic patients.
- However, TNFα serum level decreased only in 12% of died septic patients while it increased in 11% of recovered septic patients.
- Sensitivity of TNFα concentration test as prognostic indicator of sepsis was 92%, specificity was 84%, the predictive value of positive test was 88% and the predictive value of negative test was 89%.
- Efficiency of TNFα concentration test as a prognostic indicator of sepsis was 89%.

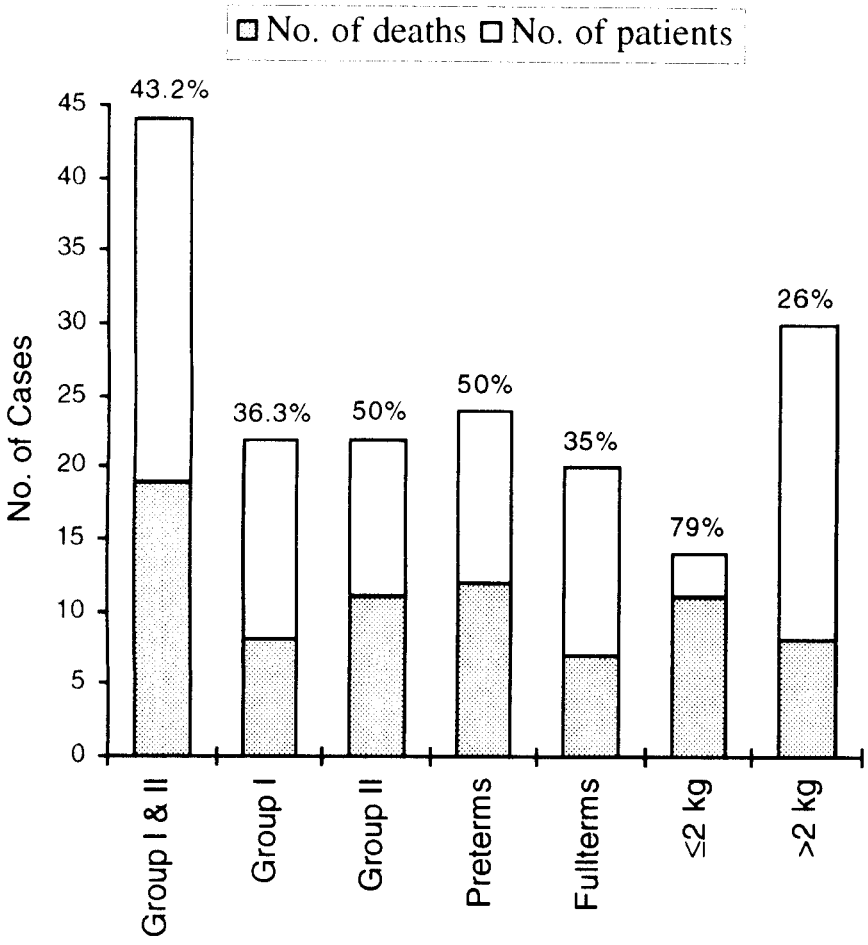


Fig. (17): Mortality rate among septic neonates.

Figure (17) shows that:

- Mortality rate among septic neonates (n=44) was 43.2%.
- Mortality rate in group I septic neonates (n=22) was 36.3%, while in group II septic neonates (n=22) it was 50%.
- Mortality rate in preterm septic neonates below 37 weeks (n=24) was 50%, while in fullterm septic neonates (n=20) it was 35%.

- Mortality rate in septic neonates with birth weight below or equal to 2 kg (n=14) was 79%, while in septic neonates with birth weight more than 2 kg (n=30) it was 26%.

Discussion

DISCUSSION

Sepsis and septic shock continue to be among the most leading causes of death in critically ill patients in spite of remarkable advances in life support systems (*Robinovici*, 1995).

The incidence of neonatal septicemia has been estimated to range from 1 to 8 per 1000 live births (*Klienaud Marcy*, 1995).

Pathophysiology of the systemic inflammatory response has advanced considerably with the improved understanding of cytokines and their effects.

Cytokines are groups of mediators whose release is triggered by antigen binding.

They directly influence cardiovascular, hemodynamics and coagulation mechanisms. They stimulate a cascade of mediator driven events involving the production and release of secondary mediators (*Roger*, 1995)

One of the cytokines that have been proposed as a major mediator in the cascade of pathophysiologic events that follow bacterial sepsis was tumour necrosis factor alpha (TNFα) (*Deforge et al.*, 1990).

A limited number of clinical studies are available on the relationship between TNFα in sepsis and septic shock among children and newborns (*Berger et al.*, 1991).

The present work was conducted on 44 neonates with sepsis divided randomly into 2 groups and 16 healthy neonates serving as a control group.

This study aimed to evaluate the involvement of TNFα in the pathogenesis of neonatal sepsis and the possible therapeutic outcome of dexamethasone therapy.

In general the symptoms of neonatal sepsis are variable and non specific (*Klein*, 1983).

No single laboratory test has been found to have acceptable specificity and sensitivity for predicting infections. Therefore the results of laboratory studies must be assessed in conjugation with presence of risk factor and clinical sign of sepsis (*Guerina*, 1991).

Multiple arrays of investigations were assessed to diagnose neonatal sepsis including different laboratory and clinical parameters. These clinical parameters were considered in the form of sepsis score (*Tollner*, 1982) and the laboratory one formed the hematological score (*Rodwell*, 1988).

In this present study among septic neonates there were 20 males and 24 females with a male to female ratio in group I was (1.2) while in group II was (0.57) ($P > 0.05$).

Premature with gestational age below 37 weeks were 54.5% of septic newborns and this increase susceptibility to infection in preterms might be related to defects in both humoral and cellular defence mechanisms.

Gotoff, (1992) stated that the most important neonatal factor predisposing to infection is prematurity.

In this study gestational age in group I ranged from 30 to 40 weeks with a mean of (34.6 weeks) compared to group II ranged between (30-40 weeks) with a mean of (35.1 weeks) with no significant difference ($P > 0.05$).

Also birth weight ranged from (1.05 to 3.8 kg) in group I with a mean of (2.55 kg) compared to group II birth weight

ranged from (1.11 to 4.4 kg) with a mean of (2.74 kg) of no significant difference between two groups (P>0.05).

Andreas et al. (1992) studied the occurrence of neonatal septicemia in 10 neonates and reported that their mean birth weight was 1.86 kg and their mean gestational age was 32 weeks.

Martens et al. (1993) in their study on 10 neonates with septicemia, reported that their mean birth weight was 2005 gms and their mean gestational age was 33 weeks.

This study use sepsis score suggested by *Tollner,* (1982) as a determinator of clinical severity of the septic response.

All cases of neonatal sepsis in our study had a sepsis score ≥10 and there was no significant difference between group I with sepsis score (mean 14.6 SD 2.34) and group II with sepsis score (mean 14.91 SD 2.91) with (P>0.05).

Also in this study, all cases of neonatal sepsis had a haematological score ≥3 and there was no statistical significant difference in hematological score between group I (mean 3.59 SD 0.73) and group II (mean 3.82 SD 0.8) (P>0.05). This finding was in agreement with the work of *Abdel Haleim et al.,* (1992).

In our study, total leucocytic count (TLC) was variable as leucopenia was found among 22.7% of cases in group I compared to 27.3% of cases in group II with no statistical significant difference (P>0.05).

Leucocytosis was demonstrated among 50% of cases in group I compared to 45.5% of cases in group II with no statistical significant difference (P>0.05).

27.3% of cases in group I had their total leucocytic count within normal range compared to 22.7% of cases in group II had normal leucocytic count.

This was in agreement with *Manero et al.*, (1979) who stated that in the early course of neonatal sepsis the leucocytic count may be normal.

A useful indicator of infections is the ratio of immature to total neutrophils (I/T ratio), the total immature neutrophil counts is divided by the total neutrophil count. The maximum normal I/T ratio is 0.12 from 5th day until the end of the first month (*Roberton,* 1992).

Philip and Hewitt, (1980) and *Rodwell* (1988) had stated that an I/T ratio >0.2 was a good marker of infections.

In this present work a significant number (90.9%) of septic newborns had an I/T ratio >0.2 and therefore it is considered a good marker of infections when present.

Thrombocytopenia was recognized as a sensitive and early indicator of bacterial sepsis.

In a study by *Gorrign,* (1974) on 72 neonates with septicemia (61%) of cases were found to be thrombocytopenic (less than 150,000/mm^3).

In this study an even much higher incidence (91%) of cases with thrombocytopenia were encountered among studied neonates with platelet count below 100,000/mm^3, and this may not occur until some time after the baby is obviously clinically septic.

However there was no statistical significant difference in platelet count in group I (91%) compared to group II (91%) with ($P<0.05$).

Among the studied septic newborns (44 neonates) all neonates had positive CRP i.e. 100% of cases. On the other hand false positive were obtained in 12.5% of normal healthy neonates of the control group.

Philip and Hewitt, (1980) used CRP as one of five indirect tests to diagnose neonatal sepsis which included (I/T, WBC, ESR, CRP, haptoglobin).

A neonate was considered septic when 2 tests or more were positive.

Seigel and McCracken, (1981) found that CRP, ESR, and haptoglobin and many other tests are unreliable indicators of neonatal sepsis when used individually.

Roberton (1992) suggested that the greatest use of CRP is in following the course of infection, falling levels strongly suggests that infection is responding to treatment.

Also *Gerdes,* (1991) stated that normalization of CRP or elevation appear to be a helpful tool in determining the response to antimicrobial therapy and the duration of treatment. Failure to mount a CRP response may be a poor prognostic sign.

An increase in the level of CRP without infections can be seen in cases with prolonged rupture of membranes, maternal fever during labour and perinatal asphyxia.

In our study blood culture was positive in 93.1% of cases of septic neonates (44 cases). Klebsiella was detected in 50% of cases while Pseudomonas was found in 15.9% of cases, E coli 9%, Staph and Strep was detected in 18.1% of cases while there was no growth in 6.8% of cases.

In another study, done at the NICU of Ain Shams University in 1993 by Ebrahim et al., it was found that blood culture was positive in all cases, klebsiella were isolated in 83.3% of cases, Pseudomonas in 10%, mixed klebsiella and Pseudomonas in 3.3% and E coli in 3.3% of cases.

TNFα was considered to be a marker of sepsis, regardless the site of infection, age of patient, or the type of organism.

Hawell et al., (1988) stated that TNFα was an early indicator of neonatal sepsis and the peak elevation of TNFα level in blood occurs 90 minutes after introduction of bacteria, which coincides with the occurrence of clinical signs and symptoms of sepsis, and half life was determined to be as short as 5–7 min.

Caplan, (1990) stated that TNFα serum level has been shown to be a good indicator of severity of bacterial infections.

In this study TNFα serum level was found highly elevated in septic neonates in group I (mean 387.05 ±183.56 pg/ml) and in group II (mean 442.9 ±276.99 pg/ml) compared to control group (mean 55.4 ±6.61 pg/ml) and this was of statistical significant difference (P>0.01).

While there was no significant difference between TNFα serum level in group I (mean 387.05 pg/ml) compared to group II (mean 442.9 pg/ml) with (P >0.05).

This was in agreement with *Bont et al.*, (1994) who found that TNFα serum level significantly higher in septic group than other group (healthy or suspected sepsis) with (P <0.001).

Bont et al., (1994) stated that when TNFα test is positive, the possibility of neonatal sepsis increases 12 fold while negative test results for TNFα or IL-6 decrease the possibility to 3 fold.

When both are positive the presence of neonatal sepsis is almost certain.

In our study considering a cut off value of TNFα serum level (65 pg/ml) it was found that all cases of group I and group II were above this cut off value and all control cases were below this value, denoting the high sensitivity and

specificity of the cut off value of TNFα serum level as a diagnostic test in neonatal sepsis.

Bont et al., (1994) study stated that the specificity of TNFα serum level as an indicator of sepsis was 94% and the sensitivity was 100% while *Shi et al.,* (1994) showed a specificity of 82%.

From the previous results of septic score, hematological score, CRP, bacteriological culture and TNFα level, it is evident that the selected two groups were septic on clinical and laboratory basis, and there were no differences regarding these parameters.

This standardization allows a valid comparison of the two groups regarding the therapeutic impact of dexamethasone.

In our study there was no significant correlation between TLC and TNFα serum level in groups I and II ($P > 0.05$).

Similar results was documented by *Ozdemier et al.,* (1994) when he compared level of TNFα, IL-1 beta and IL-6 and found no significant correlation between any of them and TLC.

This might be due to the fact that the origin of TNF is the tissue forms of mononuclear cells rather than the blood components.

In the present study there was a significant negative correlation between TNFα serum level and platelet count in group I and II ($P < 0.05$). This finding was in agreement with the work done by Abdel *Haleim et al.,* (1992) in which he found a significant negative correlation between TNFα serum level and platelet count ($P<0.05$) and this might be due to the effect of TNFα causing local intestinal platelet aggregation

factor (PAF) production which in turn play a role in the local intestinal consumption of platelets.

However *Ozdemir et al.*, (1994) found no significant correlation between TNFα level and platelet count ($P > 0.05$), this could be explained by the multiple factors causing thrombocytopenia in sepsis other than the increase of serum level of TNFα & PAF.

In our study there was a highly significant positive correlation between serum TNFα level and sepsis score prior to therapy. In group I with ($P < 0.01$) ($r = 0.6$) and in group II with ($P < 0.001$) ($r = 0.84$).

This was in agreement with the work of *Roman et al.*, (1993) in which he found a highly significant positive correlation between TNFα serum levels and sepsis score ($P < 0.001$).

Also there was a significant positive correlation between TNFα serum level and hematological score in group I with ($P < 0.001$) ($r = 0.76$) and in group II with ($P \leq 0.01$) ($r = 0.48$) and this was in agreement with the work of *Abdel Haleim et al.* (1992) in which he found a significant positive correlation between haematological score and serum TNFα levels ($P < 0.01$).

In our study there was a rise in TNFα serum level in septicemic neonates delivered by CS (mean 427.1 pg/ml) as compared to that of septicemic neonates delivered vaginally (mean 401.6 pg/ml) but of no statistical significant difference ($P > 0.05$).

This might be explained by the fact that deliveries by CS were either performed after several trials of vaginal delivery, or already done in critical cases with intrauterine fetal distress

this lead to a higher prevalence of sepsis and TNFα levels was related to the intensity of sepsis.

This was in agreement with *Roman et al.* (1993) who found that the degree of elevation of TNFα serum levels seems to be related to the severity of sepsis.

In the present study, there was no significant difference between TNFα serum level and the type of causative organism (gram-positive or negative) (P>0.05).

Bont et al. (1994) and *Roman et al.,* (1994) stated that there was no significant difference between patients with gram positive or gram negative bacterial sepsis and TNFα level and an increase in TNFα level is not a specific feature of gram negative sepsis but rather a general phenomena of severe bacterial infections.

In this study the comparison of TNFα serum level in preterm newborn <37 weeks (mean 437.2 pg/ml) and fullterm newborn (mean 388.2 pg/ml) showed no statistical significant difference (P>0.05).

Ozdemir et al., (1994) stated that both mature and premature neonates were able to produce a significant increase in blood level of cytokines in response to sepsis.

Bont et al. (1994) confirmed that TNFα serum levels were independent on gestational age.

In this study there was no significant difference in TNFα serum level between males (mean 375.2 pg/ml) and females (mean 448.1 pg/ml) in septic neonates with (P >0.05).

This finding was in agreement with the work of *Bont et al.,* (1994) in which he found that there was no relation between TNFα levels and sex of the patient.

In the present study there was a highly significant difference between TNFα serum levels pre-treatment in group I (mean 387.05 pg/ml) and that of post-treatment in group I also (mean 257.27 pg/ml) with ($P < 0.001$) Similar in group II the serum TNFα level pre-treatment was higher (mean 442.9 pg/ml) than that of post-treatment (403.4 pg/ml) but with no statistical significant difference ($P > 0.05$).

In our study TNFα serum level after treatment in group I (mean 257.2 pg/ml) was significantly lower than in group II (mean 403.4 pg/ml) with ($P > 0.05$).

This might be explained by the effect of dexamethasone as an adjunctive therapy in treatment of neonatal sepsis in group I, as being an anti-inflammatory drug, it leads to marked reduction in the level of TNFα concentration in group I than in group II of patients who received antibiotics only in the treatment of sepsis.

This was documented by *Odio et al.*, (1991) who found that dexamethasone will decrease serum TNFα level and improve outcome when it was used as an adjunctive therapy for bacterial infections in infants and children and when it was administered early with antibiotics at the time of diagnosis.

In this study the mortality rate among septic neonates (n=44) was 43.1%.

Preterm septic newborns below 37 weeks (n=24) had a mortality rate of 50% while fullterm septic newborns (n=20) had a mortality rate of 35%.

This high mortality rate among preterms might be related to defects to defects in both humoral and cellular defence mechanisms.

This finding was in agreement with the work of *Marten et al.* (1993), in which they found that preterm infants were more severely affected and had a significant higher mortality rate.

In the present study the mortality rate in septic neonates with birth weight below or equal to 2 kg was 79%, while the mortality rate in septic neonates with birth weight more than 2 kg was 26%.

This was in agreement with *Boyer et al.,* (1983), who found that the mortality rate for the group of neonatal sepsis with birth weight from 1501–2000 gms was 33% and for those with a birth weight from 2000–2500 gm was 29% and those with a birth weight more than 2500 gms, the mortality rate was 3%.

Also in our study the mortality rate among septic newborns in group I was 36.3% while in group II it was 50%.

We noticed that the mortality rate in group I was lower than that of group II and this might be explained again by the effect of dexamethasone therapy in treatment of neonatal sepsis in group I which lead to modulation of inflammation, thus improving the course of sepsis, decreasing the incidence of septic shock, and improving the survival rate and long term outcome.

In *Shumer's* study (1989) stated that there was a significant difference in mortality rate in steroid group 20% compared to 49% in non steroid group of patient.

Hinshow et al., (1987) found no significant difference in mortality rate between steroid and non steroid patient and this might be due to the late use of steroid in treatment of neonatal sepsis or due to the use of uneffective doses.

Roman et al. (1993) didn't use any score for determination of clinical severity of septic newborns, he stated

that the degree of elevation of TNFα level seems to be related to the severity of sepsis since those newborns who died had the highest level of TNFα.

In this study, the mean serum level of TNFα in patient who died with sepsis (mean 506.2 pg/ml) was significantly higher than those patients who recovered in group I (mean 115.0 pg/ml) with ($P < 0.001$). Similar results were obtained in group II.

This was in agreement with *Roman et al.*, (1993) who stated that TNFα level was higher in those who died in septic group compared to those who survived and the degree of elevation of TNFα seems to be related to the severity of sepsis.

Marten et al. (1993) stated that the increase in serum TNFα levels were significantly associated with occurrence of septic shock, organ failure, sclerma and fatal outcome.

In this present study there was a statistical significant difference in TNFα serum level among recovered patients in group I (mean 115.0 pg/ml) and recovered patients in group II (mean 198.64 pg/ml) ($P<0.01$).

However we noticed that in group I the mean TNFα level among the recovered patients was lower than that of group II, this may be due to the effect of steroid treatment in group I which lead to much reduction in TNFα level.

This finding was in agreement with *Odio et al.*, (1991) who stated that the concentration of TNFα and cytokines in blood improved rapidly within 12 hours in steroid treated patients as compared to patients given antibiotics only.

Weitzman and Berger, (1989) found that the effectiveness of corticosteroid therapy depends on the point at which they are initiated in the course of illness.

Lebel et al., (1989) stated that the adverse effect of dexamethasone were uncommon and the advantages of its utilization in treatment outweigh the possible disadvantages. However the duration of steroid administration is an important determinant of both efficacy and toxicity.

In this study there was a significant relation between prognosis and TNFα level (Chi-square 25.94; P <0.001) where TNFα serum concentration was decreased in 88% of the recovered patients while it increased in 98% of died septic patients.

This means that when TNFα level decreased, the possibility of recovery (good prognosis) increased and when TNFα level increased bad prognosis was expected.

Cannon et al., (1990) found that there was a high level of TNFα and IL-1, in severe septic patient and this correlate with the severity of illness and TNFα serum level have to be a sensitive and specific test for predicting septic shock and its clinical outcome.

In this study there was a highly significant difference between sepsis score pre and post-treatment in group I and in group II with (P ≤0.001).

Also there was a significant difference between hematological score pre and post-treatment in group I with (P <0.01).

This means that sepsis score suggested by *Tollner,* (1982) and hematological score suggested by *Rodwell,* (1988) considered as a determinator of the severity of the septic response and the improvement in patients after treatment lead to the improvement in both sepsis and hematological score. This was in agreement with the work of *Roman et al.,* (1993).

In the present study, TNFα serum level still correlates with sepsis score after treatment in group I and in group II with ($P \leq 0.001$).

Also TNFα serum level correlated with the haematological score after treatment in group I and in group II with ($P < 0.001$).

This correlation proves that the improvement of neonatal sepsis either clinical or laboratory was related to TNFα concentration which can predict the possible outcome and make TNFα serum level test an early and sensitive prognostic indicator of sepsis.

Roman et al., (1993) stated that the sensitivity of TNFα serum level as a test of prognostic indicator of sepsis was 91.3% while the specificity was 100%.

In our study the sensitivity of TNFα concentration as a test of prognostic indicator of sepsis was 92% while the specificity was 84%

Shi et al., (1994) showed a sensitivity of 83% and a specificity 82% while the positive predictive value was 83% and negative predictive value was 88%.

In our study TNFα serum level decreased in 12% of the septic patients who died while it increased in 11% of those who recovered. So, in this study the predictive value of positive test was 88% while predictive value of negative test was 89%.

However the efficiency of the TNFα concentration test as an early prognostic indicator of sepsis was 89%.

So the development of a rapid, accurate and cheap test for detection of serum TNFα is needed to allow its utilization as an early predictor of sepsis, in follow up of cases and in detecting their prognosis.

Summary and Conclusion

SUMMARY AND CONCLUSION

This work was carried out on 44 neonates with septicemia selected from the NICU of Obstetrics and Gynecology Hospital of Ain Shams University aimied to evaluate the clinical value of TNF-α serum level in follow up of cases of neonatal sepsis in steroid and nonsteroid treated patients and its correlation with clinical picture, sepsis score, haematological score, outcome and other laboratory markers of sepsis.

For this purpose, all neonates in this study were subjected to history taking, clinical examinations, laboratory investigations including (CBC, CRP, blood culture) and estimation of serum TNF-α level (using ELISA technique) at the time of diagnosis and 4 days after treatment.

In our study, there was a highly significant rise in serum TNF-α level in neonates with septicemia in group I (mean 387.0 pg/ml) and in group II (mean 442.95 pg/ml) compared to the control group (healthy neonates) (mean 55.4 pg/ml) with ($P < 0.01$).

As regard CRP, 100% of septic neonates in group I and group II had positive CRP, while it was positive in 12.5% only of cases in the control group.

There was no statistically significant difference between serum TNF-α level in group I (mean 387.0 pg/ml) and in group II (mean 442.94 pg/ml) before start of treatment at the time of diagnosis of sepsis ($P > 0.05$).

After treatment there was a significant difference between TNF-α serum level in group I (mean 257.2 pg/ml) and in group II (mean 403.4 pg/ml) ($P < 0.05$).

There was a highly significant difference in TNF-α serum level in patients who recovered in group I (mean 115.00 pg/ml) compared to patients who died in group I also (mean 506.2 pg/ml) ($P < 0.01$), and the same result was present in group II.

In this study, serum TNF-α level in neonates with sepsis who were cured in group I was significantly lower (mean 115.00 pg/ml) than those who were cured in group II (mean 198.00 pg/ml) ($P < 0.01$)

No significant correlation was found between mean TNF-α serum level of septic newborns and their gestational age, birth weight and sex. But, there was a significant correlation between TNF-α serum level of septic newborns and their sepsis score, haematological score and platelet count before and after treatment.

There was no significant correlation between TNF-α serum level of septic newborns and their total leucocytic count (TLC).

There was a significant relation between fate of neonatal sepsis (outcome) and level of TNF-α in the serum.

In this study, it was found that the sensitivity of TNFα concentration test as a prognostic indicator of sepsis was 92% while the specificity was 88%.

In our study, it was found that mortality rate in group I (steroid treatment) was lower than that of group II (nonsteroid treatment).

As a conclusion, production of TNF-α appears to be a fundamental event in the initiation and maintenance of the inflammatory response in neonatal sepsis and plays an important role in amplifying the course of sepsis and its level is related to the severity of clinical course of disease. So that

TNF-α serum level being raised in neonatal septicemia could be used as a diagnostic and prognostic parameter that helps early detection and follow up of neonates with sepsis.

Also it was found that administration of dexamethasone as adjunctive therapy with antibiotics in treatment of neonatal sepsis decreased serum level of TNFα and improved long term outcome and decreased the possibility of occurrence of complications and decreased mortality rate. So, because of its advantages which outweighed its complications, we can use dexamethasone as adjunctive therapy in treatment of neonates with septicemia.

It was found that sensitivity of the TNF-α test as prognostic indicator of sepsis was (92%) while specificity was (88%).

ABSTRACT

Modulation of Inflammation and Cachectin Activity in Relation to Treatment and Nutrition of Neonatal Sepsis

Ph.D. Degree in Medical Childhood Studies

Rehab Abdel Kader Mahmoud Moustafa

This work was carried out on 44 neonates with septicemia as well as 16 healthy neonates serving as a control group selected from the NICU of Obstetrics and Gynecology Hospital of Ain Shams University, aimed to evaluate the clinical value of TNFα serum level in follow up of cases of neonatal sepsis in steroid and non-steroid treated patients.

For this purpose patients with sepsis were randomly classified into 2 groups. All neonates were subjected to history taking, clinical examination, laboratory investigations and estimation of serum TNFα level using ELISA technique just at time of diagnosis and 4 days after treatment.

In this study it was found that there was a highly significant rise in serum TNFα in neonates with septicemia in group I (mean 387 pg/ml) and in group II (mean 442.9 pg/ml) compared to the control group.

After treatment there was a significant difference between TNFα serum level in group I (mean 257.2 pg/ml) and in group II (mean 403.4 pg/ml) and there was a highly significant difference in TNFα serum level in patients who recovered in both groups compared to patients who died.

In this study it was found that sensitivity of TNFα concentration test as a prognostic indicator of sepsis was 92%. Mortality rate in this study in group I was 36% while in group II it was 50%.

Key words: Neonatal sepsis
Tumour necrosis factor (Cachectin)

Recommendations

RECOMMENDATIONS

1. Trials should be made to decrease the risk factors of neonatal sepsis especially the prematurity since the risk of infection are higher in the preterm neonates and since it carries a worse prognosis.
2. TNF-α serum level in neonatal septicemia could be used as a diagnostic and prognostic parameter that helps early detection and follow up of patients with septicemia.
3. Development of easy and cheap applicable methods for detection of TNFα make this test a reliable and sensitive test in follow up of patients of with neonatal sepsis.
4. Early administration of dexamethasone with antibiotics as an adjunctive therapy in treatment of neonatal sepsis will improve outcome and decrease mortality rate.
5. Monoclonal antibodies against TNFα have to be tried and considered as a part of treatment trial of neonatal sepsis and septic shock.

References

REFERENCES

Abd El-Haliem A; Mokhtar G; Awad H and El Rasd M (1992):
TNF-α in NEC.
Thesis for partial fulfilment of Master Degree in Paediatrics, Ain Shams University.

Amal SI and Soliman N (1994):
Mortality and morbidity in Neonatal Intensive Care Unit of Ain Shams University Hospitals.
Thesis for partial fulfilment of Master Degree in Pediatrics, Ain Shams University.

Andreas CK (1995):
Can better measures of cytokine response be obtained to guide cytokine inhibition?
Journal of Endotoxin Research: Abstracts of Conference on sepsis/septic inflammatory response: reducing mortality to patients and suppliers, February 20–21, the Washington Court Hotel, Washington.

Andreas HS; Meiser A; Weise M (1992):
IL-6 as early mediator in neonatal sepsis.
Pediatr Infect Dis J; 11 (6) : 496–498.

Baker CJ and Edwards MS (1990):
Group-B streptococcal infections.
In: Remington JS, Klein JO (eds.). Infectious Disease of the Fetus and Newborn Infant, 32nd ed. WB Saunders Company, Philadelphia, London, Tokyo, p. 742.

Baley JE (1988):
Neonatal sepsis. The potential for immunotherapy.
Clin Perinatol; 15 : 755–771.

Baley JE and Stork EK (1988):
Neonatal neutropenia.
AJDC; 142 : 1161.

Baley JE; Kliegman RM; Boxerbaum B and Fanaroff AA (1986):
Fungal colonization in very low birth weight infant.
Pediatrics; 78 (2) : 225.

Bell NE and McGuiness GA (1982):
Suppurative central nervous system infection in the neonate.
Semin Perinatol; 6 : 1.

Bennet R; Eriksson M and Zelterstrom R (1981):
Increasing incidence of neonatal septicaemia causative organism and predisposing risk factors.
Acta Pediatr Scand; 70 : 207.

Bennet R; Eriksson M; Mord CE and Zelterstrom R (1982):
Suppression of aerobic and anaerobic faecal flora in newborns receiving parenteral gentamycin and ampicillin.
Acta Pediatr Scand; 71 : 559.

Berger M (1991):
Use of intravenously administered immunoglobulin in newborn infant (prophylaxis and treatment).
J Pediatr; 118 : 557–559.

Bergstrom S; Johson H; Ewald U and Schwan A (1992):
Neonatal septicaemia caused by pneumococci.
Acta Obst Gynaecol Scand; 7 (1) : 6–11.

Beutler B and Cerami A (1987):
Cachectin: more than a tumour necrosis factor.
N Engl J Med; 3316 : 379.

Beutler BA, Milsark JV and Cerami A (1988):
Cachectin/tumour necrosis factor: Production, distribution and metabolic rate in vivo.
J Immunol; 135 : 3972.

Bont ESJM; Martens A; Van Raan J (1993):
Tumour necrosis factor-α, interleukin-1. Beta and interleukin-6 plasma levels in neonatal sepsis.
Pediatr Res; 33 : 380–4.

Bont ESJM; Martens A; Van Raan J (1994):
Diagnostic value of plasma levels of tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in newborns with sepsis.
Acta Pediatr; 83 : 696–9.

Boride DH (1995):
Inflammatory cell: structure and function.
In: Stites DP and Terr AI, eds. Basic and Clinical Immunology. 8th ed. Lange Medical Book USA New Jersey, p 141.

Boyer KM; Gradzala CA; Burd LI; Fisher DE; Paton JB and Gotoff SP (1983):
Selective intrapartum chemoprophylaxis of neonatal group B-streptococcal early onset-disease. I. Epidemiological rational.
J Infect Dis; 148 : 795.

Cairo MS (1991):
Cytokines. A new immunotherapy. In: Polin RA and Speck WT (eds.). Clinics in Perinatalogy. Volume 18 (2). WB Saunders Company, Philadelphia, London, Tokyo, Toronto, p 343.

Cairo MS; Worcester CC; Rucker RW and Hanter S (1992):
Randomized trial of granulocyte transfusions versus intravenous immunoglobulin therapy for neonatal neutropenia and sepsis.
J Pediatr; 12 : 281.

Campognone P and Singer DB (1986):
Neonatal sepsis due to non typeable haemophilus influenza.
Am J Dis Child; 140 : 117.

Cannistra SA; Vennenga E; Groshek P (1988):
Human granulocyte-monocyte colony-stimulating factor and interleukin-3 stimulate monocyte cytotoxicity through a tumor necrosis factor-dependent mechanism.
Blood; 71 : 672.

Cannon JG; Tompkins RG; Gelfand JA; Michie HR; Stanford GG; Van der Meer JWM (1990):
Circulating interleukin-1 and tumour necrosis factor in septic shock and experimental endotoxin fever.
J Infect Dis; 161 : 79–84.

Caplan EA; Oed LJ and Kassel RL (1990):
Cytokines serum level in neonatal sepsis.
Ann Surg; 112–210.

Caput D; Beutler B; Hartog K; Brown-Scheimer S and Cerami A (1986):
Identification of a common nucleotide sequence in the 3`-untranslated region of mRNA molecules specifying inflammatory mediators.
Proc. Natl. Acad. Sci U.S.A.; 83 : 167–74.

Carter MJ; Gerstmann DR; Clark MR (1990):
High frequency oscillatory ventilation and extra-corporeal membrane oxygenation for the treatment of acute neonatal respiratory failure.
Pediatrics; 85 : 159–162.

Catherine MH; Costarino AT; Sulivan JS; Dulkerian S (1994): Cytokine elevations in critically ill infants with sepsis and necrotizing enterocolitis.
J Pediatr; 124 : 105-111.

Christensen RD (1984):
Methods of prevention and treatment of neonatal septicemia.
J Clin Apheresis; 2 (1) : 98.

Christensen RD and Rothstein G (1988):
Exhaustion of mature marrow neutrophils in neonates with sepsis.
J Pediatr; 96 : 316–318.

Christensen RD; Anstail HB and Rothstein G (1982):
Deficiencies in the neutrophil system of newborn infants and use of leukocyte transfusion in the treatment of neonatal sepsis.
J Clin Apheresis; 1 : 33.

Cole FS (1991):
Infections and immunologic defense mechanisms.
In: Taeusch HW, Ballard RA and Avery ME (eds.) : Schaffers and Avery's Disease of the Newborn, 6th ed. WB Saunders Company, Philadelphia, London, Tokyo, p 305.

Corrigan JJ Jr (1974):
Thrombocytopenia: A laboratory sign of septicemia in infants and children.
J Pediatr; 85 : 219–221.

Cumberbatch M; Fielding I and Kimber I (1994):
Modulation of epidermal Langerhan's cell frequency by TNF-α.
Immunology; 81 : 395–401.

Damas P; Ledaux D; De Groote D; Franchimont P and Lamy M (1992):
Cytokine serum level during severe sepsis in human IL-6 as a marker of severity.
Ann Surg; 215 (4) : 356–62.

Dayer JM; Beutler B and Cerami A (1985):
Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts.
J Exp Med; 162 : 2163–68.

De Forge LE; Nguyen DT; Kunkel SL and Remick DG (1990):
Regulation of the pathophysiology of TNF-α.
J Lab Clin Med; 116 : 429–36.

De Louvois J and Harvey DR (1988):
Antibiotic therapy of the newborn.
Clin Perinatol; 15 : 365–388.

Debets JMH; Kampmeijer R; Van der linder MPMH (1989):
Plasma tumor necrosis factor and mortality in critically ill septic patients.
Crit Care Med; 17 : 489.

Dinarello CA; Cannon JG; Wolff SM; Figari IS; Palladino Jr MA and O'Connor JV (1986):
Tumor necrosis factor/cachectin is an endogenous pyrogen and induces production of IL-1.
J Exp Med; 163 : 1433–50.

Dobson SRM and Baker CJ (1990):
Enterococcal sepsis in neonates: features by age of onset and occurrence of focal infection.
Pediatrics; 85 (2) : 165.

Dohyns E (1993):
Neonatal sepsis.
In: Peter RH (ed.) : Textbook of Pediatric Critical Care. 3rd ed. WB Saunders Company, Philadelphia, London, Tokyo, p 98.

Donates LA (1979):
The fetus and neonatal infection.
Obstet Gynecol; 73 : 823.

Dubowitz LMS; Dobowitz V and Goldberg C (1977):
Clinical assessment of gestational age in the newborn infant.
J Pediatr; 77 : 1.

Ebrahim HM (1993):
Role of exchange transfusion in the treatment of neonatal septicemia in the neonatal intensive care unit.
Master Thesis in Pediatrics, Ain Shams University.

El-Gamal Y; Haroun AA; El Shimi M; Abo Mosalem M and El Samahi M (1989):
A new look on buffy coat smear examination in high risk pregnancy and neonatal infection.
Egypt J Pediatr; 6 (3–4) : 301–313.

El-Maraghi S; El Saifi A and Abou Alam M (1984):
Diagnosis of neonatal septicemia by gastric aspirate examination.
J Egypt Soc; X (1) : 43–48.

Ellner JJ (1983):
Septic shock.
Pediatr Clin North Am; 30 (2) : 365–371.

Ertel W; Morrison MH; Agala A; Perrin MM and Chaudry IH (1991):
Anti-TNF monoclonal antibodies prevent haemorrhage-induced suppression of Kupffer cell antigen presentation and MHC class II antigen expression.
Immunology; 74 : 290–297.

Faidas A; Shopard D and Land James L (1993):
Magnetic resonance imaging Listeria brain stem encephalitis.
Clin Infect Dis; 16 : 186.

Feigin RD; Abramson SL; Edward MS (1987):
Fungal and protozoal infections in neonates.
In: Fanaroff AA and Martin RJ (eds.). Perinatal Medicine Diseases of the Fetus and Infant, 4th ed. The CV Mosby Company, St Louis, Washington, p 817.

Fleverstein GZ (1995):
Pulmonary TNF-α is a critical mediator in adult respiratory distress syndrome.
Journal of Endotoxin Research: Abstracts of Conference on sepsis/septic inflammatory response: reducing mortality to patients and suppliers, February 20–21, the Washington Court Hotel, Washington.

Fong Y and Lowry SF (1990):
TNF in the pathophysiology of infections and sepsis.
Clin Immunol Immunopathol 55 : 157–170.

Freedman MH; Cohen A; Grunberger T; Bumin N; Luddy RF; Saunders EF; Shahidi N; Lau A and Eshaw Z (1991):
Central role of TNF-α, GM-CSF and IL-1 in the pathogenesis of juvenile chronic myogenous leukaemia.
Br J Haematol; 80 : 40–48.

Freedman RM; Ingram DL; Gross I (1981):
A half century of neonatal sepsis at Yale.
Am J Dis Child; 135 : 140.

Freeman J (1990):
Bacterial and fungal infection in manual of neonatal care, 3rd ed. (Eds.): John P, Cloherty MD and Ann R Stark MD. Little, Brown and Company, 146.

Gabrilove JL and Jakubowski A (1989):
Granulocyte colony-stimulating factor, preclinical and clinical studies.
Hematol Oncol Clin North Am; 3 : 427.

Gaur S; Kesarwala H; Gavai M and Gupta M (1994):
Clinical immunology and infectious diseases.
Pediatr Clin N Am; 41 (4) : 745–759.

Gerdes JS (1991):
Clinicopathologic approach to the diagnosis of neonatal sepsis.
Clinics in Perinatalogy; 18 (2) : 361–374

Gibbs RS; Dinsmoor MJ and Newton ER (1988):
A randomized trail of intrapartum versus immediate postpartum treatment of woman with intra-amniotic infection.
Obstet Gynecol; 73 : 823.

Girardin E; Oyam GE; Dayer JM and Lambard PR (1988):
Infections purpura.
N Engl J Med; 319 (7) : 397–400.

Givner LB and Nagaraj SK (1993):
Hyperimmune human IgG or recombinant human granulocyte-macrophage colony stimulating factor as adjunctive therapy for group B streptococcal sepsis in newborn rats.
J Pediatr; 122 : 774–779.

Gotoff SP (1992):
The fetus and the newborn.
In: Nelson WE and Vaughan VC (eds.). Nelson Textbook of Pediatrics, 14th ed. WB Saunders Company, Philadelphia, p 501.

Grauel EL; Halle E; Ebollmann R (1989):
Neonatal septicemia, incidence, etiology and outcome.
Acta Pediatr Scand; 360 : 113–119.

Groll AH (1992):
Interleukin-6 as early mediator in neonatal sepsis.
Pediatr Infect Dis J; 11 : 496.

Gronross P; Vesikari T; Janas M (1985):
Neonatal septicemia.
Arch Dis Child; 60 : 542.

Guerina NG (1991):
Bacterial and fungal infection.
In: Cloherty JP and Stark AR (eds.). Manual of Neonatal Care. 3rd ed. Little Brown and Company, London, p 146.

Guillermin R; Vargo TM; Rossier J (1977):
Beta-endorphin and adrenatal corticotropin and secreted concomitantly by the pituitary gland.
Science; 197 : 1367–70.

Guillois B; Berthau C; Awad H (1989):
The importance of C_3d estimation in the diagnosis of generalized bacterial infections in newborn infants.
Acta Pediatr Scand; 78 : 369.

Hamada Y; Kobayashi K and Brown WR (1991):
TNF-α decreases expression of the intestinal IgG Fc binding site by HT 29-N_2 cells.
Immunology; 74 : 298–303.

Haque KH (1992):
Infection and immunity in the newborn.
In: Campbell AGM and McIntosh N (eds.). Forfar and Arneil's Textbook of Pediatrics, 4th ed. Churchill Livingstone, Edinburgh, p 302–316.

Haque KN; Zaidi MH; Haque SK and Bahakim H (1986):
Intravenous immunoglobulin for prevention of sepsis in preterm and low birth weight infants.
Pediatr Infect Dis; 5 : 622–625.

Havell EA; Fiers W and Robert J North (1988):
Anti-tumour function of TNF.
J Exp Med Rol 167 : 1067–1085.

Hesse DG; Tracey KF and Fong Y (1988):
Cytokines appearance in human endotoxemia and primate bacteremia.
Surg Gynecol Obstet; 166 : 147–153.

Hillier SL; Matius J; Krohn M (1988):
A case-control study of chorioamniotic infection and histologic chorioamnionitis in prematurity.
N Engl J Med; 319 : 972–978.

Hinshaw LB; Bellar-Todd BK and Archer LT (1987):
Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis.
Circ Shock 9 : 543–53.

Holbrook PR (1993):
Neonatal sepsis.
In: Peter RH (ed.). Textbook of Pediatric Critical Care. 3rd ed. WB Saunders Company, Philadelphia, London, Toronto, p 98–113.

Hosford D and Braquet P (1990):
The potential role of platelet-activating factor in shock and ischemia.
J Crit Care; 5 : 115.

Ismail OH; Shatla H; Ibrahim NA (1988):
Evaluation of the diagnostic value of serum haptoglobin and leucocyte alkaline phosphatase activity in neonatal bacterial infection.
Egypt J Med Sci; 9 (2) : 329.

Jacob CO (1992):
Tumor necrosis factor-α in autoimmunity: Pretty girl or old witch.
Immunology Today; 13 (4) : 122–25.

Jacobs NM (1991):
Antibacterial therapy.
In: Neonatal Microbiology. 18th ed. (Eds.): Applenton and Lange Norwalk, Connecticut, San Mates, California, p. 187.

Jarvis WR (1987):
Bacterial and fungal infection.
In: Manual of Neonatal Care, 3rd ed. (Eds.): John P, Cloherty MD and Ann R Stark MD. Little, Brown and Company, p. 145.

Joachin RH and Flores NG (1995):
Development of high risk infants following premature membrane rupture in a neonatal intensive care unit.
Gynecol-Obstet-Mey; 63 : 119–122.

Kending JW; Notter RH; Cox C (1991):
A comparison of surfactant as immediate prophylaxis and as a rescue therapy in newborns of less than 30 weeks gestation.
N Engl J Med; 324 : 865–871.

Kindler V; Sappino AP; Grau GE; Piguet PF and Vassalli P (1989):
The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection.
Cell; 56 : 731–40.

Kite P; Millar MR; Gorham P and Congdon P (1988):
Comparison of five tests used in the diagnosis of neonatal bacteremia.
Arch Dis Child; 63 : 639–643.

Klein JO and Marcy SM (1995):
Bacterial sepsis and meningitis.
In: Remington JS and Klein JO (eds.). Infectious Diseases of the Fetus and Newborn Infant, 9th ed. WB Saunders Company, Philadelphia, London, Tokyo, p 836.

Kosloske AM; Ulrich JA and Hoffman H (1978):
Fulminant necrotizing enterocolitis associated with clostridia.
Lancet; 2 : 1014.

Kreger BE; Craven DE; McCabe WR (1980):
Gram-negative bacteremia. IV. Re-evaluation of clinical features and treatment in 612 patients.
Am J Med; 68 : 344–354.

Kriegler M; Perez C; Defaz K; Albert I and Lus; D (1988):
A novel form of TNF/cachectic is a cell surface cytotoxic transmembrane protein.
Ramifications for Cells; 53 : 54.

Lebel MH; Freij BJ; Syrogiamopoulos GA (1988):
Dexamethasone therapy for bacterial infections; results of two double-blind, placebo, controlled trials.
N Engl J Med; 319 : 964–71.

Lewis CE (1992):
The Macrophage (the natural immune system).
Clin Lab Hematol; 200–270.

Liu CH; Lethan C and Speer ME (1984):
Degenerative changes in neutrophils indicator of bacterial infection.
Pediatrics; 74 (5) : 823.

Manroe BL; Weinberg AG; Rosenfeld CR and Brown R (1979):
The neonatal blood count in health and disease.
J Pediatr; 95 : 89.

Martens J; Samson WPF and Fetler AA (1993):
TNF-α, IL-6 and IL-IB plasma level in neonatal sepsis.
Pediatr Res; 33 (4) : 380–383.

McCabe WR and Olans RN (1981):
Shock in Gram-negative bacteremia. Predisposing factors, pathophysiology and treatment.
In: Remington JS, Swartz MN (eds.). Current Clinical Topics in Infectious Diseases. McGraw-Hill, New York, p 121–150.

McCabe WR; Treadwell TL and Maria AD (1983):
Pathophysiology of bacteremia.
Am J Med; 28 : 7–16.

McCracken GH (1987):
Perinatal bacterial diseases.
In: Feigin RD and Cherry JD (eds.). Textbook of Pediatric infectious Diseases, 2nd ed. WB Saunders Company, Philadelphia, London, Tokyo, p 940.

McCracken GH Jr and Lebel MH (1989):
Dexamethasone therapy for bacterial meningitis in infants and children.
Am J Dis Child; 143 : 287–9.

McDonough KH and Smith LW (1988):
Inotropic sensitivity to adrenergic stimulation of early sepsis.
Am J Physiol 25 : H699–703.

McIntosh K (1984):
Bacterial infections of the newborn.
In: Taeusch HW Ballard RA and Avery ME (eds.). Schaffer's Disease of the Newborn, 5th ed. WB Saunders Company, Philadelphia, London, Toronto, p 732.

McIntosh K (1992):
The Newborn.
In: Chanphell AGM and McIntosh N (eds.). Forfar and Arneil's Textbook of Pediatrics, 4th ed. WB Saunders Company, Philadelphia, London, Toronto, p 109.

Melby JC (1974):
Systemic corticosteroid therapy: Pharmacology and endocrinologic considerations.
Am Intern Med; 81 : 505.

Mestan F; Digel W and Mittnacht S (1988):
Antiviral effects of recombinant tumor necrosis factor in vitro.
Nature; 323 : 816–819.

Mills J and Drutz DJ (1995):
Mechanisms of immunity to infection.
In: Stites DP and Terr AI, eds. Basic and Clinical Immunology, 8th ed. Lange Medical Book, USA, p. 632–636.

Mitchell SC (1991):
Cytokines. A new immunotherapy.
Clin Perinatol; 18 (2) : 343–355.

Moffet HL (1981):
Neonatal sepsis and meningitis.
In: Moffet HL (ed.). Pediatric Infectious Disease. 1st ed. Lippincott Co., Philadelphia, Toronto, p 448–461.

Moffet HL (1989):
Neonatal sepsis and meningitis.
In: Moffet HL (ed.). Pediatric Infectious Diseases, 3rd ed. Lippincott Co., p 496–507.

O'Garra A (1989):
Interleukins and immune system.
Lancet; 106 : 943.

Odio M (1991):
The bacterial effects of early dexamethasone administration in infants and children with bacterial infection.
N Engl J Med; 324 : 350.

Okusawa S; Gelfand JA; Ikejima T (1988):
Interleukin-1 induce a shock-like state in rabbits: Synergism with tumour necrosis factor and the effect of cycloxygenase inhibitors.
J Clin Invest; 1 : 1162-1172.

Oppenheim JJ; Ruscetti FW and Faltynek C (1991):
Cytokines. In: Basic and Clinical Immunology. 7th ed. (Eds.): Stites DP, Terr AI. Appleton and Lange, Norwalk. Connecticut/California, p. 78–99.

Otsuka Y; Nagano K; Hori K; Oh-Ishi JI; Hayashi H; Watanabe N and Nitsu Y (1990):
Inhibition of neutrophil migration by tumor necrosis factor. Ex vivo and in vivo studies in comparison with in vitro effect.
J Immunol; 145 : 2639–43.

Ozdemir A; Oygur N; Gultekin M and Yegin O (1994):
Neonatal TNF-α, IL-1 alpha, IL-1 beta and IL-6 response to infection.
Am J Perinatal; 11 (4) : 282–5.

Pacheco SE and Shearer W (1994):
Laboratory aspects of immunology.
Pediatr Clin N Am; 41 (4) : 650–657

Patrik CC (1990):
Coagulase negative staphylococci; pathogens with increasing clinical significance.
J Pediatr 116 : 497–507.

Philip AGS (1980):
Neonatal infection.
In: Philip AGS (ed.). Neonatology Medical Examination. Co, Inc. Garden City, New York, p. 193–196.

Philip AGS and Hewitt JR (1980):
Early diagnosis of neonatal sepsis.
Pediatrics; 65 : 1036–1041.

Piguet PF; Collart MA; Grau GE; Sappino AP and Vassalli P (1990):
Requirement of tumor necrosis factor for development of silica-induced pulmonary fibrosis. *Nature,* 344 : 245–47.

Pizzo PA (1992):
Modulation of host defenses by cytokines evolving adjuncts in prevention and treatment of serious infections in immunocompromised hosts.
Clin Infect Dis; 15 : 508–524.

Polin RA and Ditmar MF (1989):
From Ped. Secrets Textbook. The CV Mosby Company, St. Louis, Toronto, London, p. 249.

Pursley DM and Richardson DK (1994):
Management of the normal newborn.
In: Nathan DG and Stranahan R, eds. Manual of Pediatric Therapeutics. 5th ed. Little, Brown and Company, Boston, New York, London, p. 126.

Quesenberry PJ (1992):
White blood cells: Dramatically impressive synergistic effects of cytokine combinations. In: Yearbook of Hematology. JL Spivak, WR, Bell, PM Ness, PJ Quesenberry and PH Wiernik (Eds.). Mosby, London, Philadelphia, Chapter 2, page 72.

Rabinovici R (1995):
Current understanding of sepsis.
Journal of Endotoxin Research: Abstracts of conference on sepsis/septic inflammatory response: reducing mortality to patients and suppliers. February 20–21, 1995. The Washington Court Hotel, Washington.

Ralph D; Stuart L and Morven S (1987):
Infection in the fetus and neonates.
In: Fanaroff AA and Martin RJ (eds.). Neonatal Perinatal Medicine, 4th ed. CV Mosby Co., St. Louis, Washington DC, Toronto, p 765.

Redline S (1988):
Bacterial and fungal infections.
In: Manual of Neonatal Care, 3rd ed. (Eds.): John P, Cloherty MD and Ann R Stark MD. Little, Brown and Company, p. 146.

Rench MA and Baker CJ (1993):
Neonatal sepsis caused by a new group B streptococcal serotype.
J Pediatr; 122 : 638–640.

Roberton NRC (1992):
Infection in the newborn.
In: Roberton NRC (eds.). Textbook of Neonatology, 2nd ed. Churchill Livingstone, New York and Tokyo, p 925.

Rodriquez AF; Kaplan SI and Mason EO (1990):
Cerebrospinal fluid values in the very low birth weight infant.
J Pediatr; 116 : 971–977.

Rodwell RL (1988):
Early diagnosis of neonatal sepsis using a hematologic scoring system.
J Pediatr; 122 : 761–767.

Roger CB (1995):
Pathophysiology of septic inflammatory reactions.
Journal of Endotoxin Research: Abstracts of conference on sepsis/septic inflammatory response: reducing mortality to patients and suppliers. February 20–21, 1995. The Washington Court Hotel, Washington.

Roman J; Fernandez F; Velosco F; Rajas R; Rolddan M and Torres A (1993):
Serum TNF levels in neonatal sepsis and septic shock.
Acta Pediatr; 82 : 352–354.

Saez-Liorens X; George. H and McCracken (1993):
Sepsis syndrome and septic shock in pediatrics. Current concepts of terminology, pathophysiology and management.
J Pediatr; 497 : 509.

Salmi TT (1973):
Haptoglobin levels in the plasma of newborn infant.
Acta Pediatr Scandinavica; 241 : 7–50.

Samuel (1992):
Sepsis in the newborn.
In: Krugman S, Kat SL, Gershon SL and Wilfert CM (eds.). Textbook of Infectious Disease of Children, 9th ed. The CV Mosby Company, Saint Louis, p 402.

Schreiber AD and Austen KF (1973):
Interrelationship of the fibrinolytic, coagulation kinin generating and complement systems.
Semin Hematol; 6 : 4–11.

Schumer W (1976):
Steroid in treatment of clinical septic shock.
Ann Surg; 184 : 333–41.

Seigel JD and McCracken GH (1981):
Sepsis neonatorum.
N Engl J Med; 304 : 642.

Shatla H; Bahgat N; Youssef S (1988):
Diagnostic value of alpha-1 acid glycoprotein and fibrinogen in neonatal bacterial infections.
Egypt J Pediatr; 5 : 131.

Sheagren JN (1981):
Septic shock and corticosteroids.
N Engl J Med; 305 : 465.

Sheagren JN (1985):
Glucocorticoid therapy in the Management of Severe Sepsis.
In: Septic Shock. Root RK and Sande MA (Eds.). New York: Churchill Livingstone, p. 201–18.

Shi Y; Shen C; Wang J; Li H; Qins and Liu R (1994):
Role of TNF in neonatal sepsis.
Chin Med Sci J; 1 : 45–8.

Sleigh JB ad Duguid JP (1989):
Enterobacteriaceae: Escherichia, Klebsiella, Proteus and other enterobacteria.
In: Cartney NC, Colleae JG, Duguid JP, Fraser AG and Marmion BP (eds.). Practical Medical Microbiology, 13th ed. Churchill Livingstone, p 432–455.

Sperling RS; Newton E and Gibbs RS (1988):
Intra-amniotic infections in low-birth weight infants.
J Inf Dis; 157 : 113–117.

Spriggs DR; Imamure K; Rodriguez C; Sariban E and Kufe DW (1988):
Tumor necrosis factor expression in human epithelial tumor cell lines.
J Clin Invest; 81 : 455–460.

Springer TA (1990):
Adhesion receptors of the immune system.
Nature; 364 : 425–434.

Stubitz KM; Craddock PR and Hammerschmidt DE (1981):
Corticosteroids block binding of chemotactic peptide to its receptor on granulocytes and causes disaggregation of granulocyte aggregates in vitro.
J Clin Invest; 68 : 130.

Sullivan JS; Kilpatrick L; Costarino AT and Chilee S (1992):
Correlation of plasma cytokine elevations with mortality rate in children with sepsis.
J Pediatr; 120 : 510–5.

Taeusch WH; Ballard AR and Avery EM (1991):
Bacterial infections of the newborn.
In: Taeusch WH; Ballard AR and Avery EM (eds.). Schaffer and Avery's Diseases of the Newborn. 6th ed. WB Saunders Company, Philadelphia, London, Toronto, p 350.

Tollner U (1982):
Early diagnosis of septicemia in the newborn: clinical studies and sepsis score.
Eur J Pediatr; 138 : 331–337.

Tracey KJ and Cerami A (1992):
TNF in the malnutrition (cachexia) of infection and cancer.
Am J Trop Med Hyg; 47 (1) : 2–7.

Tracey KJ; Fong and Hesse DG (1987):
Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia.
Nature; 330 : 662–64.

Tracey KJ; Valssara H and Cerami A (1989):
Cachectin/tumor necrosis factor.
Lancet; 1 : 1122–25.

Tracey KJ; Wei H; Monogue KR (1988):
Cachectin/tumor necrosis factor induces cachexia, anemia and inflammation.
J Exp Med; 167 : 1211–1227.

Truog WE; Gibson RL; Henderson WR (1990):
Tumor necrosis factor-induced neonatal pulmonary hypertension. Effects of dazmegrel pretreatment.
Pediatr Res; 27 : 466.

Vesikari T; Isolauri E and Tuppurainen N (1989):
Neonatal septicemia in Finland 1981–1985.
Acta Pediatr Scand; 78 : 44.

Vesikari T; Janas M and Gronross P (1985):
Neonatal septicemia.
Arch Dis Child; 60 : 542.

Vreugdenhil G; Lowenberg B; Vaneijk HG and Swaak AJ (1992):
TNF-α is associated with disease activity and the degree of anemia in patients with rheumatoid arthritis.
Aur J Clin Invest; 227 : 488–493.

Weitzman S and Berger S (1989):
Clinical trial design in studies of corticosteroid for bacterial infections.
Ann Intern Med; 81 : 36–42.

Wendel A (1995):
Use of G-CSF as anti-inflammatory drug.
Journal of Endotoxin Research: Abstracts of Conference on sepsis/septic inflammatory response: reducing mortality to patients and suppliers, February 20–21, the Washington Court Hotel, Washington.

Wheeler JG and Givner LB (1992):
Therapeutic use of recombinant human granulocyte-macrophage colony-stimulating factor in neonatal rate with type III group B-streptococcal sepsis.
J Infect Dis; 165 : 939–41.

Whitfield JM (1993):
Neonatal sepsis.
In: Holbrook PR (eds.). Textbook of Pediatric Critical Care 3rd ed. WB Saunders Company, Philadelphia, London, Toronto, p 98.

Windebank KP (1990):
The cytokines are coming.
Arch Dis Child; 65 : 1283–85.

Wong GHW and Goeddel DV (1986):
Tumor necrosis factors-α and β inhibit virus replication and synergize with interferons.
Nature; 323 : 819–22.

Yoder MC and Polin RA (1986):
Immunotherapy of neonatal septicaemia.
Pediatr Clin N Am; 33 (3) : 481.

Arabic Summary

الملخص العربى

رغم التقدم الهائل فى استخدام المضادات الحيوية فى العلاج ووجود العناية المركزة لكن نسبة الوفيات من حالات التسمم الدموى الجرثومى لازالت عالية.

هذه النسبة المرتفعة من الوفيات اثارت الإهتمام لإستخدام أدوية أخرى فى العلاج كمحاولة لتقليل نسبة الوفيات فى مثل هذه الحالات.

يشتمل هذا البحث على أربعة واربعين طفلا من الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى فى العناية المركزة للأطفال حديثى الولادة بمستشفى جامعة عين شمس. تم تقسيم هؤلاء الأطفال عشوائيا إلى مجموعتين:

المجموعة الأولى : تتكون من ٢٢ طفلا من المصابين بالتسمم الدموى الجرثومى تم اعطائهم ديكساميثازون ١٥ ملليجرام لكل كيلوجرام كل ٦ ساعات لمدة ٤ أيام وذلك بجانب المضادات الحيوية والعلاج المقرر لهم (المحاليل وغيرها).

المجموعة الثانية: تتكون من ٢٢ طفلا من الأطفال المصابين بتسمم الدم الجرثومى تم اعطائهم المضادات الحيوية فقط بجانب العلاج المقرر لهم (المحاليل وغيرها) وقد بدأ هذا العلاج فى خلال ساعتين من تشخيص حالات التسمم الدموى الجرثومى.

المجموعة الضابطة: تتكون من ستة عشر طفلا من الأطفال الطبيعين الأصحاء كمجموعة ضابطة.

الهدف من هذا البحث :

تهدف الدراسة إلى معرفة القيمة الإكلينيكية لمعامل تحلل الأوارم-الفا فى متابعة حالات التسمم الدموى الجرثومى فى الأطفال حديثى الولادة والتى تم استخدام الديكساميثازون فى علاجها.

ولذلك تم عمل الفحوصات المعلميةالآتية لهذه الحالات:

١- صورة دم كاملة.
٢- مزارع بكتيرية فى الدم.
٣- قياس نسبة بروتين (س) التفاعلى فى الدم.
٤- قياس معامل تحلل الأورام (الفا) فى الدم.

وقد وجد أن الأطفال المبتسرين أكثر عرضة لتسمم الدم الجرثومى وكذلك في حالات التمزق المبكر لأغشية الحمل وقد وجد أن عدد كرات الدم البيضاء وكذلك الصفائح الدموية فى المصابين بالتسمم الجرثومى أقل منه فى الأطفال الأصحاء.

أما بالنسبة لنتائج البروتين التفاعلى (س) فلقد وجد انها كانت إيجابية عند ١٠٠٪ فى الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى. أما بالنسبة للأطفال حديثى الولادة الأصحاء فقد كانت ايجابية عند حوالى ١٢,٥٪ فقط من هؤلاء الأطفال.

وكذلك المزارع البكتيرية فى الدم كانت ايجابية عند ٩٠٪ من الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى.

وكانت نسبة معامل تحلل الأورام الفا مرتفعة جدا عند الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى بالمقارنة بالأطفال الأصحاء.

وقد لوحظ ارتفاع نسبة معامل تحلل الأورام-الفا فى الأطفال المصابين بتسمم الدم الجرثومى الذين توفوا من المرض عن الأطفال الذين تم شفائهم.

ولم تكن هناك علاقة بين مستوى معامل تحلل الأورام الفا فى الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى وبين مستوى اعمارهم أو أوزانهم أو نوع الجنس.

وهناك تناسب ملحوظ بين مستوى معامل تحلل الأورام-الفا وبين حدة المرض فى الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى. وهذا يدل على أن نسبة معامل تحلل الأورام-الفا فى حالات الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى يمكن استخدامه كعلامة فى تشخيص حالات التسمم الدموى الجرثومى واكتشافه مبكرا ومتابعة هذه الحالات حتى تمام شفائها.

وقد لوحظ انخفاض نسبة الوفيات فى الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى الذين تم علاجهم بواسطة الديكساميثازون وهذا يدل على أن اضافة الديكساميثازون للمضادات الحيوية فى علاج حالات التسمم الدموى الجرثومى فى الأطفال حديثى الولادة يقلل من حدوث مضاعفات ويحسن نسبة الشفاء في مثل هذه الحالات.

مستخلص

اسم الطالب : رحاب عبد القادر محمود مصطفى

الدرجة : دكتواره الفلسفة فى دراسات الطفولة

عنوان الرسالة : دراسة القيمة الإكلينيكية لمعامل تحلل الأورام - الفا فى متابعة حالات علاج التسمم الدموى الجرثومى فى الأطفال حديثى الولادة ·

يشتمل هذا البحث على أربعة واربعين طفلاً من الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى فى العناية المركزة للأطفال حديثى الولادة بمستشفى جامعة عين شمس ·

تم تقسيم هؤلاء الأطفال عشوائياً إلى مجموعتين بالإضافة إلى مجموعة ضابطة تتكون من ١٦ طفلاً من الأطفال الأصحاء ·

تهدف الرسالة إلى معرفة القيمة الإكلينيكية لمعامل تحلل الأورام – الفا فى متابعة حالات التسمم الدموى الجرثومى فى الأطفال حديثى الولادة والتى تم أستخدام الديكسا مثازون فى علاجها ·

لذلك تم عمل عدة فحوصات معملية لهذه الحالات بالإضافة إلى قياس معامل تحلل الأورام – الفا فى الدم عند التشخيص وبعد ٤ أيام من العلاج ·

وقد وجد أن نسبة معامل تحلل الأورام – الفا كانت مرتفعة جداً عند الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى بالمقارنة بالأطفال الأصحاء أيضاً قد وجد أن نسبة معامل تحلل الأورام – الفا فى الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى كانت مرتفعه فى حالات الوفاه من المرض بالمقارنة بالأطفال الذين تم شفائهم ·

وكان هناك تناسب ملحوظ بين مستوى معامل تحلل الأورام – الفا وبين حدة المرض فى الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى ولذلك يمكن استخدامه كعلامة فى تشخيص ومتابعة حالات التسمم الدموى الجرثومى · وقد لوحظ انخفاض نسبة الوفيات فى الأطفال حديثى الولادة المصابين بتسمم الدم الجرثومى الذين تم علاجهم بواسطة الديكسامثازون بالمقارنة بالمجموعة الأخرى ، وهذا يدل على أن اضافة الديكسا مثيازون للمضادات الحيوية فى علاج حالات التسمم الدموى الجرثومى فى الأطفال حديثى الولادة يقلل من نسبة الوفيات ويحسن نسبة الشفاء ·

الكلمات المفتاحية : – التسمم الدموى الجرثومى فى الأطفال حديثى الولادة

– معامل تحلل الأورام – الفا (كاشكتين)

«جامعة عين شمس»
معهد الدراسات العليا للطفولة
قسم الدراسات الطبية

شكـــــــر

أشكر السادة الأساتذة الذين قاموا بالإشراف وهم:

١ - أ. د. حامد محمود شتلة أستاذ طب الأطفال - كلية الطب - جامعة عين شمس

٢ - أ. د. هادية حسين بسيم أستاذ مساعد الباثولوجيا الإكلينيكية - كلية الطب - جامعة عين شمس

٣ - أ. د. مدحت حسن شحاتة أستاذ مساعد بمعهد الدراسات العليا للطفولة - جامعة عين شمس

٤ - د. ممدوح عبد المقصود محمد مدرس طب الأطفال - كلية الطب - جامعة عين شمس

ثم الأشخاص الذين تعاونوا معي في البحث وهم:

١ - د. مصطفى عبد العزيز الهدهد، مدرس طب الأطفال - كلية طب جامعة عين شمس

٢ - أطباء وحدة الأطفال المبتسرين بمستشفى النساء والتوليد - جامعة عين شمس

«جامعة عين شمس»

معهد الدراسات العليا للطفولة

قسم الدراسات الطبية

صفحة العنوان

اسم الطالب: **رحاب عبد القادر محمود مصطفى**

الدرجة العلمية: دكتوراه الفلسفة في دراسات الطفولة

القسم التابع له: قسم الدراسات الطبية

اسم الكلية: معهد الدراسات العليا للطفولة

الجامعة: جامعة عين شمس

سنة التخرج: ١٩٩٦

سنة المنح: ١٩٩٦

«جامعة عين شمس»

معهد الدراسات العليا للطفولة

قسم الدراسات الطبية

رسالة دكتـوراه

اسم الطالب: رحاب عبد القادر محمود مصطفى

عنوان الرسالة: دراسة القيمة الإكلينيكية لمعامل تحلل الأورام ـ الفا في متابعة حالات علاج التسمم الدموي الجرثومي في الأطفال حديثي الولادة

اسم الدرجة: دكتوراه، الفلسفة في دراسات الطفولة

لجنة الإشراف:

١ـ **الاسم**/ أ.د. حامد محمود شتلة **الوظيفة**: أستاذ طب الأطفال ـ كلية الطب ـ جامعة عين شمس

٢ـ **الاسم**/ أ.د. هادية حسين بسيم **الوظيفة**: أستاذ مساعد الباثولوچيا الإكلينيكية ـ كلية الطب جامعة عين شمس

٣ـ **الاسم**/ أ.د. مدحت حسن شحاتة **الوظيفة**: أستاذ مساعد بمعهد الدراسات العليا للطفولة جامعة عين شمس

٤ـ **الاسم**/ د. ممدوح عبد المقصود محمد **الوظيفة**: مدرس طب الأطفال ـ كلية الطب جامعة عين شمس

تاريخ البحث: ١٠ / ٧ / ١٩٩٤

الدراسات العليا أجيزت الرسالة بتاريخ / / ١٩٩

ختم الإجازة:

/ / ١٩٩

موافقة مجلس الكلية ١٧ / ١٢ / ١٩٩٦

موافقة مجلس الجامعة / / ١٩٩

جامعة عين شمس
معهد الدراسات العليا للطفولة
قسم الدراسات الطبية

دراسة القيمة الاكلينكية لمعامل تحلل الأورام-ألفا في متابعة حالات علاج التسمم الدموي الجرثومي في الأطفال حديثي الولادة

رسـالة

مقدمة توطئة للحصول على

درجة دكتوراه الفلسفة في الدراسات الطبية

مقدمة من

الطبيبة / رحاب عبد القادر محمود مصطفى

ماجستير طب الأطفال

تحت إشـراف

الأستاذ الدكتور / حامد محمود شتلة

أستاذ طب الأطفال - كلية الطب - جامعة عين شمس

الأستاذ الدكتور / هادية حسين بسيم

أستاذ مساعد الباثولوجيا الاكلينيكية - كلية الطب - جامعة عين شمس

الأستاذ الدكتور / مدحت حسن شحاته

أستاذ مساعد بمعهد الدراسات العليا للطفولة - جامعة عين شمس

الدكتور / ممدوح عبد المقصود محمد

مدرس طب الأطفال - كلية الطب - جامعة عين شمس

١٩٩٦