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Education and Scientific Research  
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College of Medicine  
Department of Microbiology*



# **Association of interleukin-6 and interleukin- 11 with neonatal sepsis in Diyala Province**

*A Thesis*

*Submitted to the College of Medicine-University of Diyala  
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M.Sc. in Biology*

By

**Rahma Mohammad Abbas**

BVMS. (2011) - College of Veterinary Medicine - University of  
**Diyala**

Supervised by

**Assistant Professor Dr.**

**Anfal Shakir Motib**

**PhD Molecular Biology**

**Professor Dr.**

**Jalil Ibrahim Kadhim**

**FICMS, Ped**

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**1442 A.H.**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

( وَأَنْ لَيْسَ لِلْإِنْسَانِ إِلَّا مَا سَعَى )

صدق الله العظيم

سورة النجم

الآية (39)

## *Dedication*

*To my mother's soul that didn't leave me for a moment, May God have mercy on her and make her one of the women of Paradise.*

*The apple of my eyes and my refuge after God.....my father*

*To the most precious people to me... my husband (**Mohammed**),*

*he always encourages, helps and supports me in all of my life, without he I wouldn't be who I am today*

*To my support in life..... ..my brothers(**Omar, Ali, Othman**)*

*To my second mother... ..my sister (**Zainab**)*

*To my supervisors ..... **Assistant professor . Dr. Anfal Shakir Motib and Professor ....Dr. Jalil Ibrahim elezzi***

*To the pure souls of neonatal patients in any place...*

***To** everyone who supports me, even with a word, I dedicate the fruit of my humble effort this*

***Rahma***

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*I appreciate the cooperation of all doctors especially **Dr. Firas Abbas**, a pediatrician, for his help and support in this study.*

***Rahma***

## **Supervisor Certification**

I, certify that this thesis entitled (**Association of interleukin-6 and interleukin- 11 with neonatal sepsis in diyala Province**) has been conducted under my supervision at the College of Medicine, University of Diyala, as partial fulfillment of the requirements for the Master Degree of Science in Medical Microbiology.

**Assistant professor Dr.  
Anfal Shakir Motib**

**Professor Dr.  
Jalil Ibrahim Kadhim**

Given the available recommendation, I forward this thesis for debate  
by the examining committee.

### **Signature**

**Professor Dr. Luma Taha Ahmed**

Head of Microbiology Department

College of Medicine - University of Diyala

## Committee Certification

We, as the examining committee, certify that we have read this thesis and examined the student (**Rahma Mohammad Abbas**) in and its contents, found it adequate as a thesis for the Master Degree of Science in Medical Microbiology.

Professor

**Dr. -----**

Chairman

Professor

**Dr. -----**

Member

Professor

**Dr. -----**

Member

**Assistant professor Dr. Anfal Shakir Motib**

**Professor Dr. Jalil Ibrahim Kadhim**

(Supervisor)

Approved by the Council of College of Medicine

The Dean

**Professor Dr. Ismail Ibrahim Latif**

Date:

## Summary

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### Summary

Sepsis or septicemia are severe bloodstream infections that can quickly become life-threatening. Sepsis is most often caused by bacteria infection, but can also be caused by fungi, viruses, or parasites, and it is one of the major causes of mortality and morbidity in newborns (< 1 month). The aim of this study is to investigate the role of interleukin-6 (IL-6) and interleukin-11 (IL-11) in the early diagnosis of sepsis in newborns .

From November/ 2020 to April/ 2021, 100 blood samples were collected from neonates admitted to the pediatrics-neonatal wards at Al-Batool Teaching Hospital for Gynecology and Pediatrics in Baquba / Diyala Governorate who were clinically diagnosed with sepsis. The neonates ranged in age from 1 - 30 days, and blood samples were obtained to determine the levels of Interleukins IL-6 and IL-11.

The findings of this study show that (43%) of newborns have early onset sepsis (infected within the first seven days of their lives), while the remaining (17%) have late onset sepsis (infected during 7-30 days of their life). Bacterial isolation was performed on the samples collected. Bacterial culture was positive in (60%) patients versus (40%) patients revealed a negative bacterial culture. The most common types of bacteria isolated were *Staphylococcus epidermidis*, 26 isolates (43.3%) followed by *Klebsiella pneumonia*, 13 isolates (21.7%), and *Staphylococcus aureus* , 10 isolates (16.7%), *Pseudomonas aeruginosa*, 8 isolates (13.3%) and *Escherichia coli* , 3 isolates (5%). The susceptibility of bacteria to some antibiotics included in this study was determined *in vitro*, and it was showed that gentamicin, amikacin, and ceftazidime are the most efficient antibiotics against various pathogenic bacteria.

## **Summary**

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The level of Interleukins was quantified using for the immune antibodies binds to the enzyme (Sandwich ELISA test). For one hundred ninety-six samples, of which hundred were from patients and ninety-six samples from healthy ones. The Interleukin levels showed that a significant increase in the level of interleukin-6 (IL-6) for all patients males and females preterm or full-term and who their weight <2.5 or >2.5 kg compared with a control group. In terms of interleukin -11 levels, the current study found a significant increase in patients with sepsis in terms of gestational age, gender, and birth weight when compared to the control group.

In conclusion, the findings revealed that neonatal early onset sepsis is more common than late onset sepsis in NICUs in Baquba city / Diyala Governorates. The results showed that IL-6 can be a reliable marker for predicting neonatal sepsis and can be used as a good guide for early detection of sepsis in neonatal care units, in comparison to blood culture whereas the blood culture technology (the gold standard) takes at least 24–48 hours, and prenatal antibiotic use further reduces blood culture accuracy. Finally, the study found that the levels of the interleukins IL-6 and IL-11 levels increase in early time of inflammation, making them a good diagnosis marker for neonatal sepsis.



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### List of Abbreviations

NS	Neonatal Sepsis
EOS	Early onset sepsis
LOS	Late onset sepsis
LBW	Low Birth Weight
VLBW	Very Low Birth Weight
TLR	Toll-like receptor
G+	Gram positive bacteria
G-	Gram negative bacteria
TNF- $\alpha$	Tumor necrosis factor alpha
IL-6	Interleukin-6
IL-11	Interleukin-11
CARS	compensatory anti-inflammatory response syndrome
SIRS	systemic inflammatory response syndrome

HRP	Horseradish Peroxidase
KDa	Kilo Dalton
GI	Gastrointestinal tract
GUT	Genitourinary Tract
NICU	Intensive Care Unit Neonatal
EMB	Eosin Methylen Blue Agar
VP	Voges-proskauer test
KIA	Kligler iron agar
CLSI	Clinical and Laboratory Standards Institute
WHO	World Health Organization
PCT	procalcitonin
EMB	Eosin Methylene Blue
CONS	Coagulase Negative Staphyococcal Bacteria
CBC	Complete Blood Count
CSF	Cerebrospinal Fluid
CRP	C-Reactive Protein
IMVIC	Indole, Methyl red test ,Voges-proskauer test

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# **Chapter one**

## **Introduction**

## 1.1 Introduction

Neonatal bacterial septicemia is a more important syndrome of infections at early stage of infant's life Worldwide (Russell , 2015). The one of the largest causes of morbidity and mortality in infants is neonatal septicemia (Jain *et al.*,2003). Edwards (2006) classified Neonatal sepsis as early onset sepsis (EOS) that occurs at age through one to seven days or late onset sepsis (LOS) that occurs at age through eight to twenty eight days. The spectrum of infecting organisms has evolved over the last two decades from a predominance of gram negative bacteria to gram positive bacteria. Most community-acquired infections are due to *Streptococcus* spp. and *Escherichia coli*, and other Enterobacteriaceae and non-ferment Gram-negative bacteria like *Pseudomonas* and *Acinetobacter* spp. (Ramphal , 2004).

It was reported that *Streptococcus agalactiae* (Group B *Streptococcus*) is considered the main cause lead to meningitis and neonatal sepsis. Many clinical isolates of pathogenic bacterial species that can cause septicemia including, *Mycobacterium, tuberculosis, Enterococcus faecium, Staphylococcus aureus, Pseudomonas aeruginosa, Kleibsiella pneumonia, Neisseria gonorrhoea, Acinetobacter baumannii , enterobacter ,Salmonella , and Shigella spp.*, are the most antimicrobial-resistant and the problem remains out of control (Nathan and Cars, 2014). Many clinical diseases, include upper respiratory tract ; infection and urinary tract (UTI) , cystitis, pneumonia, thrombophlebitis, wound infection; meningitis, osteomyelitis can led to bacteremia, sepsis and septic shock, that may result from the bacteria entering the blood stream (Jung *et al.*, 2012; Siham and Rachid ,2016). On the other hand, the microorganisms commonly isolated with early - onset septicemia in neonate were *K. pneumonia, S. aureus, P. aeruginosa* and *Enterobacter*

spp (Baltimore *et al.*, 2001), while in late-onset septicemia, the bacteria are those acquired from human contacts or equipment's and contaminated materials and organisms from maternal genital canal. (Stoll *et al.*, 2002). There are difficulties in diagnosis of the neonate sepsis that attributed to nonspecific signs of sepsis and may observe same signs with other noninfectious cases (Al-Saady *et al.*, 2018). For example many neonate have bacteremia with absence of clinical signs (Ottolini *et al.*, 2003). Blood cultures or other fluids in the body are the typical diagnostic method in the conditions of neonatal sepsis (Chiesa *et al.*, 2004). Recently the molecular assays have been used as a substitute for blood cultures that due to direct pathogen detection and get the results within less time (Mancini *et al.*, 2010). The therapy of effective antibiotic must be used in treating sepsis and the untreated cases lead to rapidly fatal (Brocklehurst *et al.*, 2011).

Cytokines is defined as proteins (small polypeptides) secreted by variety of tissue cells, which most commonly of the immunity system, that have functions of pleotropic at the local tissue or sometimes at the systemic level. Different cell types' activation, growth and differentiation are all regulated to them. They work by binding to cytokine receptors on cell membranes, as well as plasma and receptors of tissue fluids. The molecular weight ranging from 27 to 30 KD (Paradkar *et al.*, 2014). Interleukins are the most numerous cytokines, ranging from IL-1 to IL-35, and are divided into many smaller families (Williams *et al.*, 2012). Interleukin-6 is one of the most essential multifunctional cytokines, with a molecular weight of (22-30) K D. Many types of cells generate it, including active T and B lymphocytes, monocytes, macrophages, epithelial cells, and meningeal cells in the brain (Swami Nathan, 2014). Interleukin-6 (IL-6R) receptors are widespread in the body, as they are present on hematopoietic precursors, macrophages, myeloid cells,

hepatocytes, and plasma cells (Thandavan *et al.*, 2015). In sepsis, IL-6 is a key mediator during the acute phase of the inflammatory response and several studies have looked at its therapeutic benefit in patients with different septic conditions (Takahashi and Talcahashiw, 2016). Interleukin-11 is a protein that induces platelet synthesis (megakaryocytopoiesis), as well as activating osteoclasts, inhibiting macrophage mediator production and epithelial cell apoptosis and proliferation. These roles may be especially significant in mediating interleukin 11's mucosal protective effects (Leng *et al.*, 1997). IL-11 was isolated for the first time from bone marrow-derived fibrocytic-like stromal cells. It was thought to be essential for hematopoiesis, especially megakaryocyte maturation (Paul SR *et al.*, 1990). However, in both mice and humans, it was later discovered to be redundant for platelets and other blood cell forms (Nandurkar *et al.*, 1997; Brischoux-Boucher *et al.*, 2018). It's also known as adipose genesis inhibitory factor, as the drug substance oprelvekin (Kawashima I *et al.*, 1991; Chen *et al.*, 2002). Therefore, it is important to study the role and concentration of interleukin 6 and 11 in neonatal septicemia.

### 1.1 The current study aimed to

- 1-Identify the common bacterial species which is isolated from blood samples of neonatal sepsis cases.
- 2-Determine the antibiotic Susceptibility of bacterial isolates.
- 3-Detect the level of interleukin 6 and the level of interleukin 11 in newborns serum with septicemia and to compare the level of them with their levels in healthy newborns.
- 4- Study the correlation of the levels of interleukin 6 and 11 with neonatal ages, gender, and weight.