Inter-alpha-Inhibitor Proteins as a Marker of Neonatal Sepsis

Thesis

Submitted for the Partial Fulfillment of Master Degree in Pediatrics

By

Maged Zakaria Mahmoud

M.B.B.Ch, 2006

Ain-Shams University

Under the Supervision of

Prof. Dr. Hoda Lotfy Al-Sayed

Professor of Pediatrics

Faculty of Medicine, Ain-Shams University

Dr. Dina Adel Fouad

Assist. Professor of Clinical Pathology

Faculty of Medicine, Ain-Shams University

Dr. Rania Hamed Mahmoud Shatla

Lecturer of Pediatrics

Faculty of Medicine, Ain-Shams University

Faculty of Medicine

Ain-Shams University

2011

Inter-alpha-Inhibitor Proteins as a Marker of Neonatal Sepsis

Thesis

Submitted for the Partial Fulfillment of Master Degree in Pediatrics

By

Maged Zakaria Mahmoud

M.B.B.Ch, 2006

Ain-Shams University

Under the Supervision of

Prof. Dr. Hoda Lotfy Al-Sayed

Professor of Pediatrics

Faculty of Medicine, Ain-Shams University

Dr. Dina Adel Foad

Assist. Professor of Clinical Pathology

Faculty of Medicine, Ain-Shams University

Dr. Rania Hamed Mahmoud Shatla

Lecturer of Pediatrics

Faculty of Medicine, Ain-Shams University

Faculty of Medicine

Ain-Shams University

2 0 1 0

بروتينـــــــات انـــــــترـ الفـــا المثبطـــــــة
كدلالـــة على الخمج في حديثي الولادة

رســـالة

توطئـــة للحصول على درجة الماجستير في طب الأطفال

مقدمة من

الطبيب / ماجـــد زكـريـا محمـــود

بكالوريوس الطب والجراحةـ جامعة عين شمس ٢٠٠٦

تحت إشراف

أ.د. هُـــــدَى لُـــــطفِي السَيـــــــد

أستـــــاذ طـــــــب الأطفـــــال

كليـــــــة الطـــــب – جـــــامعة عين شمس

د. دينـــــــا عـــــادل فـــــــؤاد

استاذ مساعد الباثولوجيا الإكلينيكيةـ كلية الطبـ جامعة عين شمس

د. رانيا حامد محمود شتلة

مدرس طب الأطفالـ كلية الطبـ جامعة عين شمس

كليـــــــة الطـــــب – جـــــامعة عين شمس

٢٠١١

Summary and Conclusion

Early and late onset systemic bacterial infection remains a devastating complication and an important cause of morbidity and mortality in neonates.

Neonatal sepsis is hard to differentiate from noninfectious diseases because of its nonspecific symptoms. At present high quality diagnostic tools are still being required. As well as giving accurate pre-opinion about prognosis, these tools are expected to be highly specific, sensitive and fast.

The Inter-alpha inhibitor proteins (IaIp) family is a group of plasma-associated serine protease inhibitors synthesized mainly in the liver. IaIp are involved in numerous biologic activities, including an anti-inflammatory and regulatory role in infection.

The aim of our study was to evaluate the serum concentration of inter-alpha inhibitor proteins (IaIp) in neonate infants as a diagnostic and prognostic marker of neonatal sepsis.

This study was conducted on 25 neonates (7 full-term and 18 preterm infants) diagnosed as having sepsis (confirmed with culture) and 25 healthy neonates (7 full-term and 18 preterm

Introduction

Neonatal sepsis is an important cause of morbidity and mortality despite the major advances in neonatal management **(Stoll et al., 2004)**. Early institution of an appropriate antimicrobial regimen in infected patients is associated with a better outcome **(Kollef et al., 1999)**, and hence early diagnosis of bacterial infection is of primary importance. However, some patients with an infection have minimal or even no symptoms or signs. Not all patients who appear septic demonstrate an infection, and the widespread administration of antibiotics to all these patients carries problems of antibiotic resistance, of drug toxicity, and of increased medical costs **(Chan et al., 2003)**. Currently, there are no reliable biochemical markers for confirmation of sepsis **(Mishra et al., 2006)**. Blood culture tests are still considered the gold standard for the diagnosis of sepsis although results are not available until at least 48 hours. There is a need for an effective and accurate biochemical marker to support or exclude the diagnosis of infection.

Hematologic indices, acute phase reactants, protein markers, and cytokines have been extensively examined as adjunctive tests for diagnosis of sepsis. None have shown

sensitivity, specificity, positive predictive values (PPV), or negative predictive values (NPV) that can sufficiently guide clinical management **(Bhandari et al., 2008)**.

The Inter-alpha inhibitor proteins (IaIp) family is a group of plasma-associated serine protease inhibitors synthesized mainly in the liver **(Kobayashi et al., 2006)**. IaIp are involved in numerous biologic activities, including tumor invasion, extracellular matrix stabilization, inflammation, and wound healing, and play an important anti-inflammatory and regulatory role in infection **(Fries and Blom, 2000).** Correlation between mortality and the levels of inter-alpha inhibitors in the plasma of patients with severe sepsis has recently been investigated; plasma levels are significantly decreased (by 20%-90%) and inversely correlated with unfavorable outcome **(Lim et al., 2003)**.

Aim of the Work

The present study aims at evaluation of the serum concentration of inter-alpha inhibitor proteins (IaIp) in newborn infants as a diagnostic and prognostic marker of neonatal sepsis.

Subjects and Methods

The present study is a prospective study using a sample of neonate infants admitted to the NICU of Ain-Shams University Maternity Hospital. The study is approved by the administration of the NICU unit. Informed consent will be waived in view of the lack of need for additional blood sampling.

The subjects under study are **25** neonates of different gestational ages with culture-proved neonatal sepsis whose serum IaIp will be compared with another **25** non-septic control newborn infants matched for gestational age.

For all neonates included in the study the following will be performed:

1. **History taking:**
    - Prenatal and natal history to detect risk factors for neonatal sepsis:
        - Gestational age.
        - Sex.
        - Premature or prolonged (>18 h) rupture of membranes.
        - Maternal peripartum fever (≥38°C) or infection; chorioamnionitis.

- Meconium-stained or foul-smelling, cloudy amniotic fluid.
- Multiple gestation.
- Invasive respiratory support, indwelling central venous lines or parenteral hyperalimentation especially if includes lipids.

- Present history which may include symptoms of sepsis as poor feeding or vomiting.
- History of antibiotics given if any (type, route of administration, number of doses and duration).

2. Clinical Examination:

- Weight, length and skull circumference.
- Gestational age according to last menstrual period date and new Ballard score **(Ballard et al., 1991)**.
- Vital signs (heart rate, body temperature, blood pressure and respiratory rate).
- Examination for clinical signs of sepsis **(Richard and Joan, 2008)**:
  - Temperature instability (<37°C or >38.5°C).

- Respiratory signs: increased oxygen requirement, apnea, cyanosis, intercostal retraction, tachypnea or grunting (chest x-ray will be done if clinically indicated).
- Circulatory signs: weak pulses, prolonged capillary refilling time, hypotension, tachycardia or shock.
- GIT signs: abdominal distension, diarrhea, bloody stool, feeding intolerance, hepatomegaly or jaundice.
- Neurological signs: irritability, hypotonia or lethargy.
- Hypoglycemia or hyperglycemia.
- Petechiae, bleeding (with thrombocytopenia) or DIC.

3. Laboratory Investigations:

The following laboratory investigations will be done upon the first evaluation for neonatal sepsis then they will be repeated after two weeks.

- Complete blood count (CBC) with differential leucocytic count. Previously validated hematologic criteria will be used as indicators of sepsis **(Rodwell et al., 1988):** total leucocytic count ≤ 5000 cells / mm^3 or ≥ 21.000 cells / mm^3 at day 2 of life onwards, total neutrophils count < 1750 cells / mm^3 or > 8500 cells / mm^3, immature neutrophils count > 400 cells / mm^3, maximum immature/total

neutrophil ratio ≥ 0.2, Degenerative changes in neutrophils and platelet count <150 000 cells/mm^3.

- CRP semi-quantitative assay.
- Blood culture (considered the gold standard for diagnosis of sepsis).
- Tracheal aspirate or other body fluid culture, when clinically indicated.
- Plasma IaIp levels quantitatively by an enzyme-linked *immunosorbent* assay with a monoclonal antibody against human IaIp (purchased from ProThera Biologics, USA). This was done twice, at the time of diagnosis and 14 days after treatment.

Statistical analysis of the results will be done using standardized computer programs.

References

Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL and Lipp R. (1991): New Ballard Score expanded to include extremely premature infants. *J Pediatr; 119 (3): 417-23.*

Bhandari V, Wang C, Rinder C, Rinder H (2008): Hematologic profile of sepsis in neonates: neutrophil CD64 as a diagnostic marker. *Pediatrics;121:129-34.*

Chan YL, Tseng CP, Tsay PK, Chang SS, Chiu TF, Chen JC (2003): Procalcitonin as a marker of bacterial infection in the emergency department: an observational study. *Crit Care;8(1):R12-20.*

Fries E and Blom AM (2000): Bikunin—not just a plasma proteinase inhibitor. *Int J Biochem Cell Biol ;32:125-37.*

Kobayashi H (2006): Endogenous anti-inflammatory substances, inter-alpha-inhibitor and bikunin. Biol Chem;387:1545-9.

Kollef MH, Sherman G, Ward S, Fraser VJ (1999): Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. *Chest; 115:462-474.*

Lim YP, Bendelja K, Opal SM, Siryaporn E, Hixson DC, Palardy JE (2003): Correlation between mortality and the levels of inter-alpha inhibitors in the plasma of patients with severe sepsis. *J Infect Dis;188:919-26.*

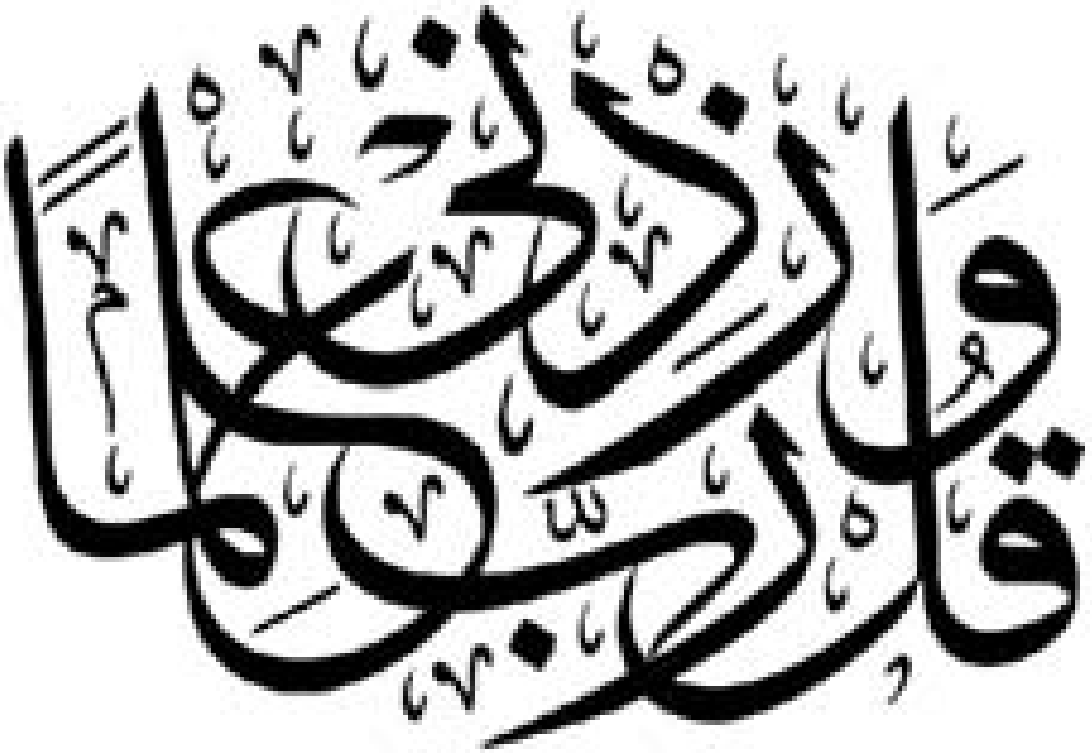
Mishra UK, Jacobs SE, Doyle LW, Garland SM (2006): Newer approaches to the diagnosis of early onset neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed;91:F208-12.*

Richard AP and Joan AR (2008): Early onset sepsis. In: Neonatology, 1st ed. *Cambridge University Press, 283-92.*

Rodwell RL, Leise AL and Tudehope DJ (1988): Early diagnosis of sepsis using a hematologic scoring system. *J Pediatr.; 12:P 761.*

Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. (2004): Neurodevelopmental and growth impairment among extremely low-birthweight infants with neonatal infection. *JAMA;292:2357-65.*

بسم الله الرحمن الرحيم



بروتينـــــات انـــــترـ الفـــا المثبطـــــة
كدلالـــة على الخمج في حديثي الولادة

رســـالة
توطئـــة للحصول على درجة الماجستير في طب الأطفال

مقدمة من

الطبيب / ماجـــد زكـريـا محمـــود

بكالوريوس الطب والجراحةـ جامعة عين شمس ٢٠٠٦

تحت إشراف

أ.د. هُـــــدَى لُـــــطفِي السَيـــــــد

أستـــــاذ طـــــــب الأطفـــــال

كليـــــــة الطـــــب – جـــــامعة عين شمس

د. دينـــــــا عـــــادل فـــــــؤاد

استاذ مساعد الباثولوجيا الإكلينيكيةـ كلية الطبـ جامعة عين شمس

د. رانيا حامد محمود شتلة

مدرس طب الأطفالـ كلية الطبـ جامعة عين شمس

كليـــــــة الطـــــب – جـــــامعة عين شمس

٢٠١٠