Plasma IP-10 as a predictor of Serious Bacterial Infection in neonates and young infants

Salem A. Sallam[1], Gihan M. Babrs[1], Mohamed Said,[2] and Heba M.Taghian[3]

[1]Pediatrics Department, Faculty of Medicine, El-Minia University
[2] Microbiology and Immunology Department, Faculty of Medicine, El-Minia University
[3] Pediatrics Department, El-Minia General hospital
gihanbabrs@hotmail.com

Abstract: Abstract: Background: Early diagnosis of SBI in young infants is a difficult problem by clinical symptoms and signs. IP-10 has been identified to play an important role during infectious and inflammatory processes. The goal of this study was to evaluate the value of plasma IP-10 in early diagnosis of SBI in young infants. **Patients & Methods:** 100 patients with clinical suspicion to have SBI were admitted in Pediatric department and NICU and subjected to clinical examination and investigations (complete blood count, C-reactive protein and plasma IP-10 levels and microbiological cultures). **Results:** SBI proved by positive culture in 45 infants and had higher plasma IP-10 levels than those infants without SBI (n=55) (435.1±31 versus 25.5±26.9, *P*=0.0001) after adjusting age. A plasma IP-10 level > 43.5 ng/ml has the best diagnostic accuracy for indicating SBI (Sensitivity 82%, Specificity 90%). **Conclusion:** Plasma IP-10 is a valuable laboratory test in diagnosis of SBI and may serve as a better diagnostic marker of SBI than total WBC count, CRP, ANC and IT ratio.

[Salem A. Sallam, Gihan M. Babrs, Mohamed Said and Heba M. Taghian. **Plasma IP-10 as a predictor of Serious Bacterial Infection in neonates and young infants.** Journal of American Science 2012; 8(4):190-194]. (ISSN: 1545-1003). http://www.americanscience.org. 26

Key word: Serious bacterial infection, IP-10, chemokines

1. Introduction

Despite improved neonatal care over the past decades, infections remain common and sometimes life-threatening in neonates admitted to the neonatal intensive care unit (NICU) and young infants **(1)**.

Fever is the most common chief symptom in infants younger than three months visiting emergency departments or outpatient clinics. The causes of fever in these infants vary from mild viral infections to serious bacterial infections (SBIs) (such as urinary tract infections [UTIs], bacteremia, sepsis, pneumonia and bacterial meningitis) which are progressive and lead to permanent neurological sequelae or death without proper antibiotic treatment **(2).** It is a big problem in pediatric patients, especially in young infants; infants with SBI must be identified and differentiated from non-bacterial infected patients to start the treatment with antibiotics without delay.

However, the symptoms and signs of febrile infants who are suffering from SBIs are usually nonspecific and can not differentiated from mild viral infections, especially in the early phase of SBI **(3).** The temperature of the infant with SBI_S may be elevated, depressed or normal **(4).** Infants with SBI may be suffered from respiratory distress (tachypnea, grunting, cyanosis or apnea), gastrointestinal disturbances including anorexia, regurgitation or vomiting, diarrhea and abdominal distention. Also, they may be suffered from neurologic signs as poor tone, tremors, twitching or convulsions. But these manifestations are not specific and may be subtle or insidious **(5).** Definite identification of SBI requires a positive culture of CSF, blood or urine. But clinicians must choose appropriate management by history, physical examination and initial laboratory tests until cultures results. As antibiotic treatment for treating SBI based only on clinical symptoms and signs may result in over treatment that can be solved by a test or panel of tests able to identify infants with SBI accurately and rapidly in order to obtain an early diagnosis and develop a specific effective treatment for a successful outcome.

Chemokines play an important role at various stages throughout the infectious process**(*6*).**

IP-10 is one of the Chemokines (10kDa interferon –induced protein) that induced by interferon in several cell types as monocytes, neutrophils, fibroblasts and endothelial cells and act as chemo attractant for monocytes and T cells or allow T cell adhesion to endothelial cells (*7*).

2. Material and Methods

This study was done over the period from June 2010 to June 2011in Pediatric Department at Children and Obstetric hospital, Minia University. The study was conducted on 100 pediatric patients less than 4 months of age with a clinical suspicion to have SBI admitted in neonatal intensive care unit (NICU) or complete nursing unit of Pediatric Department.

In all enrolled patients, diagnostic work up was performed to identify or rule out bacterial infection and antibiotic therapy for all patients was performed. SBI was defined as any pathogen isolated from the CSF, blood or urine. For all patients, the followings were done:

1- History taking for detection of the risk factors especially obstetric history as previous still birth, prenatal history as diabetes mellitus and maternal fever, postnatal as low Apgar score at 1 and 5 minutes and present history for symptoms of SBI.
2- Clinical examination for temperature, respiratory distress, abdominal distension, circulatory disturbance and neurological irritability.
3- Laboratory investigations for diagnosis of SBI as the samples were blood, urine and cerebro spinal fluid (CSF). All the samples were collected from patients before starting antibiotics under complete aseptic technique. CSF was withdrawn by lumbar puncture between L3 and L4 under complete aseptic condition and examined physically for aspect and pressure, chemically for protein and glucose and microbiological by culture on different and suitable media. Urine was collected in sterile tubes and sent to the microbiological laboratory for culture as acute urinary tract infections diagnosed as a urine culture with a single pathogen growth >104CFUml-1 from a bladder catheterization or >105CFUml-1 collected from sterile collection bag after sterile preparations . Finally, blood was collected and divided into different tubes for analysis of complete blood count (C.B.C) by Sysnex K12, C- reactive protein by latex agglutination (Spinnreact, Espina), part of the blood was put on highly enriched broth for culture (Salix, Egypt) and subculture was done on suitable media. The last part of blood was centrifuged and frozed at-80C untill measurement of IP10 was performed by specific ELISA kit (Qunitikine, U.S.A). All the procedures were done according to the manufacture instructions. The media and biochemical reactions used in the paper included different types as nutrient agar (Oxoid, England), blood agar, chocolate agar, mac conkey agar (Oxoid, England), triple sugar test, methyl red and indole test.

Statistical Analysis:

All data were recorded in a special chart for every patient. The statistical work of this study was done by a package of computer programs (SPSS version 11 for windows i.e. Statistical Package for the Social Science). *P*-value <0.05 was considered statistically significant.

3. Results

In the current study, the definite identification of SBI requires a positive culture of CSF or blood or urine and the patients were classified into 2 groups according to the result of the culture.

1- Positive culture group included 45 patients
2- Negative culture group included 55 patients

Infants with positive culture (n=45) were older than infants with negative culture (n=55) as the mean age was 52.6 days versus 34.4 days, *p*-value about 0.01. But, there was no significant difference in sex, weight and length of hospital stay between both groups as shown in table 1

Table (1): Demographic characteristics of the patients

Variable		Infants with Positive culture (n=45)	Infants with Negative culture (n=55)	*P*-value
Age (days)	Mean±SD	52.6±37.9	34.4±39.3	0.01*
	Median(Range)	50(2-120)	12(1-120)	
Sex	Male	24 (53.3%)	26 (47.3%)	0.68
	Female	21 (46.7%)	29 (52.7%)	
Weight (kg)	Mean±SD	3.68±2.07	3.85±1.72	0.66
	Median(Range)	3.1(1-8)	3.9(0.8-8)	
Length of hospital stay (days)	Mean±SD	9.9±5.1	9±3.8	0.50
	Median(Range)	9(3-25)	8(4-22)	

* means significant

Table (2): Comparison of clinical findings in the studied groups

Variable	Infants with positive culture (n=45)	Infants with negative culture (n=55)	*P*-value
Fever	36 (80%)	24 (43.6%)	0.001*
Poor appetite	31 (68.9%)	17 (30.9%)	0.001*
Hepatosplenomegaly	13 (28.9%)	3 (5.5%)	0.002*
Respiratory distress	20 (44.4%)	25 (45.5%)	0.98
Gastrointestinal symptoms and signs	22 (48.9%)	22 (40%)	0.42
Disturbed level of consciousness (DLC)	5 (11.1%)	4 (7.3%)	0.72
Convulsions	9 (20%)	12 (21.8%)	0.98

● means significant

As shown in Table 2, there were significant difference between both groups in relation to fever, poor appetite, and hepatosplenomegaly and no significant difference between both groups as regards to respiratory distress, gastrointestinal symptoms and signs, DLC and convulsions.

Laboratory diagnosis of SBI showed the results of blood cultures in which 19 cases with bacteremia (3 *Klebsiella pneumonia*, 6 Enterobacter, 6 group B beta hemolytic Streptococcus, 2 *Salmonella*, and 2 *Staphylococcus aureus*), the urine cultures showed 24 with UTI (13 *Escherichia coli*, 4 *Proteus mirabilis*, 1 *Salmonella* and 6 Enterobacter), and there were 2 cases with bacteremia accompanied with meningitis as shown in table 3. Also, there were significant relations in IT ratio and in the level of IP-10 between 2 groups as *p* value 0.0002 and 0.001, respectively as shown in table 4.

Table (3): Microbial species in infants with positive culture.

Microbial species		Type of culture			Total
		Blood culture	Urine culture	CSF	
Klebsiella pneumonia	**No.**	3	-	-	3
	Percent	6.7%	-	-	6.7%
Enterobacter	**No.**	6	6	-	7
	Percent	13.4%	13.2%	-	26.6%
Streptococcus B	**No.**	6	-	-	6
	Percent	13.3%	-	-	13.3%
Salmonella	**No.**	2	1	-	3
	Percent	4.4%	2.2%	-	6.7%
Staphyllococcus	**No.**	2	-	-	2
	Percent	4.4%	-	-	4.4%
Escherichia coli	**No.**	-	13	-	13
	Percent	-	28.9%	-	28.9%
Proteus mirabilis	**No.**	-	4	-	4
	Percent	-	8.9%	-	8.9%
Meningococcus	**No.**	-	-	2	2
	Percent	-	-	4.5%	4.5%
Total	**No.**	19	24	2	45
	Percent	42.22%	53.33%	4.5%	100%

* means significant

Table (4): Comparison of laboratory data in the studied groups

Variable		Infants with positive culture (n=45)	Infants with negative culture (n=55)	ANCOVA analysis, *P*-value
Corrected WBC counts (mm^{-3})	**Mean±SD**	14018±5907	13139±3527	0.14
	Median (Range)	13400(3800-31200)	13000(6500-22000)	
ANC (mm^{-3})	**Mean±SD**	4227.5±3825	4967.6±4599.7	0.41
	Median (Range)	3500 (450-18500)	3700 (1000-20900)	
CRP (µg/ ml)	**Mean±SD**	17.7±24.3	14.3±19.8	0.42
	Median(Range)	5 (0-121)	0 (0-61)	
IT ratio	**Mean±SD**	0.19±0.13	0.11±0.06	0.0002*
	Median(Range)	0.14(0-0.70)	0.10(0-0.25)	
IP-10 (ng/ ml)	**Mean±SD**	435.1±311	25.5±26.9	0.0001*
	Median(Range)	333 (0-1130)	20(0-125)	

* means significant

Table (5): Correlation of IP-10 with other criteria in the positive group study.

	r-value	*P*-value
Age	0.11	0.24
Corrected WBC count	0.08	0.59
ANC	0.08	0.56
IT ratio	-0.10	0.50
CRP	0.08	0.56

Correlation of IP-10 with other criteria in the infants with positive culture showed no correlation of plasma IP-10 to corrected WBC count, ANC, IT ratio and CRP as shown in table 5.

ROC curve for various cut-off plasma levels of IP-10, CRP, IT ratio, corrected WBC count and ANC in differentiating between presence and absence of SBI were determineded. Based on the ROC analysis, values were identified for each variable that maximized both the sensitivity and specificity. The best cut-off value provides both the highest sensitivity and the highest specificity, located on the ROC curve by finding the highest point on the vertical axis and the furthest to the left on the horizontal axis (upper left corner).

The sensitivity, specificity, accuracy for the best determined cut-off points with areas under the ROC curves (AUC) for predictors of SBI were shown in Table (6). At a cut-off value of 43.5 (ng /ml), IP-10 yielded a sensitivity of 82%, specificity of 90% and accuracy of 86% for differentiating between presence and absence of SBI.

Table (6): Predictors of infants with positive culture.

Variable	IP-10 (ng/ ml)	CRP (µg /ml)	IT ratio	Corrected WBC count (mm-3)	ANC
Cut-off value	43.5	3.5	0.13	13100	2750
Sensitivity (%)	82	53	71	55	64
Specificity (%)	90	60	73	55	40
Accuracy (%)	86	56	72	55	52
AUC	0.91(0.86-0.97)	0.64(0.53-0.75)	0.69(0.58-80)	0.52(0.40-0.64)	0.47(0.35-0.58)

4. Discussion:

Total WBC count was one of the first indicators of SBI. There were several published researches that confirmed total WBC count was very important in diagnosis of SBI as Baker *et al.,*[8], while others found WBC count was not a reliable indicator of SBI in febrile infants (9).

In our study, the mean total WBC counts were not significantly different between those with and without SBI (mean ± SD, 14018 ± 5907 mm^3 versus 13139 ± 3527mm^3 , p =0.14) and at a cut-off level of 13100, total WBC counts just yield a sensitivity of 55%, a specificity of 55%. It suggested that a total WBC was not good predictor between infants with and without SBI.

Our result was in agreement with Bonsu and Harper, [10] who found that there was an inverse relationship between the height of the peripheral WBC count and the likelihood of acute bacterial meningitis relative to bacteremia.

In our study, ANC had been considered to be better than total WBC counts in predicting SBI. But, the overall data of ANC was similar to that of total WBC counts and came in agreement with Hsiu-lin *et al.,* [11]. However, results noted by Isaacman and Burke reported that an ANC cut-off of 10 600 cells/mm^3 was the single best predictor of SBI in children 3 – 36 months of age.[12]

In this study, IT ratio was significantly higher in infants with SBI when compared to infants without SBI (median 0.14 versus 0.10, p-value = 0.0002, sensitivity 71% and specificity 73%) but poor predictor.

CRP is synthesized within six to eight hours of exposure to an infective process or tissue damage. It has a half life of 19 hours and may increase more than 1000-fold during an acute phase response.

In our study, CRP levels were higher in SBI group than non- SBI group but there was no statistatical significance. The concentration of CRP just had a sensitivity of 53% and a specificity of 60% with a cut-off value of (3.5µgm-1) p-value was 0.42 in this study.

Chemokine IP-10 had been identified to play an important role during infectious and inflammatory process such as chemo-attraction for monocytes and T cells or promotion of T cell adhesion to endothelial cells and in TH1- type inflammatory diseases (Manes *et al.,*[7].

In our study, plasma chemokine IP-10 concentrations were significantly higher in SBI group than non- SBI group p-value (0.0001). At cut-off level of (43.5ngml-1), IP-10 levels yielded a sensitivity of (82%), a specificity of (90%), AUC 0.91(0.86-0.97) for differentiation between presence and absence of SBI.

Ng and his associates, 2007[13] found IP-10 and other chemokines (IL8, MIG, and MCP-1) were significantly higher in infected group than non-infected group in the initial evaluation of sepsis. Concentrations of all studied inflammatory mediators (except IL-1beta and RANTES) were significantly higher in the infected than in the non infected group at 0 h, but the levels decreased precipitously by 24h. IP-10 with a plasma cut-off concentration > or = 1250 pg/mL could identify all septicemic and had the highest overall sensitivity (93%) and specificity (89%) at 0 h. They concluded that preterm infants had the ability to induce a robust chemokine and cytokine response during sepsis, and IP-10 is a sensitive early marker of infection.

From the results of our study we concluded that plasma IP-10 is a valuable laboratory test in the assessment of infants aged <4 months old with

suspicion of SBI and may serve as a better diagnostic marker of SBI than total WBC count,CRP, ANC and IT ratio.

Based on these results it is recommend that Serum IP-10 level should be done in every infants with suspicion to have SBI.

Corresponding author
Gihan M. Babrs
Pediatrics Department, Faculty of Medicine, El-Minia University
gihanbabrs@hotmail.com

References

1. Stoll BJ, Hansen N. and Higgins RD. (2005): Very low birth weight preterm infants with early onset neonatal sepsis: the predominance of gram-negative infections continues in the NICHD. Pediatr Infect Dis J.;24 (7):635-9.
2. Luszczak M (2001): Evaluation and management of infants and young children with fever. Am Fam Physician; 64(7): 1219-26.
3. Macias CG (2006); Prospective evaluation of the risk of serious bacterial infection in children who present to the emergency department with hyperpyrexia (temperature of 106°F or higher). Pediatrics, 118: 34-40.
4. Weisman LE, Stoll BJ and Cruess DF (2001): Early-onset group B streptococcal sepsis: a current assessment. J Pediatr.; 121: 428-33.
5. Klein JO and Remington JS (2001): Current concepts of infections of the fetus and newborn infant. In: Klein JO, Remington JS (eds). Infectious Diseases of the Fetus and Newborn Infant. 5th ed. WB Saunders;5: 943-998
6. CalandraT.,HollandS.(2004):Infection in immuonocompermised host. Innate and acquired host defences against infection. In: Cohen J,Powderly WG(eds). Infections Diseases, 2nd end. Vol 1;pp.1033_43.
7. Manes TD, Pober JS and Kluger MS (2006): Endothelial T cell lymphocyte interactions: Ip10 stimulates rapid transendothelial migration of human effector but not central memory CD_4 T cells. Transplantation, 82:S9-14 Ed;91:208-212.
8. Baker MD, Bell LM, Avner JR (1993): Outpatient management without antibiotics of fever in selected infants. N Engl J Med.; 329:1437–1441
9. Baraff LJ (2000): Management of fever without source in infants and children. Ann Emerg Med.; 36:602–614.
10. Bonsu BK and Harper MB (2003): Identifying febrile young infants with bacteremia: is the peripheral white blood cell count an accurate screen? Ann Emerg Med.; 42:216–225
11. Hsiu-lin, Chih-Hasing Hong, Rci-Cheng (2008):Plasma IP-10 as a predictor of serious bacterial Infection in infants less than 4 months of Age Journal of Tropical Pediatrics ;55:103-107
12. Isaacman DJ , Burke BL (2002):Utility of the serum C-reactive protein for detection of occult bacterial infection in children . Arch. Pediatr. Adolesc. Med .; 156 : 905 – 9 .
13. Ng PC, Li K, Chui KM, Leung TF, Wong RP, Chu WC, Wong E and Fok TF (2007): IP-10 is an early diagnostic marker for identification of late-onset bacterial infection in preterm infants. Pediatr Res., 61:93-98.

3/13/2012