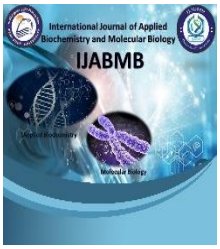
International Journal of Applied Biochemistry and Molecular Biology (IJABMB)



Interferon-gamma inducible protein-10 role in neonatal sepsis

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Running Title: Interferon-gamma inducible protein-10 in neonatal sepsis

Abstract

Background: Sepsis continues to be a major cause of neonatal mortality and morbidity, especially in low- and middle-income countries. Because neonatal sepsis has hazy signs and symptoms, tests are required to make the diagnosis. Important treatment decisions may be postponed due to the time-consuming nature of the classic techniques for culture utilized in current diagnostic procedures.

Objective: The study sought to evaluate the diagnostic utility of interferon-gamma inducible protein 10 (IP-10) in newborn sepsis.

Patients and methods: There were two groups in the study: group (1) consisted of thirty newborns who were diagnosed with neonatal sepsis based on laboratory and clinical data, and group (2) consisted of fifteen healthy neonates who showed neither clinical nor laboratory indicators of sepsis. The enzyme-linked immunosorbent assay (ELIZA) was used to determine the serum level of IP-10.

Results: According to the findings, there was a statistically significant variation in IP-10 between the two groups under study. Except for age, which showed a strong positive association with IP10, other patient factors showed no significant link with IP10. According to the Roc curve analysis, the IP-10's sensitivity and specificity as a marker to differentiate between patients and controls were 93.3% sensitivity and 100% specificity, with a cutoff of 133.

Conclusion: In comparison to other regularly used laboratory markers, IP-10 demonstrated superiority in detecting neonatal sepsis when added to the panel of screening tests for the condition. It is an easy, quick, and predictive tool (CBC and CRP).

Keywords

Neonate, Sepsis, biomarkers, diagnosis, IP-10.

Introduction

The systemic reaction to infection in neonates has been referred to as neonatal sepsis. It was once thought to mostly involve bacterial infections. It is now understood, although, that a wide range of microorganisms other than bacteria could be in charge (1).

For neonates, particularly those born before term, infections due to bacteria are a major cause of morbidity and mortality (2). Since untreated sepsis can have a 50% fatality rate, most clinicians start treatment while they wait for culture findings (2).

The cellular element that makes up the defense system attaches and lyses the bacteria after it has passed past chemical and physical barriers in neonatal sepsis, causing the neutrophils and monocytes to release a range of inflammatory mediators. Additionally, they trigger the coagulation, complement, and fibrinolytic cascades, which results in the production of pro-inflammatory and vasoactive mediators (3). Numerous cytokines are also produced by macrophages and other cells; among these, tumor necrosis factor-alpha (TNF-α), interleukin-Ib (IL-Ib), IL-6, IL-8, and IL-10 are significant mediators of sepsis. Most of these mediators can either stimulate or inhibit the release of other mediators or themselves (4).

The doctor's clinical judgment determines if sepsis neonatorum is diagnosed. Symptoms and indicators could be deceptive and ambiguous. To establish his diagnosis, the doctor must be guided by a comprehensive prenatal history, a comprehensive physical examination, as well as a proper laboratory workup (5).

CXCL10, also known as IP-10 (interferon-gamma inducible protein 10 kDa), was first discovered to be an IFN-gamma-inducible gene. It is triggered in a range of cells in reaction to lipopolysaccharide (LPS) and IFN-gamma. Unlike similar CXC (the cysteine (amino acid), x, cysteine) cytokines, IP-10 cannot chemotactically interact with neutrophils. This pleiotropic molecule seems to target monocytes and activated T cells (6).

IP-10 suppresses angiogenesis and bone marrow colony development (7). Additionally, it can control T cell maturation, promote NK and T cell movement, and alter the expression of adhesion molecules (8). According to its aa sequence, IP-10 belongs to a subclass of CXC cytokines that do not have the ELR domain. Similarly, IP-10 and MIG (cytokines produced by interferon-gamma) bind to CXCR3 (9)

In line with earlier findings that CD4 T cells from IFN-gamma mice exhibited more antigen-specific proliferative responses when compared to wild-type mice, the interaction of CXCR3 and IP-10 to increase IFN-gamma synthesis and block T-cell proliferation suggests that IFN-gamma could exert antiproliferative impacts and minimize the proliferation of CD4 pathogenic T cells (10).

In contrast to IP-10, which stimulates the IFN-gamma gene strongly through antigen or polyclonal stimulation, IP-10 was described as a downstream molecule that is directly activated by IFN-gamma. It is reasonable to hypothesize that CXCR3/IP-10 and IFN-gamma could have a positive amplified loop in vivo. Such input amplification would emphasize the critical function of IP-10 in the pathophysiology of Th-1-dependent disorders and aid in the explanation of the mechanisms behind inflammatory conditions (11).

In order to evaluate the diagnostic utility of protein -10 in instances of newborn sepsis using the enzyme-linked immunosorbent assay (ELIZA), case-control research was conducted in the newborn Intensive Care Unit at the University Hospital of Beni-Suef.

Patients and Methods

Thirty newborns with a diagnosis of neonatal sepsis according to laboratory and clinical examination results were included in group (1) of the study, while fifteen healthy neonates without any clinical symptoms or laboratory indications of sepsis were included in group (2).

Apnea, tachypnea, breathing difficulty, cyanosis, hypotonia, convulsions, humble skin color, inadequate perfusion, anxiety, apathy, poor nutrition, hepatomegaly, splenomegaly, distension of the abdomen, hypothermia, hyperthermia, as well babies with a positive CRP (6 mg/dl or more) were among the criteria for inclusion. Babies with congenital infections suspected inborn metabolic errors, prenatal hypoxia, congenital malformations, chromosomal abnormalities, and negative CRP were among the exclusion criteria.

Methods:

Both groups' newborns underwent the following tests: Prenatal, and natal, as well as family histories are taken, with special attention paid to sex, newborn weight, age at conception, postnatal age, and delivery method. thorough clinical examination with a focus on cyanosis, residual gastric aspirate, abdominal distension, fever, gestational age, and responsiveness to oral feeding, among other factors.

Investigations in the lab included whole blood count and differential count the ratio of immature neutrophils to total neutrophils (I/T ratio) of 0.2 or higher, and a quantitative CRP of 6 mg/dl or above is regarded as a case. Blood culture: 8 milliliters of nutrient broth were mixed with 5 milliliters of sterilely extracted blood, which was then incubated on solid media at 37 degrees Celsius for identification and culture. Estimation of level of protein- 10 on the first day of diagnosis.

On day one of the sepsis examination, all newborns will be included at the same time of clinical presentation. The immunological test, ELISA intended to assess human IP-10 in serum. Using a serum tube, we let the samples coagulate for half an hour before centrifuging them for fifteen minutes each at 1000Xg to extract the serum. The samples were then refrigerated at -20°C. Steer clear of repeated freezing cycles. The appropriate test will be used to statistically assess the results.

Principles of Assay:

A quantitative sandwiches enzyme immunoassay approach is used in the assay's concepts. A microplate has been pre-coated with an IP-10-specific monoclonal antibody. Any IP-10 that exists is attached by the antibodies that have been immobilized after the specimens and standards have been poured into the wells. An IP-10-specific enzyme-linked polyclonal antibody is added to the wells after any unbound materials have been cleaned out. After washing to get rid of any unattached antibody-enzyme reagent, the wells are filled with a substrate solution, and the color changes according to how much IP-10 was bound in the first place. The color's intensity is measured following the color evolution is halted. DIP 100/SIP100/PDIP100 is the catalog number for the Quantikine human CXCL/IP-10 immunoassay.

Statistical Methods

The data was coded and entered using version 21 of the Statistical Software for the Social Sciences (SPSS). The data were statistically characterized using the median, the standard deviation, frequency ratios (percentages), and the absolute frequency (number of cases), where appropriate. Quantitative factors were compared using ANOVA, and a multiple comparisons post hoc test was utilized. Data categories were compared using the Chi-square test. An exact test had been employed in its place when the expected incidence was less than five. The frequency of genotypes and alleles among the patient groups was analyzed using chi-square tests. The 95 percent confidence interval (CI) and odds ratio (OR) were calculated. A value for probability (p-value) of lower than 0.05 was found to be statistically significant.

Results

This study was carried out on 30 subjects with an age mean of 14.6 days and sex of 20 (66.7%) males and 10 (33.3%) females in the Faculty of Medicine NICU, Beni Suef. On the other hand, a group of 15 healthy served as controls.

Table 1 showed a statistically significant difference between the two groups studied as regards laboratory findings which were highly significant with Plt and IP10 (Figure 1).

Table (1): Comparison between patient and control as regards laboratory findings

	Patient Mean±SD	Control Mean±SD	P value	Sig.
Hb (gm/dl)	14.6±2.2	16.4±2.0	0.013	S
Tlc	12.3±9.7	6.2±1.5	0.021	S
Plt	140.9±102.3	235.3±44.2	0.001	HS
IP10 (Pgm)	651.7±696.5	82.4±28.1	0.003	HS

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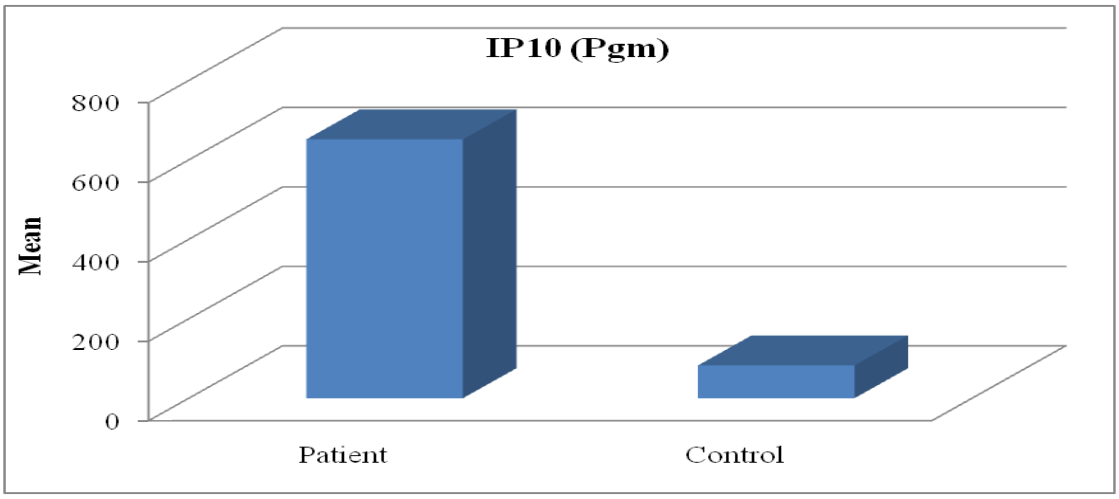

Figure 1: Comparison between patient and control as regards IP 10

Table 2 showed the correlation between IP10 and all parameters in patients which was not significant except age shows a significant positive correlation with IP10, correlation coefficient 0.4, p-value <0.05.

Table (2): Correlation between IP10 and all parameters in patients

	IP10 (Pgm)		Sig.
	r	P value	
Age (Day)	**0.4**	**0.030**	S
Stay in NICU (Day)	0.3	0.064	NS
Gestational age (Weak)	0.1	0.504	NS
Weight (kg)	0.1	0.616	NS
Hb	-0.2	0.350	NS
Tlc	-0.1	0.435	NS
Plt	0.1	0.689	NS
CRP	0.2	0.247	NS

r: Correlation coefficient

Table 3 and Figure 2 showed the sensitivity and specificity of the IP10 (Pgm) as a marker of patients (the ability of IP10 (Pgm) to distinguish between patients and control) were found to be 93.3% sensitivity and 100% specificity, and cutoff (>133). Figure 3 showed a statistically significant difference between patients and control groups as regards IP10 (Pgm) which was highly significant, we note that abnormal IP10 was more frequent among patients (93.3%), while abnormal IP10 was low frequent among control (0%). There is no statistically significant difference between the two studied groups normal and abnormal IP10 (Pgm) as regards demographic data and laboratory findings except CRP.

Table (3): Cut off, sensitivity and specificity for IP10 between patient and control

	IP10 (Pgm)
Cut off	**>133**
Sensitivity	93.3
Specificity	100
+PV	100
-PV	88.2
Prevalence of disease	66.7
Accuracy	95.6

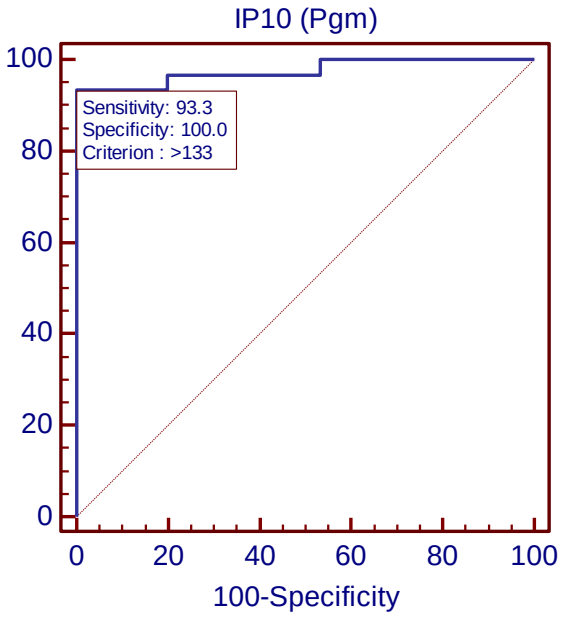

Figure (2): ROC curve for IP10

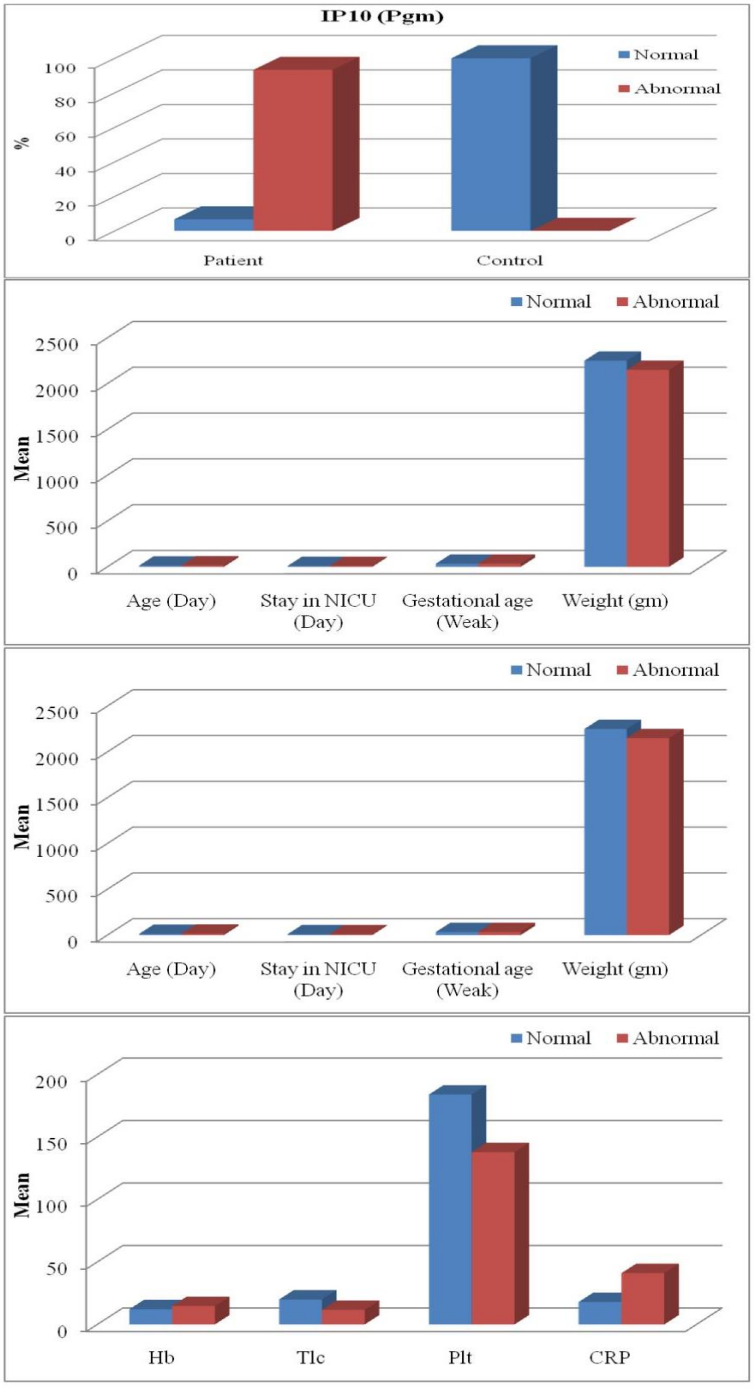

Figure 3. normal and abnormal levels of IP 10

Discussion

Systemic inflammation and extensive tissue damage are hallmarks of sepsis, a clinical state that exacerbates severe infection (12). Although there are several contributing factors, almost any pathogenic organism can cause sepsis. One of the main causes of newborn mortality and morbidity in the world is infection. The diagnosis is made based on clinical suspicion and septic clinical signs, and it is confirmed by positive microbiologic results from cultures a few days following the start of empirical therapy. Quick objective in vitro tests are desperately needed to diagnose infection in neonates exhibiting clinical instability (13).

Serum cytokine and chemokine immunoassays may be employed as an infection marker for prognostic and diagnostic reasons. Leukocytes are drawn to inflammatory or microbial invasion sites as a result of a quick and well-coordinated series of host reactions brought on by exposure to microbes and their byproducts. Chemoattractant cytokines, also known as chemokines, are primarily responsible for controlling the activity and transport of leukocytes into particular bodily regions (13).

An increase (or reduction) in the levels of these mediators in the blood may serve as an early warning sign or biomarker of systemic newborn septicemia since these mediators are up-regulated (or down-regulated) at an early stage in the inflammatory process. Only a small number of these mediators are sufficiently sensitive and specific to be considered as helpful diagnostic markers of infection, even though the circulation concentrations of several chemokines and cytokines are probably altered in healthy and infected people (14).

The chemokine IP-10 was found to be crucial in inflammatory and infectious processes, including Th1-type inflammatory illness, chemoattraction of monocytes and T cells, and the enhancement of T-cell attachment to endothelial cells. (15).
The purpose of this study was to assess IP-10's diagnostic utility in newborn sepsis. Forty-five newborns (thirty patients and 15 controls) were recruited for the study. The average gestational age of the patient cases in our study was 33.7 weeks. For confirmed cases, the average newborn weight was 2.157 kg. According to statistical analysis, the groups differed significantly in terms of birth weight and gestational age. There were twenty males as well as ten females in the groups with proven sepsis, and ten males as well as 5 females in the control groups.

According to statistical analysis, there was not a significant variation in the clinical features between the infected and noninfected groups concerning sex (p-value 0.635). However, there was a statistically significant difference in weight (p-value 0.007), and gestational age between the two groups. This is consistent with NG et al., 2007 (14). Additionally, Sallam et al. (2012) (16) and Chen et al. (2011) (17) showed that older infants with sepsis were compared to healthy ones (p-value = 0.01). However, the two groups' weight and sex did not differ significantly.

However, as we found in our study, there is a highly significant relationship between sepsis and weight (p-value 0.007), as well as research by De Benedetti et al., 2007 (18) and Gomella et al., 2004 (1) also found that a decreased birth weight has been associated with a higher likelihood of sepsis. Premature birth or low birth weight is the most significant neonatal characteristic that predisposes to infection, as the incidence of infection is 3–10 times higher in preterm infants than in full-term, normal-birth-weight infants (19).

Additionally, our study supported the findings of Gerdes (2004) (20) that males had a significantly higher frequency of newborn sepsis, and Rehan (2002) (21) that male infants are four times more likely than females to develop sepsis, as we found that males experience sepsis twice as frequently as females. There is a hypothesis that a gene on the X chromosome is involved in immunoglobulin synthesis or the function of the thymus.

Shah (2006) (22). One hundred newborn sepsis cases and one hundred control neonates made up the study. A variety of neonatal sepsis risk variables were evaluated in each of these instances. Although they were not statistically significant, maternal illiteracy, primi-gravida moms, PROM, eclampsia, and UTIs all modestly increased the risk of developing sepsis. Although the p-value was very significant and prematurity had a 4.85-fold increased risk for sepsis, in our analysis, three cases had hypertension, and six cases had PROM.

Additionally, poor feeding was the most common symptom in our study's patient group, followed by trouble breathing and poor skin tone. Temperature instability, poor eating, and evidence of respiratory distress were the most common symptoms and indicators of sepsis in the identical study by Shah, 2006 (22). According to a study by Korang et al. (2019) (23), the most prevalent symptom among infants receiving antibiotic treatment for clinical signs of infection and positive screening tests was respiratory distress, which was followed by sclerema and poor activity.

According to a study by Elizabeth et al. (2004), the most common signs and symptoms of sepsis were respiratory distress, cyanosis, or apnea. The most prevalent clinical indicators in each group were temperature instability and poor feeding, according to a study conducted by Hajiehe and Sedigheh in 2005 (25) on 200 newborns to assess the clinical state in neonatal sepsis. In the confirmed group, neurologic symptoms such convulsions, lethargy, and fontaneal bulging were more prevalent. The most common skin appearance in the identified group was 47%, compared to 0.7% within the healthy group, affecting a total of 14 newborns.

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CRP levels in the septic group were higher than those in the non-septic group in our study, with a range of 6 to 96 mg/dl. This is consistent with a study by Carrigan et al. (2004) that found that CRP concentrations in septic neonates ranged from 12 to 159 mg/ml. This slight variation in both the highest and lowest levels of CRP is caused by variations in laboratory procedures.

However, CRP is not a highly sensitive early indicator of infection, as shown by NG et al., 2007 (14) and Sallam et al., 2012 (16). This is explained by the fact that CRP is produced 6–8 hours after being exposed to an infectious process or tissue injury, has a 19-hour half-life, and can rise by more than 1000 times throughout an acute phase reaction.

The cytokine IP-10 was identified to be better than the total WBC count in the study by NG et al., 2007 (14) because the role of White blood cells in the detection of SBI produced only a sensitivity of 63.2%, a specificity of 72.5%, and an accuracy of 66%. This means that the IP-10 is a more accurate diagnostic tool for diagnosing SBI, having a sensitivity 93.3%, and a specificity 100%, and an accuracy of 95.6%.

SBIs support the findings of Ng et al. (2007) (14), Sallam et al. (2012) (16), and CHEN et al. (2011) (17), who found no significant difference in mean total white blood cell counts between individuals exhibiting and not exhibiting SBIs and proposed that a total WBC count was not a reliable indicator of whether an infant had SBIs or not.

However, Baker et al. (2000) (26), who affirmed that the total count of white blood cells was crucial in identifying the presence of SBIs, differed from Ng et al. 2007 (14).

Twenty-eight of the thirty cases in our investigation had positive blood cultures (16 had klebsiella, five had Acinetobacter, four had coagulase-negative staph (cons), one developed staph aureus, one developed pseudomonas, and one developed MRSA). The initial administration of antibiotics in cases of early clinical suspicion is responsible for this. This is in line with Hilgendorff et al., 2005 (27), who stated that repeated cultures of blood are required to provide an accurate diagnosis because it is commonly acknowledged that early-stage neonatal sepsis is frequently a clinical diagnosis due to the possibility of false negative blood cultures following antenatal antibiotic administration. Another reason could be that the baby's blood sample wasn't enough for a culture or that the technique wasn't done correctly (28, 29).

About 90% of the cases in the current study had positive blood cultures; however, Procianoy and Silveire's 2004 study (30) revealed that only 18 out of 85 cases (21%) of newborn sepsis had positive blood cultures. Poor progress was indicated by the elevated blood concentration of IP-10, which was positively connected with the seriousness of the infection process, the incidence of septic shock, multisystem organ failure, and death (31).

According to the ROC curve, the IP-10 cut-off for sepsis diagnosis in our investigation was 133 pg/ml, having a sensitivity of 93.3% as well as a specificity of 100%. The association between IP-10 and late-stage Sepsis caused by bacteria and NEC in premature babies was examined in the study by Ng et al., 2007 (14). At 0 hours, the plasma IP-10 cut-off was 1250 pg/ml, with 80% sensitivity and 75% specificity. The reason for the discrepancy in IP-10 levels between our study and the one above is that we employed human IP-10 ELISA, whereas they used cytometric bead array kits and flow cytometry as their analysis method.

The IP-10 cut-off value was 48.2 pg\ml, with sensitivity = 81% and specificity = 94%, in other studies by CHEN et al. (2011) (17), who investigated the function of plasma IP-10 as a marker of severe infections with bacteria in neonates and young infants. Similarly, Sallam et al. (2012) (16) investigated the function of IP-10 as a prognostic marker of serious infection in newborns and young infants, with a cut-off of 43.5 pg\ml, with sensitivity = 82% and specificity = 90%. These discrepancies were also brought on by early sample withdrawal (samples were taken at 0 hours before the initiation of antibiotic therapy) and variances in laboratory standards.

The current study showed that newborns with neonatal septicemia had plasma IP-10 levels that were noticeably greater than those of healthy neonates. IP-10 was deemed a

highly accurate diagnostic test for sepsis in our Roc curve analysis, with a diagnostic accuracy of 95.6%, a sensitivity of 93.3%, a specificity of 100% cut off at 133 pg/ml, a PPV of 100%, and an NPV of 88.2%.

Conclusion

According to our research, newborns with neonatal septicemia had plasma IP-10 levels that were noticeably greater than those of healthy neonates. IP-10 was deemed a highly accurate diagnostic test for sepsis in our Roc curve analysis, with a diagnostic accuracy of 95.6%, a sensitivity of 93.3%, a specificity of 100% cut off at 133 pg/ml, a PPV of 100%, and an NPV of 88.2%.

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