Original Article

Estimation Of Interleukin 27 And Interleukin 33 Serum Levels In Iraqi Neonatal Sepsis

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Abstract

Bloodstream infections in neonates are one of the most life-threatening infections in them . In this study, a total of 140 blood samples were collected from neonates at AL-Batoul Teaching Hospital for Gynecology and Pediatrics in Baqubah/Diyala, their ages ranging from 1 day to 28 days during the period from November 2021 to March 2022. The samples were subjected to a culture study as well as hematological and immunological tests (CRP, measuring the level of IL-27 and IL-33). Out of total cultured samples 45 give a positive blood culture, 45 specimens from blood culture negative was chosen to become (control group) to be comparable to positive culture specimens. The results of the CRP test showed in the Gram-negative bacteria group, the positive result was 76.92% (10 of 13). In the Gram-positive bacteria group, the positive CRP percentage was 84.85% (28 of 33). While in the control group the positive result was 13.33% (6 of 45) , significant differences appeared between the compared groups. The results showed the mean levels of IL-27 in the sepsis patient group and control group were (21.14 ± 1.01 and 14.49 ± 0.34 pg/ml, respectively). also showed the mean levels of IL-33 were in the sepsis patient group and control group (250.28 ± 15.75 and 101.16 ± 3.38 pg/ml, respectively). There was a significant increased level of IL-27 and IL-33 in the sepsis patients' group compared to the control group.

Keywords: Sepsis, CRP, IL-27, IL-33.

INTRODUCTION

Sepsis results from an injury associated with systemic inflammation, a response with the production of many inflammatory mediators such as cytokines that consider potent inflammatory mediators (Aziz et al., 2013). When cytokines levels in serum increase during infection, changes will occur in the molecules of inflammatory stimuli. Therefore, these cytokines are considered potential markers diagnosis of neonatal infection (Skirecki et al., 2012; Opdal, 2018). Additionally, the ensuing immunosuppression brought on by sepsis makes patients more vulnerable to getting secondary infections and their measurement provides new options for the follow-up and diagnosis of neonates with sepsis, thus enabling treatment early and, as a result, increased neonatal survival (Hotchkiss et al., 2013).

Cytokines are tiny proteins that are released by immune system stimulated cells in order to affect other cells, they could also be polypeptides or glycoproteins, by attaching to receptors on target cells, cytokines influence the nature, strength, and duration of the immune response. Hematopoiesis is also regulated by cytokines. Fibroblasts and other immune stromal cells and endothelial cells can produce them and regulate cell growth. Survival, differentiation, immune cell activation, cell migration, and death are all processes that occur in the body (Zamarron and Chen, 2011).

Cytokines play a role in almost all aspects of immunity and inflammation. The stimulation of the innate immune response, the creation of cytotoxic T cells, and the formation of antibodies by the humoral immune system are all examples of effects. There are four types of cytokines: interleukins, interferons, chemokines, and tumor necrosis factors. Interleukins have a key role in immune system cell proliferation, differentiation, and maturation (Steinke and Borishu, 2006; Brocker et al., 2010).

Interleukins (IL) are a family of cytokines that were first discovered in leukocytes. However, it was eventually discovered that they were created by a variety of immune system cells. They stimulate immune cell proliferation, differentiation, maturation, migration, and adhesion. They have both pro- and anti-inflammatory properties.

Interleukins can have an autocrine or paracrine effect. There are variety discovered interleukins, varying from IL-1 to IL-40 , and each one has a significant different role in the immune response (Justiz and Qurie, 2018).

In the present study, we aimed to evaluate the relationship between Interleukin 27 (IL-27) and IL-33 role and septicemia in the neonates. IL-27 is a heterogeneous cytokine belonging to IL-12 family, has been discovered to act as a biomodulator of inflammatory immune responses (Hunter and Kastelein, 2012; Cao et al., 2014). The Epstein-Barr virus gene 3 (EBI3), a subunit of IL-27, was demonstrated to have high prediction potential for bacterial infection using genome-wide expression analysis (Wong et al., 2012). IL-27 has also been found to be an effective biomarker for predicting the likelihood of blood bacterial infection in critically ill children and adults (Hanna et al., 2015). However, there is no information on how IL-27 levels alter in newborn sepsis.

Interleukin-33 (IL-33) is a member of IL-1 family that promotes the production of cytokines associated with T helper 2 (Th2) cells (Yagami et al.,2010). IL-33 expressed by varsity of cells such as fibroblasts, mast cells, macrophages, osteoblasts, endothelial cells, and epithelial cells (Mirchandani et al., 2012).

IL-33 has variety functions as a nuclear factor inside the cell and as a cytokine outside the cell (Baekkevold et al., 2003). Thus, the effects of IL-33 are either pro- or anti-inflammatory depending on the disease. In addition to acting as a nuclear factor that regulates gene transcription, IL-33 has the capacity to operate as a typical cytokine, it's intended to function as an "alarmin" that released after a cell dies to alert the immune system for tissue's injury or stress. (Miller, 2011).

MATERIALS AND METHODS

The current study was done in the laboratories of AL-Batoul Teaching Hospital for Gynecology and Pediatrics in Baqubah, Diyala governorate, including 140 blood specimens collected from the neonates admitted to the hospital with suspected sepsis, 45 of 140 samples were negative to blood culture chosen as control group, the ages of the both groups ranging from 1 day to 28 days. Blood specimens were collected during the period from November 2021 to March 2022.

Five ml of blood drawn from the infants and divided into three parts, 2 ml were injected to do a complete blood count (CBC) test, and 1 ml placed in a gel tube to assess the immunological tests (C-reactive protein, Interleukin-27 and Interleukin-33), and the last 2 ml were injected into the blood culturing vial that used in another part of the study under publishing.

The IBM SPSS version 28.0 was used to analyze the data statistically, the data expressed as mean, standard error of mean for parametric data, and the probability calculated by student's t-test, ANOVA table. While the non-parametric data are expressed as frequency and frequency percentages, the probability was calculated by using Pearson's chi-square. The probability was significant when it was less than 0.05.

RESULTS

A total of 140 patients' blood samples were collected and cultured after inclusion and exclusion criteria. Out of total cultured samples 45 give a positive blood culture while 95 give negative blood culture result.The results showed that the percentage of neonates' males was 68.8% while female 31.1%. Regarding age the results in table 1 showed the means of age between patients and control groups (7.84 ± 1.347.84 ± 1.34 and 10.52 ± 1.52 day) in males respectively, (7.77 ± 1.90 and 7.79 ± 1.38) in females respectively. there was no significant difference between the means

Table 1: Age comparison between patients and control groups according gender

Gender	Mean ± SE of Age (days)		Probability
	Patients	Control	
Males	7.84 ± 1.34	10.52 ± 1.52	P > 0.05
Females	7.77 ± 1.90	7.79 ± 1.38	P > 0.05
Total	7.82 ± 1.09	9.67 ± 1.14	P > 0.05
Probability	P > 0.05	P > 0.05	

Complete blood counts assessment between patients and control groups

The results in table 2 showed the values of complete blood counts between the sepsis patients and control groups. There was a significant increasing mean level of Hb in sepsis patients' group compared to control group (15.57 ± 0.35 versus 14.41 ± 0.39 g/dl, respectively) (Table 2).

Also, there was a significant increasing mean of WBCs count in sepsis patients' group compared to control group (13.61 ± 0.68 versus 8.54 ± 0.39 $x10^3/mm^3$, respectively) (Table 2). While the results of platelets count mean were not significantly decreased in sepsis patients' group compared to control group (257.98 ± 15.16 versus 269.51 ± 9.46 $x10^9/L$) (Table 2).

Table 2: Complete blood counts between sepsis patients and control groups

	CBCs mean ± SE		
Group	**WBCs count (x 10^3/ mm^3)**	**Hb level (g/dl)**	**PLTs (x 10^9/L)**
Patients	13.61 ± 0.68	15.57 ± 0.35	257.98 ± 15.16
Control	8.54 ± 0.39	14.41 ± 0.39	269.51 ± 9.46
P-value	**0.0001** **	**0.0304** *	0.502 NS

Ns: Non-significant (P > 0.05), *: Significant at P < 0.05, **: Significant at P < 0.001

CRP assessment between patients and control groups

The results of CRP assessment showed that the positive result percentage was 13.33 (6 of 45) and the negative result percentage was 86.67 (39 of 45) in control group. While the sepsis patients' group was divided into two group according to gram stain results, gram-positive and gram-negative bacterial isolates' groups. In gram negative bacterial isolates group, the positive result percentage was 76.92 (10 of 13). While in gram positive group, the positive CRP percentage was 84.85 (28 of 33). There was a significant difference appeared between the compared groups (Table 3).

Table 3: Distribution the CRP frequencies percentages according to the studies groups

Group	CRP frequency (%)		P-Value
	Positive	Negative	
Control (Negative blood culture)	6 (13.33%)	39 (86.67%)	**0.0001** **
Gram-Negative	10 (76.92%)	3 (23.98%)	**0.0001** **
Gram-Positive	28 (84.85%)	5 (15.15%)	**0.0001** **

**: Significant at P < 0.001

IL-27 and IL-33 serum levels between sepsis patients' and control groups

The results of IL-27 and IL-33 serum levels in table 4, showed a significant increased level of IL-27 in sepsis patients' group compared to control group (21.14 ± 1.01 and 14.49 ± 0.34 pg/ml, respectively). Also, similar results of IL-33 were appeared (250.28 ± 15.75 and 101.16 ± 3.38 pg/ml, respectively) (Table 4).

Table 4: IL-27 and IL-33 serum level between sepsis patients' and control groups

	Mean ± SE (pg/ml)	
Groups	**IL-27**	**IL-33**
Patients	21.14 ± 1.01	250.28 ± 15.75
Control	14.49 ± 0.34	101.16 ± 3.38
P-value	**0.0001** **	**0.0001** **

**: Significant at P < 0.001

Also, the results in table 5 showed the mean of IL-27 serum level between the sepsis patients' group and control group according to the aged groups. In the sepsis neonate patients' group, there was a significant increased level of IL-27 in the aged group 0 – 3 days compared to the age group 4 – 28-days (24.92 ± 1.97 *vs.*18.85 ± 0.86 pg/ml, respectively). While in control groups, the results showed no significant differences in the level of IL-27 between the two aged groups (14.76 ± 0.82 *vs.* 14.43 ± 0.38 pg/ml, respectively).

Table 5: Distribution of IL-27 serum level between the patients and control groups according to aged groups

	IL-27 serum level mean ± SE (pg/ml)		P-value
Group	**Age groups**		
	(0-3) days	**(4-28) days**	
Patients	24.92 ±1.97	18.85 ±0.86	**0.003** *
Control	14.76 ±0.82	14.43 ±0.38	0.625 NS

Ns: Non-significant (P > 0.05), *: Significant at P < 0.05, **: Significant at P < 0.001

IL-33 serum level mean between sepsis patients' and control groups according to the aged group

Furthermore, the results in table 6 showed the mean of IL-33 serum level between the sepsis neonate patients group compared to the neonate control group according to the aged groups. The results appeared a significant increased level of IL-33 between the aged group 0 – 3 days and 4 – 28 days in the sepsis patients' group (299.54 ± 30.52 *vs.* 220.37 ±15.08 pg/ml, respectively), while there was no significant difference appeared between the two aged group in the control group (93.47 ± 7.47 *vs.* 103.08 ± 3.78 pg/ml, respectively).

Table 6: Distribution of IL-33 serum level mean between sepsis patients' and control groups according to the aged groups

Group	IL-33 serum level mean ± SE (pg/ml)		P-value
	Aged groups		
	(0-3) days	**(4-28) days**	
Patients	299.54 ±30.52	220.37 ±15.08	**0.013** *
Control	93.47 ±7.47	103.08 ±3.78	0.261 NS

Ns: Non-significant (P > 0.05), *: Significant at P < 0.05

DISCUSSIONS

Our results showed that the percentage of neonates' males is higher than female (68.8% *vs.* 31.1%, respectively). These results in agreement with study in Syria by Noha *et al*., (2022) who showed that most of patients were males (65.5%). While, the results of another study from Oman by Abdellatif *et al*., (2019) appeared that 55.2% were males, resembling the results reported by Abbas, (2021) in Diyala, who mentioned that 53.8% were males. In addition, our results agreed with the result of Jemal *et al*., (2021) from Ethiopia, who showed that 53.5% of patients were males. Specific pathogenic differences have unknown causes, and the mechanism underlying them is unclear and poorly understood. It is potentially complex, influenced by genetic, immune and hormonal factors.

The results of current study showed a significant increased count of WBCs in sepsis patients group compared to control group (13.61 ± 0.68 *vs.* 8.54-± 0.39 $x10^3/mm^3$, respectively). This result in agreement with a pervious study in Baghdad by Abood (2021) who revealed that a difference between the mean of WBCs count in sepsis patients group compared to control group. Also, another study by Yang *et al*., (2016) showed the mean count of WBCs was higher in the sepsis group

than non-sepsis group.
In addition, the mean level of Hb in the present results showed a significant increase in the sepsis patients group compared to control group (15.57 ± 0.35 and 14.41 ± 0.39, respectively). While, the results of platelets count showed no significant difference appeared between in sepsis patients and control groups (257.98 ± 15.16 and 269.51 ± 9.46, respectively). These results were partially comparable to the results of Mostafa Kamel *et al*. study (2021) in Egypt, who showed that the mean of Hb level and PLTs count was lower in the sepsis group compared to control group.
Sepsis can cause anemia through bone marrow dysfunction or bleeding (petechiae, purpura, oozing) and hemolysis (Kliegman *et al*., 2016). Also, platelets count may drop hours to days before clinical sepsis develops, but it usually remains elevated until the neonate is sick for a one or two days. Platelets count is often not helpful in evaluating a neonate for sepsis; because of the timing of these changes thus hematological marker used alone are inaccurate to define neonatal sepsis in the absence of a positive blood culture (Wynn *et al*., 2014).
The results of current study showed an increased frequency percentage of positive CRP in neonatal septicemia patients in both gram-positive and gram-negative bacteria compared to control (84.85%, 76.92% and 13.33%, respectively). In addition, the results showed significant differences between the positive and negative of CRP results for all groups.

These results were comparable to pervious results reported by Akhmaltdinova *et al.* (2021) and Hanaganahalli *et al*., (2018), who showed significant differences in CRP results between the patients and control group. Liu et al., (2019) evaluated the diagnostic value of the CRP test in detecting neonatal septicemia, and suggested that the CRP test has a good diagnostic marker for neonatal sepsis.
The serum CRP levels are usually relatively low, nevertheless, when a person is infected with bacteria, endogenous neurotransmitters are released by WBCs and other inflammatory cells to excite liver cells. CRP is synthesized in 4–6 hours and peaks at 36–50 hours, hence the inflammatory process starts 6–12 hours after CRP is detected (Magrini *et al.*, 2014). Neonatal sepsis, in which germs infiltrate the bloodstream, release toxins, and trigger a systemic inflammatory response, can result in an increase in CRP (McWilliam and Riordan, 2010). However, CRP cannot be recommended as a sole indicator of sepsis, but it may be used as part of a sepsis workup and in combination with other laboratory tests.

IL-27 levels between patients and control groups

The current study results of IL-27 level mean were 21.14 ± 1.01 pg/ml in the neonates patients' group compared to 14.49 ± 0.34 pg/ml in the control group. In addition, the mean of IL-27 was 24.92 ± 1.97 pg/ml in neonates' group with age range between 0 – 3 days, while it was 18.85 ± 0.86 pg/ml in neonates' group with age range between 4 – 28 days. The present results of IL-27 were compatible with the results of Akattabet *et al.*, (2022) from Egypt and Tosson *et al.*, (2022), who showed that the level of IL-27 was raised in neonatal sepsis, and there was a significant relationship between the level of IL-27 and the age. Another study from Egypt by Abo El Magd *et al.*, (2018) indicated that elevated IL-27 correlated well with bacterial sepsis among neonatal patients with bloodstream infections.
Because of their underdeveloped immunity, newborns are more susceptible to bacterial sepsis. IL-27, an immunosuppressive cytokine, has been found to be raised early in life. Increased levels of IL-27 may expose sepsis and exposes the infant population to more serious infections, according to the current research. During infection in neonatal sepsis, systemic IL-27 levels continued to rise, and it is commonly linked to a reduction in inflammation (Abo El Magd *et al*., 2018; Seman *et al*., 2020).
A meta-analysis applied by Wang *et al.*, (2021) concluded that IL-27 is a potential candidate biomarker for the diagnosis of sepsis in humans. Even though IL-27 may be used clinically to detect sepsis in critically ill patients, the test results for IL-27 should be interpreted in the context of other clinical and test criteria.
Seman *et al.*, (2020) defined the cell and tissue sources of cytokines, and described the effects of high early-life IL-27 on the host response in a neonatal infection model, IL-27 is typically linked to reduced inflammatory response, by directly compromising the control of the bacteria that trigger the inflammatory response, however, can show how IL-27 indirectly encourages an inflammatory cytokine response during newborn sepsis.
The IL-27-p28 and EBI3 subunits of IL-27, a heterodimeric cytokine that belongs to the IL-6 and IL-12 families of cytokines, are generated by antigen-presenting cells in response to exposure to microbial products and inflammatory stimuli (Wojno and Hunter, 2012). In a mouse model of septic peritonitis, IL-27, a T-cell regulator with pro- and anti-inflammatory actions, is rapidly increased (Pflanz *et al*., 2002, Villarino *et al*., 2005). In addition, in a mouse model of septic peritonitis, genetic deletion of EBI3 or neutralization of IL-27 via a soluble IL-27 receptor fusion protein is protective (Wirtz *et al*., 2006).

IL-33 levels between patients and control groups

The results in current study displayed the mean of IL-33 in neonates group compared to control group (250.28 ± 15.75 versus 101.16 ± 3.38 pg/ml). In addition, the mean of IL-33 was 299.54 ± 30.52 pg/ml in neonates' group with age range between 0 – 3 days, while it was 220.37 ± 15.08 pg/ml in neonates' group with age range between 4 – 28 days.
The current results corresponded with another study in Turkey by Halil *et al.*, (2018) done on septic neonates, which revealed similar results with a sharp rising of IL-33 level on the first day following diagnosis, followed by a steady decline over time (days 3 and 7). Our study also agreed with Yang *et al.*, (2020) study that compared the EOS group with the non-EOS group, which had significantly higher median levels of IL-33.
A wide variety of immunocompetent cells had their activity modulated by IL-33, which plays a crucial role in this process. By encouraging an early neutrophil response and lowering T lymphocyte apoptosis, IL-33 defends against sepsis (Li *et al*., 2016). The preservation of adipose tissue cells, innate and adaptive immune responses in mucosal organs and

cardioprotection have all been linked to crucial functions. In the last ten years, several research have assessed the usefulness of IL-33 as a biomarker in both inflammatory and non-inflammatory disorders (Erfurt *et al*., 2021).

CONCLUSION

The current study including 140 blood specimens collected from the neonates admitted to the hospital with suspected sepsis, the ages of the both groups ranging from 1 day to 28 days. Blood specimens were collected during the period from November 2021 to March 2022, and the study was done in the laboratories of AL-Batoul Teaching Hospital for Gynecology and Pediatrics in Baqubah, Diyala governorate. A complete blood count (CBC) test, and immunological tests (C-reactive protein, Interleukin-27 and Interleukin-33) were assessed.

Cytokines are tiny proteins that are released by immune system stimulated cells in order to affect other cells. Cytokines play a role in almost all aspects of immunity and inflammation, There are four types of cytokines: interleukins, interferons, chemokines, and tumor necrosis factors. Interleukins have a key role in immune system cell proliferation, differentiation, and maturation The present results concluded the possibility of CBC, IL-27 and IL-33 adoption as earlier markers in neonates to diagnose sepsis.

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