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ORIGINAL ARTICLE

The Effects of Different Infectious Organisms on Platelet Counts and Thrombopiotin Level in Neonates with Late Onset Sepsis: an Organism-Specific Response

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ABSTRACT

Background: *Late-onset sepsis (LOS) is well-defined as onset of sepsis more than 72 hours of age. Late onset thrombocytopenia occurs frequently due to sepsis.* ***Objective:*** *The aim of our study was to learn the incidence of thrombocytopenia in neonates with late onset nosocomial sepsis and to study the effects of different infectious organisms on platelet counts and thrombopiotin (Tpo) Level in Neonates with LOS.* ***Methodology:*** *This study was performed prospectively on 60 neonates from Neonatal Intensive Care Unit (NICU). The sample eligibility criterion was the presence of documented nosocomial late onset sepsis (LOS). Sixty non septicemic neonates from the same NICU were included in the study as a control group. All neonates were subjected to the following: Complete blood cell count, C-reactive protein assessment, blood culture and assesment of thrombopoietin level.* ***Results:*** *Our study showed that platelet counts were significantly lower among case group compared with control group ($p<0.01$). This study showed that more than half of the cases had severe thrombocytopenia (53.13%) and the majority of them had bacteria culture test positive (83.3%) with high mortality among them (53.13%). The most common isolated organism was klebsiella pneumoniae followed by Staph. aureus then Coagulase Negative Staphylococcus (CONS). In our study, there was statistically significant association between platelet count in thrombocytopenic neonates and blood culture resultes among studied cases. There was significant inverse correlation between platelet count and Tpo level in thrombocytopenic group.* ***Conclusion:*** *Thrombocytopenia is an early marker of sepsis and can be used as a screening procedure for early detection of sepsis, especially in NICU. Type of organism in blood culture results affect both platlet count and Tpo level.*

INTRODUCTION

Late-onset sepsis (LOS) is well-defined as onset of sepsis more than 72 hours of age. LOS is a main cause of mortality and morbidity in the neonatal intensive care unit (NICU) and occurs in nearly 15% of all neonates[1].

Thrombocytopenia is one of the commonest hematological disorders in the neonatal period, affecting up to a third of those admitted to neonatal intensive care units[2].

Late onset thrombocytopenia occurs frequently due to sepsis and or necrotizing enterocolitis. Any organism capable of producing sepsis can cause thrombocytopenia. Thrombocytopenia can occur in 80% of gram-negative septicemia and in 65% of gram-positive septicemia. Recently, sepsis induced by fungi is progressively associated with thrombocytopenia[3]. Thrombocytopenia is an early marker of sepsis and can be used as a screening procedure for early detection of sepsis, especially in NICU[4].

Thrombopoietin (Tpo) is the recently discovered physiological regulator of platelet production. In thrombocytopenic patients, several investigators have described an inverse relationship between circulating Tpo levels and marrow megakaryocyte mass[5].

The aim of our study was to learn the incidence of thrombocytopenia in neonates with late onset nosocomial sepsis and to study the effects of different infectious organisms on platelet counts and thrombopiotin Level in Neonates with Late Onset Sepsis. We also studied the relation between platlet count and thrombopiotin level in infected neonates.

METHODOLOGY

This research work was carried out in Medical Microbiology & Immunology Department and Neonatal intensive care, Faculty of Medicine, Zagazig University, during the period from January 2011 to May 2013.

The study was performed prospectively on 60 neonates from Neonatal Intensive Care Unit. The

sample eligibility criterion was the presence of documented nosocomial late onset sepsis, defined by the presence of clinical features of sepsis along (respiratory distress, poor feeding, lethargy, shock, disseminated intravascular coagulation, temperature instability) with a positive blood culture. Neonates with early onset thrombocytopenia (in the first 72 hours) were excluded from the study. Sixty non septicemic neonates (no clinical manifestation of sepsis, C-reactive protein (CRP) negative and sterile blood culture results) from the same NICU were included in the study as a control group. An informed consent was taken from the parents before neonates enrollment in the study.

All neonates were subjected to the following:

I. **Full history taking**: To detect risk factors for sepsis including obstetric history, prenatal history, and natal history.

II. **Full clinical examination:** Gestational age assessment, detection of nosocomial risk factors (delayed enteral feeding, total parental nutrition (TPN), mechanical ventilation, surgical procedure, exchange transfusion) and the clinical signs of sepsis (eg. pallor, jaundice, cyanosis).

III. Laboratory investigations:

a. *Complete blood cell count (CBC):* CBCs were performed by using Coulter counter (Sismax ST 3000). Thrombocytopenia was defined as a platelet count <150 000/mm^3 and was classified by severity (mild if the platelet count was bettween 100000 and 150000/mm3, moderate if the count was between 50000 and 100000/mm3, and severe if the platelet count was <50000[2].

b. *C-reactive protein:* by latex agglutination test for the qualitative screening of C Reactive Protein (CRP) in human serum according to manufacture instruction (Egyptian Company for Biotechnology Obour city, Cairo. Egypt)

IV. Blood culture

Blood from a single collection site was inoculated into pediatric blood culture bottles (bioMérieux, Inc., Durham, USA). The optimum volume for neonate was 4ml of blood for each bottle. Standard antisepsis procedures were recommended for use during blood collection. The blood was mixed with the broth without delay. The bottles were labeled with the name, the number of the patient and the date of collection.

The blood culture bottles were incubated at 37°C. Blind subculture was done after the first night incubation on Blood agar, Chocolate agar, MacConkey agar, and Sabouraud's dextrose agar (Oxoid, UK).

All plates were examined after the incubation period for growth. Negative blood culture bottles were checked daily for evidence of macroscopic growth (e.g. hemolysis, turbidity, gas production and the presence of visible colonies or a layer of growth over the fluid surface). If no evidence of microbial growth after 10 days of incubation, gram stains and blind subcultures were done before considering the culture negative[6].

Identification of bacterial isolates was done by colonial morphology, microscopic examination of gram stained films. API 20 and API 20 E Strep system (Bio-Merieux. Marcy L Etoile. France) were used for identification of the isolates according to the manufacturer's instructions. Sabouraud's dextrose agar plates were observed every two days for fungal growth and up to 6 weeks before considering them negative. Growth was recognized by gross examination of color and growth rate[7].

V. Assesment of thrombopoietin level (Tpo):

Blood samples were attained by direct venipuncture. Blood was centrifuged at 1500 rpm for 10 min. The serum was separated and stored frozen until analyze. Serum thrombopoietin levels were measured by a commercially available enzyme linked immunosorbant assay (ELISA) kit (QuantikineTM Human TPO Immunoassay, R&D Systems, Minneapolis, MN, USA). Samples were incubated for 3 h at 4°C into the wells of a microplate coated with a monoclonal anti-Tpo. After washing and adding a horseradish peroxidase-conjugated anti-TPO monoclonal antibody for another hour, bound TPO was quantified colourimetrically at 450 nm. The detection limit of the assay was less than 10 pg/ml.

Statistical analysis:

The collected data were coded and analyzed by computer using a data base software program, Statistical Package for Social Science version 19 (SPSS). For quantitative variables mean, standard deviation, and range were computed. Independent t-test was used for quantitative normally distributed data for detection difference between two different groups. Kruskal wallis test used for detection difference between non normally distributed quantitative data in more than two groups. Qualitative data were represented as frequencies and percents. Chi square test were used to detect relation between different qualitative variable.

RESULTS

This study involved sixty neonates of nosocomial sepsis (case group). Sixty non septicemic neonates from the same NICU were included in the study (control group).

In our study the newborns of the case group had significantly lower birth weight and gestational age than the control group, while there was no significant difference with regard to gender, mode of delivery, maternal age and parity between the two groups (table 1).

Our study showed that there was high statistically significant difference ($p<0.01$) between both case and control groups with regard to all clinical manifestations of sepsis lethargy, temperature instability, pallor respiratory distress, poor reflexes, and feeding intolerance except for bleeding tendency in which there is no significant difference between them ($p>0.05$).

Table 1: Demographic characteristics of studied groups

Parameters	Cases (N=60)		Control (N=60)		test	P value
	n	%	n	%	χ^2	
Gender						
- Male	39	65.0	27	45.0	2.5	>0.05
- Female	21	35.0	33	55.0		**(NS)**
Mode of delivery						
- Vaginal	25	41.7	27	45.0	0.068	>0.05
- CS	35	58.3	33	55.0		**(NS)**
Gestational age (weeks)					**t-test**	
- Mean±SD	35.73±3.76		37.95±1.32			**<0.05**
- range	(28-40)		(35-40)		2.57	
Birth weight (Kg)						
- Mean±SD	2.29±0.92		2. 84±0.41		2.609	**<0.05**
- Range	(0.9-4.5)		(2.4-3.8)			
Maternal age (years)						
- Mean±SD	28.7±5.21		29.25±5.28		0.408	>0.05
- Range	(18-42)		(20-40)			**(NS)**
Parity						
- Mean±SD	3.08±1.97		2.8±1.85		0.56	>0.05
- Range	(1-8)		(1-7)			**(NS)**

t: Student t- test *χ^2 : Chi-square test* *NS: Non significant*

Our study showed that platelets count was significantly lower among case group compared with control group ($p<0.01$) while there is no significant difference between both groups in total leucocytic count and hemoglobin level (table 2).

Table 2: Complete blood cell count measurements in studied groups.

Parameters	Cases (N=60)	Control (N=60)	t- test	p value
Total leucocytic count				
- Mean±SD	15.03±3.88	11.23±3.46	1.54	>0.05
- Range	(2.5-61.9)	(5.3-18.6)		**(NS)**
Hemoglobin (Hb) level (gm/dl)	13.26±3.35	14.27±1.8	1.29	>0.05
- Mean±SD	(6.5-23.6)	(10.8-17.3)		**(NS)**
- Range				
Platelets count				
- Mean±SD	175.2±15.53	430.35±185.58	6.185	**<0.01****
- Range	(3-590)	(147-813)		

N: number *SD: Standard deviation* *NS: Non significant* *t: Student t- test* ***: highly significant*

This study showed that that majority of cases (70%) had positive CRP. Normal leucocytic count was found in 73.3%. More than half cases had thrombocytopenia (53.3%) and normal hemoglobin level (51.7%) (table 3).

Table 3: CRP results and laboratory measurements in cases (N=60).

Parameter	N.	%
Total leucocytic count		
- normal	44	73.3
- leucopenia	4	6.7
- leucocytosis	12	20.0
C-reactive protein (CRP)		
- positive (+ve)	42	70.0
- Negative (-ve)	18	30.0
Hemoglobin (Hb) level (gm/dl)		
- normal	31	51.7
- Anemic	29	48.3
Platelets count		
- normal	28	46.7
- thrombocytopenia	32	53.3

This study showed that more than half of the cases had severe thrombocytopenia (53.13%) and the majority of them had bacteria culture test positive (83.3%) with high mortality among them (53.13%). Among the thrombocytopenic patients, 9 (28.1%) were diagnosed as mild, 6 (18.7%) as moderate, and 17 (53.1%) as severe. (table 4) (figure 1).

Table 4: Onset and degree of thrombocytopenia in cases (N=32)

Parameter	N.	%
Onset by days		
- Mean±SD	10.63±5.03	
- Range	(4-25)	
degree		
- Mild	9	28.12
- Moderate	6	18.75
- Severe	17	53.13
Culture test		
- Bacterial	50	83.3
- Fungal	2	3.7
- Mixed	8	13.3
Mortality	17	53.13

In our study, the most common isolated organism is *klebsiella pneumoniae* followed by *Staph. aureus* then *Coagulase Negative Staphylococcus (CONS).* There were 8 cases having mixed infection. These cases had combined fungal (candida in all cases) and bacterial sepsis (*klebsiella* 5, *staph Cons* 1, *pseudomonas* 1 and *E-Coli* 1) (table 5).

Table 5: Types of organisms in culture of cases

Parameter	N.	%
Klebsiella	15	25
Staph. Aureus	13	21.66
CONS	10	16.66
Pseudomonas	6	10
E- coli	3	5
Enterobacter	2	3.33
Streptococcus viridians	1	1.67
Candida albicans	2	3.33
Mixed infection	8	13.33

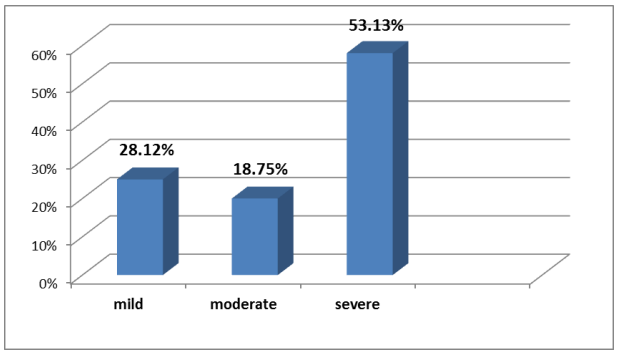


Fig. 1: Bar chart showing degree of thrombocytopenia among cases (n=32)

Our study showed that 73.3% of *klebsiella pneumoniae* cases develop thrombocytopenia and 40% of them are severe. 62.5% of cases having mixed infection developed thrombocytopenia, 37.5% of them were severe. Among cases infected with *Staph aureus*, 46.16% develop thrombocytopenia, and about 23% of them are severe. Among cases infected with *CONS*, 40% develop thrombocytopenia, and about 30% of them are severe (Table 6).

Our study showed that there was high statistically significant difference between case and control groups as regard Tpo level. There was also high statistically significant difference between thrombocytopenic and non thrombocytopenic cases as regard Tpo level.(p=0.001) (Table 7 & Figure2).

Table 6: Organism-specific response to thrombocytopenia

Type of organism	No thrombocytopenia (n=28)		Mild (n=9)		Moderate (n=6)		Severe (n=17)	
	N.	%	N.	%	N.	%	N.	%
Klebsiella (n=15)	4	26.67	3	20.0	2	13.3	6	40.0
Staph. Aureus(n=13)	7	53.84	3	23.08	0	0.0	3	23.08
Staph ConS (n=10)	6	60.0	0	0.0	1	10.0	3	30.0
Pseudomonas (n=6)	5	83.33	0	0.0	0	0.0	1	16.67
E- coli (n=3)	1	33.33	2	66.67	0	0.0	0	0.0
Enterobacter (n=2)	1	50.0	0	0.0	0	0.0	1	50.0
Streptococcus viridians (n=1)	0	0.0	0	0.0	1	100.0	0	0.0
Candida albicans (n=2)	1	50.0	0	0.0	1	50.0	0	0.0
Mixed infection (n=8)	3	37.5	1	12.5	1	12.5	3	37.5

Table 7: Thrrombopiotin measurement in studied groups.

	Case (Sepsis) N=60		Control N=60	Kruskal wallis	P value	LSD
Mean ±SD Range	**Thrombocyopenic N=32**	**Non thrombocytopenic N=28**				
	430±1078 (520-1780)	100± 68.51 (162-430)	40.33±105.3 (41-162)	100.3	0.001	P1 0.04 P2 0.006 P3 0.002

LSD : Least significant difference *P1: Thrombocytopenic versus non thrombocytopenic*
p2: thrombocytopenic versus control *p3: non thrombocytopenic versus control group*

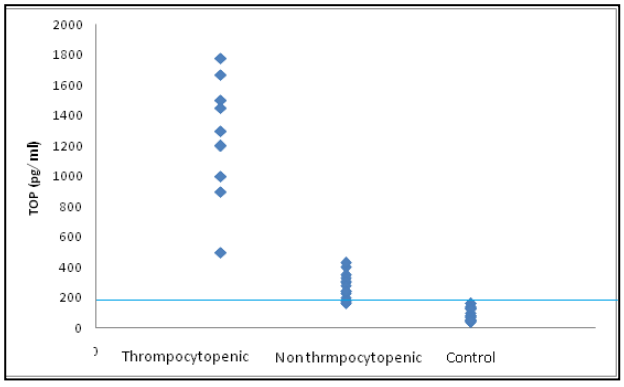


Fig. 2: Tpo levels in different study groups

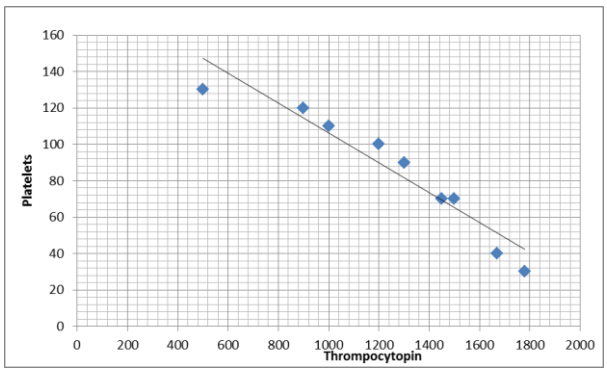
Our study showed that there was significant inverse correlation between platlet count and Tpo level in Thrombocytopenic cases (r =0.955, p <0.001) (table 8) (figure 3).

In our study, platlet count showed lowest count in G –ve bacteria, followed by G +ve bacteria, mixed infection, and finally fungi. (Table 9) (Figure4). However, Tpo level were higher in G –ve bacteria, followed by G +ve bacteria, mixed infection, and finally fungi (table 10) (figure 5).

Our study showed that mortality was significantly higher among cases with severe thrombocytopenia compared with cases of normal platelet count (figure 6).

Table 8: Correlation between TPO level and platelets count.

Variable	Thrombocytopenic N=32	
	r	P value
Platelets	**-0.955**	**<0.001**



Fig, 3: Correlation between Tpo level and platelets count.

Table 9: Relation between platelets count in thrombocytopenic patients and blood culture results.

Variable	3 Platelets <150*10		Kruskal wallis	P value
	Mean± SD	Range		
Blood culture				
G -ve (15)	70.8± 90.8	(62-100)	0.14	0.93
G +ve (11)	80.0± 70.6	(90-140)		
Mixed infection (5)	93.7±100.3	(70-110)		
Fungi (1)	110.5±99.8	(70-140)		

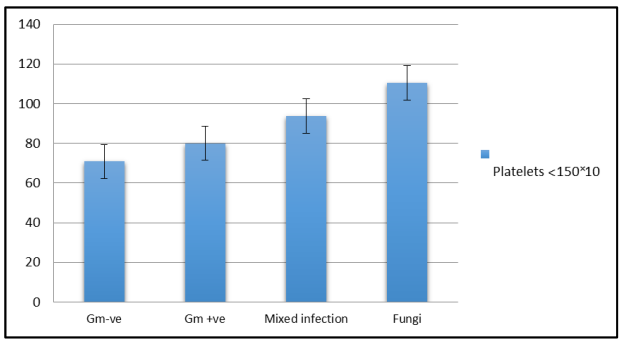


Fig. 4: Relation between platelets count in thrombocytopenic pt and blood culture results

Table 10: Relation between Tpo level in thrombocytopenic patients and blood culture results.

Variable	TPO (Pg/ml)		Kruskal wallis	p value
	Mean± SD	Range		
Blood culture				
G -ve (15)	780.8±1087	(1200-2000)	0.34	0.79
G +ve (11)	530.5±430.0	(560-1000)		
Mixed	440.0±680.7	(1000-1700)		
infection (5)	300.8±430.2	(520-560)		
Fungi (1)				

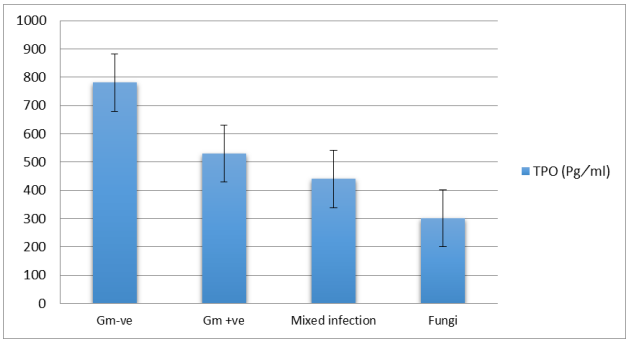


Fig. 5: Relation between Tpo level in thrombocytopenic patients and blood culture results

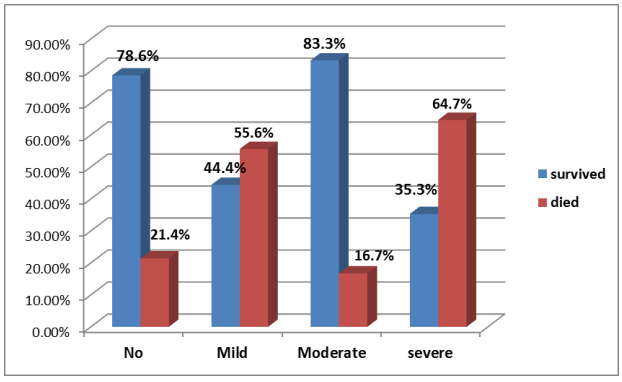


Fig. 6: Bar chart showing mortality in different study groups among cases according to thrombocytopenia

Our study showed that there was statistically significant direct correlation between platelet count and both gestational age and birth weight in cases($p<0.05$). However it showed insignificant inverse correlation between platelet count with total leucocytic count. In our study there was statistically insignificant inverse correlation between platelet count and gestational age, birth weight and total leucocytic count) of controls ($p>0.05$). Our study showed that there was statistically insignificant direct correlation between cases with thrombocytopenia and gestational age, birth weight, sepsis manifestation, CRP and total leucocytic count ($p>0.05$) (figure 7 a,b) (table 10).

Table 11: Correlations between platelet count in thrombocytopenic cases and clinical and laboratory data (n=32).

Parameter	Thrombocytopenia <150 x10^3/CM	
	r	p value
Gestational age	0.14	>0.05
Birth weight	0.09	>0.05
Sepsis manifestation:		
- Apnea	0.33	>0.05
- Poor reflexes	0.09	>0.05
- Poor perfusion	0.32	>0.05
Laboratory measurements		
- CRP	0.13	>0.05
- TLC	0.06	>0.05

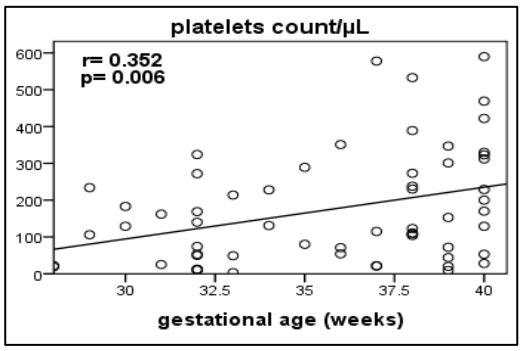


Fig. 7a: Shows significant direct correlation between gestational age and platelet count of cases (n= 60) ($p<0.05$).

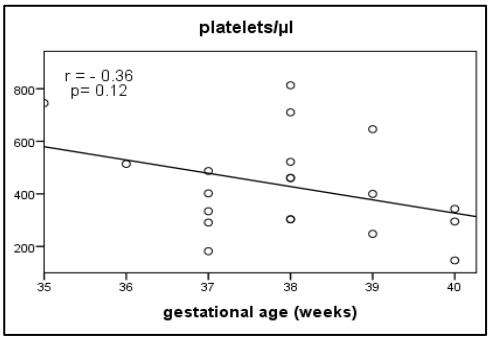


Fig. 7b: Shows insignificant inverse correlation between gestational age and platelet count of controls (n= 60) ($p>0.05$).

DISCUSSION

Sepsis is a common complication in the neonatal intensive care unit. It is most common in the smallest and most premature infants, in whom the clinical presentation can be subtle and nonspecific. Disseminated intravascular coagulation and thrombocytopenia are well-known complications of sepsis[8].

We conducted this study to learn the incidence of thrombocytopenia in neonates with late onset nosocomial sepsis and to study the influence of type of blood culture on platelet response and thrombopiotin level. We also studied the relation between platlets count and thrombopiotin level.

Thrombocytopenia was defined as a platelets count <150 000/mm^3 [9]. We observed that 53.3% of infants with documented sepsis developed a platelets count <150×000/ mm^3. of them, 15% had mild, 10% had moderate, and 28.3% had severe thrombocytopenia this was in accordance with Bashir et al.[9] who found that 59.5% of infants with documented sepsis developed a platelet count <150×109/L, while *Griffin et al.,2003* found that 71% of the sepsis episodes were associated with a platelet count <150 000/mm^3 [10].

We compared demographic and clinical data between patients and found that gestational age is significantly lower among cases with severe thrombocytopenia compared with cases of normal platelet count ($p<0.05$) and a positive significant correlation between gestational age, birth weight and platelet count ($p<0.05$), while *Bashir et al.* was also found that the severity of thrombocytopenia was directly related to gestational age and birth weight.[9] We also found no relation between gender, mode of delivery and thrombocytopenia, which conforms with the results found by Griffin et al.[11].

The predominant organism in our study was *klebsiella* which accounted for 25% of culture results and found also in another 5cases which had *klebsiella and candida* in their blood culture resulting in a form of mixed infection, followed by *staphylococcus aureus* which accounted for 21.67%, all of them were pure bacterial. These results were consistent with the results of other studies but with different ratios[12]. *Bashir et al., 2009* found that 62.5% were infected with *klebsiella pneumonia*[9]. On other hand *Rabie et al*. in a study in Egypt found that gram-positive bacteria was the predominant organism at 48%, and the majority of them were CONS[12]. In two separate studies Boghossian et al. and Hammoud et al. found that Coagulase-negative *staphylococci* were the predominant pathogens of LOS, accounting for 53.2%–77.9% of LOS in industrialized countries and 35.5%–47.4% in some developing regions[13,14]. This variation in the distribution pattern of causative pathogens may be explained by changing across regions and also change over time within the same hospital due to demographic characteristics of patients, microflora colonisation of the nosocomial environment and the policy of antibiotics regimens[15].

In our study, there was statistically significant association between platelet count in thrombocytopenic neonates and blood culture among studied cases. Severe thrombocytopenia was observed in Gram –ve bacteria followed by Gram +ve group then those of mixed infection and finally fungi. On the other hand TPO levels were higher in fungi group. This was in accordance with many studies[16,17]. However, there was no statistically significant association between platelet count in thrombocytopenic neonates and blood culture among studied cases ($p>0.05$), which conforms with the results found by Mei-Yin et al. as they found the difference was not significant when clustering for sepsis caused by Gram+Ve and Gram–ve organisms and they concluded that caution should be maintained in relating a low platelet count to any infectious agent[18].

CRP was positive in 70% of cases and negative in 30% of cases and there was an insignificant direct correlation betwen platelet count in thrombocytopenic cases and CRP results. In a study by Mei-Yin et al. (25.1%) of septic neonates had CRP negative and they concluded that a considerable proportion of neonatal blood culture proven sepsis had a normal or low initial CRP level[18].

The overall mortality rate in our study population was 38% as we had 37 surviving cases, and 23 died. We found that 28 non-thrombocytopenic cases (22 survived, 6 died), 9 mild thrombocytopenic cases (4survived, 5died), 6 moderate thrombocytopenic cases (5 survived, 1 died), and 17 severe thrombocytopenic cases (6 survived, 11 died). Rabie et al. found a pit higher mortality rate in mansoura hospitals of 42.9%[12].

In our study, we only found that mortality is significantly higher among cases with severe thrombocytopenia compared with cases of normal platelet count ($p<0.05$). No statistical difference is observed in mortality rates between cases with mild and moderate thrombocytopenia compared with cases of normal platelet count ($p>0.05$). Meanwhile, mortality rate observed by Bashir et al. was 20.5%, and mortality was significantly higher in patients with thrombocytopenia than in the non thrombocytopenic, and mortality was highest in patients with severe thrombocytopenia as compared to patients with moderate thrombocytopenia and mild thrombocytopenia[9].

Thrombopoietin is the principal physiologic regulator of megakaryocyte synthesis and platelet manufacture. The role of thrombopoietin in the pathophysiology of neonatal thrombocytopenia is unidentified[19]. In our study, we measured thrombopiotin level in case and control groups by quantitative ELISA. We found that Tpo level was statistically significant higher in neonates with thrombocytopenia than other

groups (p<0,001). This was in accordance with *Martha et al*. who found that Tpo level was higher in thrombocytopenic neonates in comparison to non-thrombocytopenic patients[20].

In our study, there was significant inverse correlation between platelet count and Tpo level in thrombocytopenic group. Our results were similar to those obtained by *Colarizi etal*. who found that Tpo concentrations markedly decreased (median value 46 pg/ml, range 0–625) in relation to platelet count[21].

CONCLUSION

Late onset sepsis (onset of sepsis >72 hours of age or nosocomial sepsis) is an important cause of morbidity and mortality in the neonatal intensive care unit. Blood culture is the gold standard for diagnosis of sepsis. Thrombocytopenia is an early marker of sepsis and can be used as a screening procedure for early detection of sepsis, especially in NICU. Thrombopoietin is the principal physiologic regulator of megakaryocyte synthesis and platelet manufacture. Type of organism in blood culture results affect both platlet count and Tpo level. Neonates with LOS have high circulating TPO levels in the face of low platelet counts. Whether larger TPO concentrations following exogenous administration of recombinant TPO would restore the number of circulating platelets needs further investigation.

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