Research Article

Study of risk factors for neonatal thrombocytopenia in preterm infants

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Abstract

Background: Thrombocytopenia is a common hematological problem in neonatal care units. Neonatal thrombocytopenia has been defined as platelet count less than 150x109 /L, regardless of gestational age.
Objectives: To determine the frequency and assess the severity of neonatal thrombocytopenia in preterms, and the maternal and neonatal conditions as risk factors.
Patients and methods: A cross sectional study was carried out in the neonatal care unit of Child Central Teaching Hospital/ Baghdad, over a period of six months (30th of June to 31st of December 2013). Study group included only preterms who had thrombocytopenia. Data of neonates was collected by direct interviewing of the mothers or other family members, clinical assessment and examination and relevant investigations were done.
Results: The frequency of preterm neonatal thrombocytopenia was 95 (13.04%), out of 728 neonates admitted to neonatal care unit. Male to female ratio was 1.37:1, male gender was significantly associated with prematurity and mild to moderate severity thrombocytopenia (P 0.016, P 0.019). Prematurity was significantly associated with late onset neonatal thrombocytopenia (P 0.035). Late-onset thrombocytopenia, and 32- <37 wk gestational age group were significantly associated with mild to moderate severity thrombocytopenia group (P 0.008, and 0.004 respectively). Sepsis was a frequently associated risk factor in thrombocytopenic preterms, and found in 70 (73.68%) cases of preterm thrombocytopenia (with only 8 cases were culture positive and 62 cases were clinical based diagnosis). Also birth asphyxia, respiratory distress syndrome, and Rh incompatibility were significantly associated with thrombocytopenic prematures (P 0.026, 0.001, 0.008 and 0.036 respectively). Birth asphyxia, respiratory distress syndrome, sepsis and Rh incompatibility were significantly associated with moderate to severe thrombocytopenia (P 0.001, 0.001, 0.003 and 0.011 respectively). There was no significant difference between the presence of maternal disease and gestational age to the severity of neonatal thrombocytopenia (P 0.458, 0.698 respectively).
Conclusions: Preterm thrombocytopenia is relatively common in neonatal care units. Sepsis, respiratory distress syndrome and birth asphyxia were significant neonatal risk factors of thrombocytopenia at lower gestational age preterms. Most episodes were late onset with mild or moderate severity.

Key words: Neonatal thrombocytopenia, sepsis, prematurity.

INTRODUCTION

Platelets are important component in the first phase of homeostasis (plug formation), and defects in number or function may lead to bleeding which usually involve skin and mucous membranes. (1) platelets first appear in the prenatal life by 5th -6th weeks of life.(2) Normal platelet count is 150-450 x 109 /L(3). Thrombopoietin is now widely recognized as stimulator of platelets production, acting by promoting the proliferation of megakaryocyte progenitors (the cells that multiply and give rise to megakaryocytes), and the maturation of the megakaryocytes, that generate and release new platelets into the circulation. (4,5) Neonatal TCP is defined as platelet count less than 150x109 /L, regardless gestational age (6,7). Thrombocytopenia (TCP) affects up to 35% of all patients admitted to the neonatal intensive care unit. (8,9) The risk of developing TCP is

inversely proportional to gestational age. (10) The causes of TCP in neonates include immune and non-immune disorders, most cases are non-immune,(2) and are mostly due to increased destruction rather than decreased production by bone marrow depression. (7, 11) TCP that presents in the first 72 hours of life (early) is usually secondary to placental insufficiency and caused by reduced platelet production, usually mild or moderate in severity, and seen in infants of mother with pre-eclampsia, diabetes mellitus, or intrauterine growth retardation. (12) TCP that presents after 72 hours of life (late), mostly is caused by sepsis and necrotizing colitis in >80% of cases. (13,14)

Aims of the study

1-To determine the severity of neonatal TCP in preterms.

2-Detection of maternal and neonatal conditions as risk factors of neonatal TCP.

PATIENTS AND METHODS

A cross sectional descriptive study of 95 preterm neonates with TCP who were admitted to the neonatal care unit of Child Central Teaching Hospital/ Baghdad, over the period of six months (from 30th June to 31st December 2013). Their age was ranging between 1- 28 days. The study group included only singleton preterm deliveries (between 28 and <37 weeks of gestation) with TCP admitted for any cause (after exclusion of those with incomplete data, investigations, early death or discharge from hospital by parents). Data of mothers was collected including; last menstrual period, maternal hypertension during pregnancy, diabetes mellitus, idiopathic thrombocytopenic purpura, rash and fever during pregnancy. Data of neonates was collected by direct interviewing of the mothers, clinical examination and relevant investigations, including; age on admission, gender, weight, blood group of baby and mother, cause of admission. Preterms were defined by WHO as live born infants delivered before 37 weeks from the first day of last menstrual period. (3) The gestational age of the preterm newborn was calculated depending on mother's menstrual history, prenatal ultrasonography, and external physical criteria using the new Ballard score for neuromuscular and physical maturity. (15)

The weight was measured (naked), using standard beam scale (Seca 16 kg. maximum weight, Germany made), and plotted on growth charts of preterms.

Those preterm babies were divided into two groups: (younger gestational age) for those with gestational age 28 - <32 weeks and (older gestational age) for those with gestational age 32 - <37 weeks.

Preterm neonates were screened through complete blood counts for TCP, and blood samples were obtained by drawing blood for platelet automated calculation through Abbott instrument. Mild TCP was defined as platelet count of 100×109/L - <150 ×109/L, moderate TCP with platelets count of 50×109/L - <100 ×109/L, and severe TCP with platelets counts <50 ×109/L. (15)

TCP that presents in the first 72 hours of life is considered as early, while TCP that presents after 72 hours of life is considered as a late one.(12) Blood cultures, Torch test, blood groups, blood biochemistry, radiological, ultrasound tests were conducted accordingly.Diagnosis of sepsis was (diagnosed clinically depending on the following findings; temperature instability, mottled skin, metabolic acidosis, irritability, apnea, seizures, lethargy, feeding intolerance or blood culture proved).

Statistical analysis was performed using SPSS version 20, and P value was statistically significant if below (0.05) using Fisher exact test.(16)

RESULT

The total number of neonates admitted to NCU is 728, only 95 of them are the preterm neonates with TCP (13.04%).We found a larger proportion of male neonates (1.37:1 male to female ratio). Males with TCP was significantly associated with older gestational age (32- <37 weeks), and also late onset TCP (P value 0.016, 0.035 respectively) as shown in table 1.

Table 1: The demographic distribution of gender, and onset of thrombocytopenia according to gestational age in 95 preterm neonates admitted to CCTH.

		Gestational age					
		Younger gestation 30 (31.57%)		Older gestation 65 (68.42%)		Total/(%) 95/(100%)	P value
		No.	%	No.	%		
Gender type	Male	12	40.00%	43	66.15%	55(57.89)	0.016
	Female	18	60.00%	22	33.85%	40(42.11)	
	Total	30	100.00%	65	100.00%	95(100.00)	
Onset of TCP	Early	17	56.67%	22	33.85%	39(41.05)	0.035
	Late	13	43.33%	43	66.15%	56(58.95)	
	Total	30	100.00%	65	100.00%	95(100.00)	

Also male gender was significantly associated with TCP whatever its severity (p 0.016) . late onset TCP and older gestational age were significant with mild to moderate TCP (p 0.035, 0.024), in contrast to severe TCP which is more significantly associated with early onset one and younger gestational age (p 0.008, 0.004) as shown in table 2

Among multiple neonatal risk factors; sepsis was the most frequently associated risk factor in thrombocytopenic preterms, and found in 70 cases (73.68%) of preterm TCP (with only 8 cases were culture positive and 62 cases were clinical based diagnosis). Table 3 shows birth asphyxia, RDS, sepsis and Rh incompatibility were significantly associated with prematurity in patients with TCP.

NEC (necrotizing enterocolitis), TORCH infection (toxoplasmosis, rubella, cytomegalo virus, and herpes simplex virus), Rh (rhesus factor)

Birth asphyxia was significantly associated with mild TCP (P 0.001), while respiratory distress syndrome, sepsis and Rh incompatibility were significantly associated with moderate to severe TCP (P 0.001, 0.003 and 0.011 respectively) as shown in table 4

NEC (necrotizing enterocolitis), TORCH infection (toxoplasmosis, rubella, cytomegalo virus, and herpes simplex virus), Rh (rhesus factor)

There was no significant association between the presence of maternal disease and gestational age of thrombocytopenic preterms as shown in Table 5

Diabetes mellitus(DM), Hypertension(HT), Idiopathic thrombocytopnoic purpura (ITP)

There was no significant association found between the presence of maternal disease and the severity of neonatal TCP Table 6

Table 2. The severity of neonatal thrombocytopenia according to gender, onset of thrombocytopenia and gestational age in 95 preterm neonates admitted to CCTH.

		Range							
		Mild 25(26.31%)		**Moderate 53(55.78%)**		**Severe 17(17.89%)**		**Total 95**	**P value**
		No.	**%**	**No.**	**%**	**No.**	**%**		
Gender type	Female	7	28.00%	29	54.72%	4	23.53%	40	0.019
	Male	18	72.00%	24	45.28%	13	76.47%	55	
Onset of TCP	Early	4	16.00%	25	47.17%	10	58.82%	39	0.008
	Late	21	84.00%	28	52.83%	7	41.18%	56	
Gestational Age	28- <32 wk	7	28.00%	12	22.64%	11	64.71%	30	0.004
	32- <37 wk	18	72.00%	41	77.36%	6	35.29%	65	

Table 3. The frequency and correlation between neonatal risk factors of thrombocytopenia and gestational age in 95 preterm neonate admitted to CCTH.

		Gestational age					
		Younger gestation 30(31.57%)		**Older gestation 65(68.42%)**		**Total No. 95**	**P value**
		No.	**%**	**No.**	**%**		
Birth asphyxia	Yes	21	70.00%	6	9.23%	27	0.001
	No	9	30.00%	59	90.77%	68	
Respiratory distress syndrome	Yes	25	83.33%	36	55.38%	61	0.008
	No	5	16.67%	29	44.62%	34	
NEC	Yes	3	10.00%	1	1.54%	4	0.064
	No	27	90.00%	64	98.46%	91	
Sepsis	Yes	17	56.67%	53	81.54%	70	0.026
	No	13	43.33%	12	18.46%	25	
TORCH infection	Yes	1	3.33%	2	3.08%	3	0.948
	No	29	96.67%	63	96.92%	92	
Rh factor	Compatible	21	70.00%	57	87.69%	78	0.036
	Incompatible	9	30.00%	8	12.31%	17	

Table 4. The correlation between neonatal risk factors and the severity of thrombocytopenia in 95 preterm neonate admitted to CCTH

		Thrombocytopenia Range							
		Mild 25(26.31%)		**Moderate 53(55.78%)**		**Severe 17(17.89%)**		**Total 95**	**P value**
		No.	**%**	**No.**	**%**	**No.**	**%**		
Birth asphyxia	Yes	15	60.00%	12	22.64%	0	0.00%	27	0.001
	No	10	40.00%	41	77.36%	17	100.00%	68	
Respiratory distress syndrome	Yes	12	48.00%	39	73.58%	10	58.82%	61	0.001
	No	13	52.00%	14	26.42%	7	41.18%	34	
NEC	Yes	0	0.00%	2	3.77%	1	5.88%	3	0.523
	No	25	100.00%	51	96.23%	16	94.12%	92	
Sepsis	Yes	22	88.00%	35	66.03%	13	76.47%	70	0.003
	No	3	12.00%	18	33.96%	4	23.53%	25	
TORCH infection	Yes	1	4.00%	2	3.77%	0	0.00%	3	0.718
	No	24	96.00%	51	96.23%	17	100.00%	92	
Rh factor	Compatible	20	80.00%	48	90.57%	10	58.82%	78	0.011
	Incompatible	5	20.00%	5	9.43%	7	41.18%	17	

Table 5. The correlation between the presence of maternal disease and gestational age of thrombocytopenic preterms.

Maternal disease	Gestational age					
	28- <32 weeks 30(31.57%)		**32- <37 weeks 65(68.42%)**		**Total 95(%)**	**P value**
	No.	**%**	**No.**	**%**		
No disease	23	76.67%	54	83.08%	77(81.05%)	0.458
DM	1	3.33%	2	3.07%	3(3.16%)	
HT	5	16.67%	8	12.31%	13(13.68%)	
ITP	1	3.33%	1	1.54%	2(2.11%)	

Table 6. The correlation between the presence of maternal disease and the severity of neonatal thrombocytopenia Diabetes mellitus(DM), Hypertension(HT), Idiopathic thrombocytopnoic purpura(ITP)

Maternal disease	Thrombocytopenia range							
	Mild 25(26.31%)		**Moderate 53(55.78%)**		**Severe 17(17.89%)**		**Total 95(%)**	**P value**
	No.	**%**	**No.**	**No.**	**%**	**No.**		
No	20	80.00%	43	81.13%	14	73.68%	77(81.05%)	0.698
DM	0	0.00%	2	3.77%	1	5.88%	3(3.16%)	
HT	5	20.00%	6	11.32%	2	11.76%	13(13.68%)	
ITP	0	0.00%	2	3.77%	0	0.00%	2(2.11%)	

DISCUSSION

A cross sectional study of 728 neonates, only 95 preterm neonates of them (13.04%) had TCP, fulfilling the criteria and were considered in the study group. This finding was close to Roberts I. et al (17) study (25%) and Beiner ME et. al (18) study (31%). The discrepancy in percentages is most likely due to the spectrum of gestational age as including criteria in these studies, and age of preterms at time of admission in the intensive eonatal care units, Naieri F. et al, (19) Bolat F et al, (20).

A study by Pour N. M. et al (21) reported no relation between the occurrence of TCP and gender, and Anil K.G. et al (22) found that sex doesn't predict the TCP among neonatal intensive care unit neonates, while this study showed higher frequency and significance of preterm male sex and severity (P 0.016, 0.019) of TCP probably due to the social taboos towards predominant male sex priority for caring.

Late onset TCP presented with significant difference than early onset, which was mostly of moderate severity (P 0.035) and more frequent in preterms with low gestational age. This is close to Petrova A. et al (23) study who found the majority of TCP (67.0%) was of late and were moderate to severity. Chakravorty S. (24) found in preterm neonates, early-onset TCP is usually

secondary to antenatal causes, while late-onset TCP in preterm neonates is nearly always due to acquired bacterial infection and/or necrotizing enterocolitis, leading to severe TCP.

In this study there is no accurate time of the onset of neonatal TCP or actual timing of severity its severity, because our NCU receives neonates at different ages.

In this study among many neonatal risk factor of TCP, sepsis was the most common neonatal risk factor. Bolat F et al (20), found sepsis, hypoxia, intrauterine growth retardation, and disseminated intravascular coagulation were predisposing factors for TCP. Beiner ME et. al,(18) found thrombocytopenic preterms are more likely to suffer of sepsis (p 0.002).

There is a significant association between sepsis and moderate to severe TCP and prematurity (P 0.003, 0.026), a finding was closely similar to a study done by Zaccheaus A. J.(25), who reported that neonatal sepsis is largely contributed to the causes of most severe TCP cases, Charoo BA. et al (26) found that 59.5% of patients had sepsis induced TCP, and was more common among low birth weight preterm babies. Bolat F et al (20), found sepsis was a predisposing factor for TCP.

Respiratory distress syndrome was a significant neonatal risk factor of moderate TCP (P 0.001) and prematurity (P 0.008), which is similar to Jack. (27) study who reported respiratory distress syndrome as one of the common causes of neonatal TCP (34.8%).

There was a significant association between birth asphyxia and lower gestational age (P 0.001) of preterms TCP and of mild severity (P 0.001). Bolat F et al (20) and Sola MC (28) reported birth asphyxia was commonly encountered in neonatal TCP, the mechanism is uncertain but hypoxia have a role in the mechanism (6) and they are more deficient of surfactant factor with decreasing gestational age. (3)

There was no significant association between NEC and gestational age of thrombocytopenic preterms, finding is similar with Ververidis M. et al (29) who found that monitoring the platelet count during the course of NEC is useful; yet, it cannot be used in isolation to predict the extent of the disease.

Disseminated intravascular coagulation (DIC) had no significant association with gestational age nor the severity of TCP, yet such cases may usually present as a complication of sepsis, a finding similar to Sola et al (6) who found that septic neonates develop TCP on the basis of DIC.

There was a significant association between Rh incompatibility and prematurity (P 0.036) and the severity of TCP (P 0.011), similar finding to study by Roberts I., Murray NA(12), and Mirjam EA. et al(30) study, who considered Rh incompatibility as risk factor of neonatal TCP.

Regarding maternal disease in thrombocytopenic neonates (18.94%); there was no significant association with neonatal TCP. Hypertension was uncommon (13.6%), and was more associating mild to moderate neonatal TCP. Roberts I., Murray NA(12) reported that TCP was common in infants of mothers with pre-eclampsia, and was mostly mild to moderate severity TCP. Also Sola et al(6) reported that the incidence of TCP associated with pre-eclapmsia was more likely to occur in preterm infant, our finding may be related to small sample size. TCP mechanism may be related to thrombopoietin (Tpo) system, which is regulating normal neonatal platelet regulation and neonatal thrombocytopenia is not well understood. (31)

The TCP associated with placental insufficiency (pregnancy-induced hypertension and diabetes) is almost always mild to moderate and caused by decreased platelet production as the main mechanism and decreased concentrations of circulating megakaryocyte progenitors. (32)

Three of the mothers had gestational diabetes mellitus, yet was not significantly associated. A study done by Roberts and Murray NA (12) reported that maternal diabetes may lead to early onset neonatal TCP due to placental insufficiency. The difference between this study and other studies regarding the frequency of maternal diseases be explained probably by the fact that many mothers had irregular anti-natal care visits and checking during pregnancy, so many of them didn't know or denied that they had any disease. In addition to the fact that we have no idea about the number of preterm babies of diabetic mothers with no TCP (to be used as control group).

Conclusions

1-Preterm TCP is relatively common in neonatal care units.

2-Sepsis was significantly associated with neonatal TCP in preterm infants.

3-Respiratory distress syndrome and birth asphyxia were significant neonatal risk factors of TCP at lower gestational age preterms.

4-Most episodes were late onset with mild or moderate severity TCP.

Recommendations

1.Good antenatal follow up and proper post-natal management helps ensuring healthy term deliveries,

with minimal post-natal complications encouraging TCP.

2. Screening of neonates with risk factors of neonatal TCP for platelets count is beneficial in the early diagnosis and management of TCP.

REFERENCES

1. Bussel J. Disorders of platelets. In: Philip Lanzkowsky (editor). Manual of Pediatric Hematology and Oncology 5th ed, vol.1 Elsevier: 2011;12:321-343.
2. Gibson BES, Letsky E (editors). Development of blood in fetus. In: Hann IM. fetal and neonatal hematology 1st ed 2002: 541-564.
3. Waldemar A Carlo. The Fetus and the Neonatal Infant. In: Robert M. Kliegman et al (editors). Nelson Textbook of Pediatrics 19th ed, Saunders, Philadelphia 2012; 95.3:581-590.
4. Junt T, Schulze H, Chen Z, et al. Dynamic visualization of thrombopoiesis within bone marrow. Science. 2007; 317:1767–70. [PubMed]
5. Patel SR, Hartwig JH, Italiano JE., Jr The biogenesis of platelets from megakaryocyte proplatelets. J Clin Invest. 2005; 115:3348–54. [PubMed]
6. Sola MC, Del Vechio A, Rimsza K. Evaluation and treatment of thrombocytopenia in neonatal intensive care unit. Clin Perinatology 2000; 27:655-670.
7. Sola-Visner M, Saxonhouse MA, Brown RE. Neonatal thrombocytopenia: what we do and don't know. Early Hum Dev 2008 Aug; 84(8):499-506.
8. Roberts I, Murray NA. Neonatal thrombocytopenia. Fetal Neonatal Med 2008 Aug; 13(4):256-64.
9. Martha Sola-Visner, MD, Hannes Sallmon, BSc, and Rachel Brown, MD. New insights into the mechanisms of non-immune thrombocytopenia in neonates. Semin Perinatol. 2009 February; 33(1): 43–51
10. Stanworth SJ. Thrombocytopenia, bleeding and use of platelet transfusions in sick neonates. Hematology Am Soc Hematol Educ program 2012:512-6.
11. Gail A. Bagwell. Hematologic System. In: Carole Kenner, Judy Wright Lott (editors). Comprehensive neonatal care 4th ed. St. Louis, Saunders, Missouri 2007; 10: 221-253.
12. Roberts I and Murray NA. Neonatal thrombocytopenia: causes and management. Arch of dis in child. Fetal and neonatal ed. 2003; 88:359-364.
13. Murray NA, Howarth LJ, Letsky EA et al. Platelet transfusion in the management of severe thrombocytopenia in neonatal intensive care unit patients. Transfusion Medicine 2002; 12:35-41.
14. Roberts IAG, Murray NA. Neonatal thrombocytopenia: new insights into pathogenesis and implication for clinical management. Current opinion in pediatrics 2001; 13:16-21.
15. Ballard JL, Koury JC, Wedig K, et al. New Ballard score, expanded to induce extremely premature infants. J Pediatrics 1991; 102: 99-103.
16. Nourusis MJ. APSS statistical software. SPSS-base and advanced statistics 18.0. Chicago, SPSS INC, 2009
17. Roberts I, Stanworth S, Murray NA. Thrombocytopenia in the neonate. Blood Rev 2008 Jul; 22(4):173-86.
18. Beiner ME, Simchen MJ, Sivan E, Chetrit A, Kuint J, Schiff E. Risk factors for neonatal thrombocytopenia in preterm infants. Am J Perinatol. 2003 Jan; 20(1):49-54.
19. Naieri F, Goudarzi R, Olumi Z, et al. Thrombocytopenia frequency and risk factors in neonatal intensive care unit in Vali-Asr Hospital. Sci J IRAN Blood Transfuse Org. 2010;7(2):63-69.
20. Bolat F, Kılıç SÇ, Oflaz MB, Gülhan E, et al. The prevalence and outcomes of thrombocytopenia in a neonatal intensive care unit: a three-year report. Pediatr Hematol Oncol. 2012 Nov; 29(8): 710-20.
21. Pour NM, Eslami Z, Lookzadeh MH, et al. Thrombocytopenia and associated factors in neonates admitted to NICU during years 2010-2011. Iranian Journal of Pediatric Hematology Oncology 2013 Nov; V.3:24-28.
22. Anil K. G, Kumeri S, Singhal A. et al. Neonatal thrombocytopenia and platelet transfusion science. Asian Journal of transfusion science Jul-Dec 2012; 6(2): 41-42.
23. Petrova A, Bonifacio L, Nanjundaswamy S, Mehta R. Thrombocytopenia related neonatal outcome in preterms. Indian J Pediatr 2007Mar; 74(3): 269-74.
24. Chakravorty S, Roberts I. How I manage neonatal thrombocytopenia. In Br J Haematol. 2012 Jan; 156(2):155-62.
25. Zaccheaus A J, Oburu JE. Pattern and prevalence of neonatal thrombocytopenia in port Harcourt Nigeria. Pathology and Laboratory Medicine International 2010; 2: 27-31.
26. Charoo BA, Iqbal JI, Mushtaq S, et al. Nosocomial sepsis-induced late onset thrombocytopenia in a neonatal tertiary care unit: a prospective study. Hematol oncol stem cell ther 2009; 2(2):349-53.
27. Jack Ph.D. Analysis of neonatal thrombocytopenia in 356 infant in First Hospital of Jilin University. Pediatr Res 2013Aug; 28:148.
28. Sola MC, Rimsza LM. Mechanisms underlying thrombocytopenia in the neonatal intensive care unit. Acta Paediatr Suppl. 2002;91(438):66-73.
29. Ververidis M, Kiely EM, Spitzl, et al. The clinical significance of thrombocytopenia in neonates with NEC. J Pediatr Surg 2001; 36:799.
30. Mirijam EA, Rath and Viviane EHJ, Erik W Van Zwet, et al. Thrombocytopenia at birth in neonates with red cell alloimmune hemolytic disease. Voxasang 2011; 7:84-92.
31. Albert TS, Meng YG, Simms P, Cohen RL, Phibbs RH. Thrombopoietin in the thrombocytopenic term and preterm newborn. Pediatrics. 2000 Jun;105(6):1286-91
32. Saxonhouse MA, Rimsza LM, Stevens G, Jouei N, et al. Effects of hypoxia on megakaryocyte progenitors obtained from the umbilical cord blood of term and preterm neonates. Biol Neonate. 2006; 89(2):104-8.