PLATELET PARAMETERS AS A PROGNOSTIC VALUE IN NEONATAL SEPSIS

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ABSTRACT

Background: Neonatal septicemia is one of the major health problems throughout the world. Despite improved neonatal care over the past decades, infections remain common and sometimes life-threatening in neonates admitted to the neonatal intensive care unit (NICU). Thrombocytopenia is also a common manifestation of neonatal septicemia. Neonatal septicemia requires rapid, accurate diagnosis and treatment for better prognosis. Thrombocytopenia occurs in early course of septicemia, therefore platelet count and its indices may be considered as early predictor for the diagnosis of septicemia.

Aim of the study: to find out the occurrence of thrombocytopenia, the mean platelet volume(MPV) and platelet distribution width(PDW) among the cases of probable and culture proven neonatal sepsis, and to ascertain whether any association is present between counts and mortality rate.

Patients and Methods: This study was comparative cross sectional study. It was conducted on 80 neonates with proven or probable neonatal sepsis admitted to NICU of Bab Alsheiryah Hospital (Al-Azhar University). The study carried out from May 2015 to February 2016. They were divided into 2 groups; group I consists of 26 neonates with culture proved sepsis neonatal sepsis and group II consists of 54 neonates with probable sepsis. For all neonates; History taking (to detect risk factors for sepsis), thorough clinical examination (to detect clinical signs of sepsis) and laboratory investigations (CBC, Platelets count, MPV, PDW, CRP and blood cultures) were done for all patients.

Results: Our results revealed that the most common risk factor of neonatal sepsis was PROM>18h. Also poor feeding was the most common symptom followed by respiratory distress. There was negative correlation between platelet count and CRP. In the current study there was reduction in the platelet count in died cases. Also mean platelet volume (MPV) and platelet distribution width (PDW) are significantly higher in cases with neonatal sepsis (p value = 0.001). There was no significant difference in the platelet count in cultural proved sepsis group and suspected sepsis (p value = 0.334). There was no significant difference between thrombocytopenic and non-thrombocytopenic group according to blood culture.

Conclusion: Thrombocytopenia is considered an early but nonspecific indicator of septicemia but other causes of neonatal thrombocytopenia should also be ruled out. Cases with thrombocytopenia have higher mortality rate. Low blood level of platelets are associated with bad prognosis, so follow up of levels of the platelets , MPV and PDW may act as a prognostic factors in neonatal sepsis.

Key words: Neonatal Sepsis – Platelets -Mean Platelet Volume (MPV) - Platelet Distribution width (PDW) - Outcome.

INTRODUCTION

Sepsis in neonatal population is one of the leading causes of morbidity and mortality. Thrombocytopenia is a frequent challenging for neonatologists as it affect 22 to 35% of infants admitted to the neonatal intensive care unit ***(Eslami et al., 2013)***.

Hematological changes induced by culture proven and probable neonatal sepsis have been used to make an early diagnosis and to detect complications. Beside other hematological findings, changes in platelet count and platelet indices induced by neonatal sepsis have been the focus of many studies. Thrombocytopenia is one of the early but non- specific indicators of neonatal sepsis with or without DIC. The overall prevalence of thrombocytopenia in neonatal age group varies from 1-5%, and is reported to be much higher in newborns admitted to intensive care units, i.e. ranging from 22% to 35%. Severe Thrombocytopenia (<50000/mm^3) was found to be present in 2.4% patients admitted in NICU. Bleeding is a major complication of Thrombocytopenia but is generally limited to infants with count (<30000/mm^3) ***(Baer et al., 2009)***.

Studies have shown that approximately 50% cases of culture proven sepsis get thrombocytopenia. Changes in other platelet indices, like MPV (mean Platelet volume) and PDW (platelet distribution width) have been examined in relationship to neonatal sepsis in some studies ***(Mannan, 2010)***.

Thrombocytopenia has been used as an early indicator but a nonspecific laboratory for sepsis ***(Ververidis et al., 2001)***.

Increased platelet volume (MPV) indicates an increased proportion of young platelets in the circulation. Platelet decreases in the size as they become older in age. The presence of high platelet volume suggests increase platelet production and/or destruction. In neonatal period, MPV range from 10-12 fl, and can be some time helpful in determining whether the decrease in platelet is primarily caused by the decrease in produc-

tion (small MPV) or increased destruction (normal to large MPV) ***(Patrick and Lazer, 1990)***.

Aim of the Work: to find out the prevalence of thrombocytopenia, the mean platelet volume (MPV) and platelet distribution width (PDW) among the cases of probable and culture proven neonatal sepsis and to ascertain whether any association is present between counts, morbidity and mortality rate.

PATIENTS AND METHODS

This comparative cross sectional study was conducted on 80 neonates with proven or probable neonatal sepsis admitted to NICU of Bab Alsheiryah Hospital (Al-Azhar University). The study carried out from May 2015 to February 2016.

The studied cases divided into 2 groups:

1. ***Group A (culture proved neonatal sepsis group)*** It included 26 neonates (a case aged from birth to 28 days, presenting with clinical signs and symptoms of sepsis with isolation of pathogen from blood, CSF or urine.
2. ***Group B (cases with probable neonatal sepsis group):*** It included 54 neonates with clinical signs and symptoms of sepsis, without growth of any pathogen from blood, CSF or urine, but with one or more of these criteria:-
   1. Presence of leukocyte count above 30000/mm^3,or leukocyte count below 5000/mm^3, or CRP> 6 µg/ml.
   2. Presence of predisposing factors i.e. maternal fever or foul smelling liquors or prolonged rupture of membranes (> 18hours).

Exclusion criteria:

1. Patients with congenital cardiovascular diseases.
2. Patients with congenital anomalies.
3. Patients with hypoxic-encephalopathy.
4. Patients with hyaline membrane disease.

All neonates included in the study were subjected to:

1. **History taking** (to detect risk factors for sepsis), **thorough clinical examination** (to detect clinical signs of sepsis) were done for all patients.
2. **Clinical systematic and general evaluation for neonatal sepsis.**

3. Laboratory investigation:

a) Complete blood count:

Two ml of the venous blood was taken from each neonate on 20 ml EDETA solution. The evaluation was done using coulter T660. Differential count was done on Leishman stained peripheral blood smear (RBCs WBCs Platelet count MPV (range values 10-12 fl) PDW (Normal values 10% - 17.9%) **(Farias et al., 2010)**).

b) C-reactive protein:

Semi-quantitative CRP was measured by latex agglutination test, which is an immune chemical-reaction between CRP and antibodies against CRP bound to latex particles. When the reagent containing antihuman CRP-ligand complexes that clump and precipitated, which can be visualized and measured ***(Joan et al., 2003).***

c) Cultures:

● ***Blood cultures:***

After proper sterilization, about 1 ml of blood was obtained to broth bottle suitable for blood culture that bottle contained 5-10 ml of culture media. All blood cultures were observed for at least 72 hours before reported as sterile. Then it was possible to detect bacterial growth within 12-24 hours by using improved bacteriological techniques can detect bacteria as a concentration of 1-2 colony forming unit (CFU) per ml ***(Sankar et al ., 2008).***

● Other cultures: Urine culture or CSF culture if indicated.

4-Statistical analysis:

Statistical analysis was performed with Epi - info software, version 6.04 which in public domain. Descriptive statistics including the mean and standard deviation for each group were calculated. Descriptive analysis of the presented data was used through tables. For comparing categorical data, Chi square (χ^2) test was performed. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-linear relations. The minimum significant level adopted was 5% (0.05) and P value< 0.001 was considered highly significant.

RESULTS

Our results are demonstrated in the following tables and figures:

Table (1): Demographic and Characteristic Data of the Studied Cases (n=80).

Parameters	Min.	Max.	Mean ± S. D
Gestational age (weeks)	33.00	38.00	35.31 ± 1.18
Mother age (years)	18.00	38.00	30.50 ± 5.03
Weight (kg)	1.60	3.80	2.24 ± 0.46
Length (cm)	40.00	48.00	44.44 ± 1.93
H. C (cm)	30.00	37.00	33.38 ± 1.60
Sex	Male (%)	49 (61.3)	
	Female (%)	31 (38.8)	
Mode of delivery	NVD (%)	42 (52.5)	
	CS (%)	38 (47.5)	

n = number

Neonatal sepsis was occurred more in male, preterm and low birth weight**.**

Table (2): Most Frequent Symptoms of Neonatal Sepsis in the Studied Cases.

Parameter	Patients (n = 80)	%
Poor feeding	27	33.8
Skin mottling	17	21.3
Jaundice	10	12.5
Respiratory distress	23	28.5
Convulsions	14	17.5
Others	15	18.75

n =number

Poor feeding (33.8%) was the most common symptom of neonatal sepsis followed by respiratory distress (28.5%).

Table (3): The Correlation between Platelets Count and Culture Proved Sepsis and Suspected Sepsis.

PLT (10^3/mm^3)	+ve culture (n=26)	-ve culture (n=54)
Range	8 – 280	10 – 300
Mean ± SD	122.6 ± 74.5	103.9 ± 93.5
T. test	0.893	
P. value	0.375	

P value <0.05: significant

There was no significant difference in the platelet count in culture proved sepsis and suspected sepsis.

Table (4): The Correlation between Platelets Parameters and Outcome.

Outcome / Parameters	Improved	Died	T. test	P. value
PLT (10^3/mm^3)				
Range	150 – 300	8 – 31	23.882	**0.001***
Mean ± SD	210.39 ± 46.21	15.77 ± 6.96		
MPV (9-11 fl)				
Range	7.2 – 10.3	11 – 12	54.003	**0.001***
Mean ± SD	8.78 ± 0.99	11.44 ± 0.29		
PDW (8-10 fl)				
Range	7 – 9.7	9.1 – 11.1	58.664	**0.001***
Mean ± SD	8.03 ± 0.75	10.17 ± 0.45		

P value <0.05: significant. **P value <0.001: highly significant**.

There was highly significant reduction in the platelet count in the died cases. The MPV and PDW were also significantly higher in the died cases.

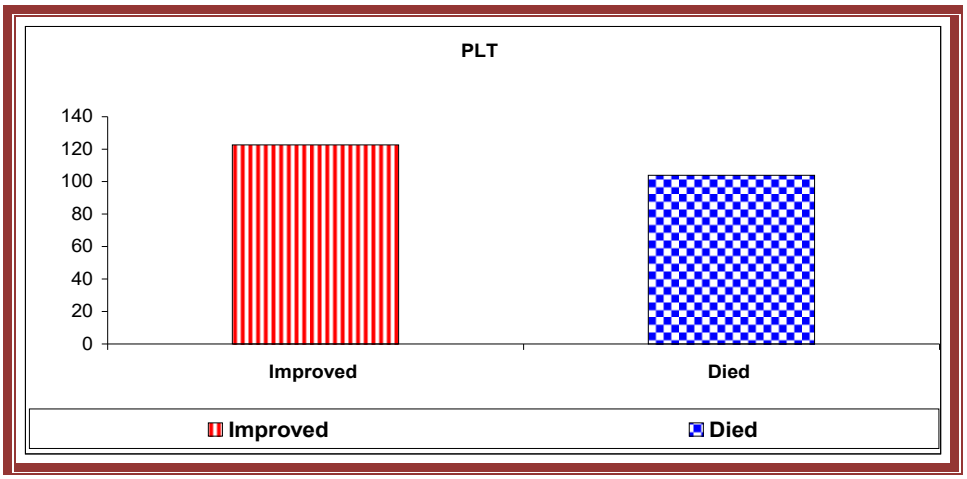

Figure (1): The mean Number of Platelet in Culture Proved Sepsis and Suspected Sepsis.

Table (5): The Correlation between Platelet Count and CRP.

Parameter	CRP	
	r.	p
PLT	- 0.546	0.001*

P value <0.001: highly significant.

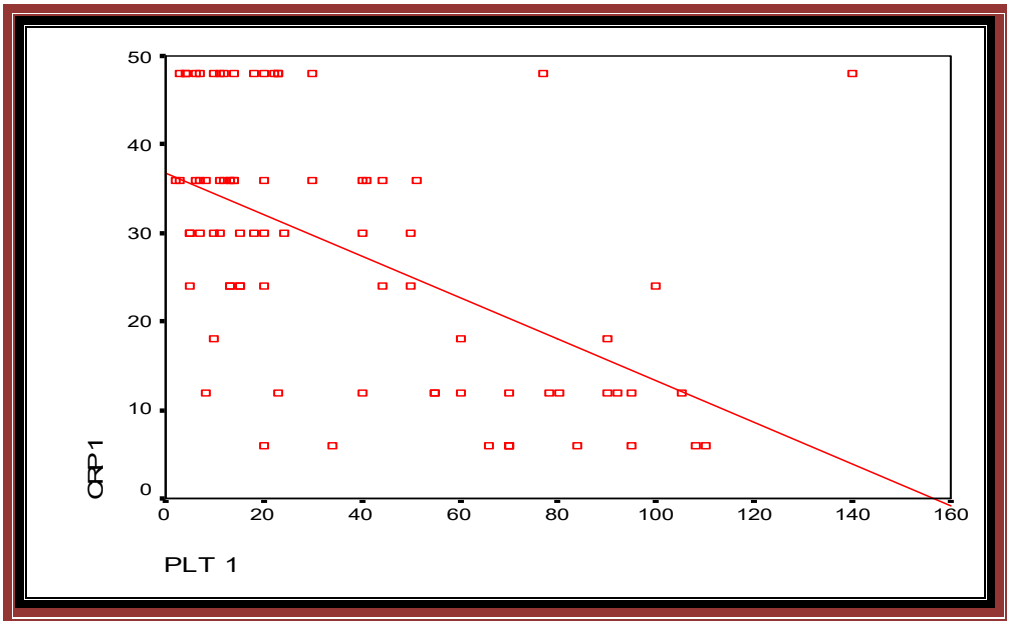

Figure (2): The Correlation between Platelet Count and CRP.

There was statistically significant negative correlation between platelet count and CRP i.e.: The more the increase in CRP the more the decrease in platelet count.

Table (6): The Comparison between Thrombocytopenic and Non Thrombocytopenic Groups According to Outcome.

Outcome			Thrombocytopenic (n=55)	Non-thrombocytopenic (n=25)	Total
Improved		N	35	23	58
		%	63.6%	92.0%	72.5%
Died		N	20	2	22
		%	36.4%	8.0%	27.5%
Chi-square	X^2		6.935		
	P-value		0.008*		

P value<0.05: significant. **P value<0.001:highly significant.**

There was increasing mortality among thrombocytopenic group.

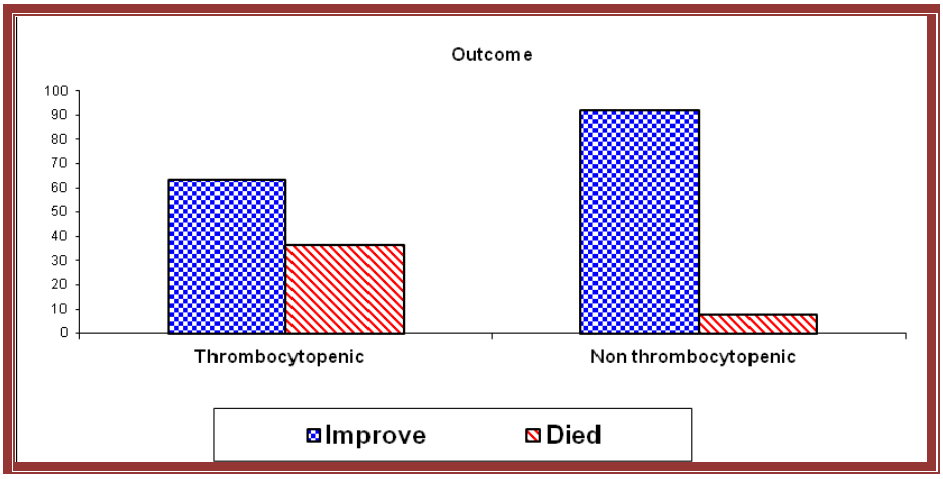

Figure (3): The Outcome of Thrombocytopenic and Non Thrombocytopenic Groups.

Table (7): The Comparison between Thrombocytopenic and Non-Thrombocytopenic Groups according to Blood Culture.

Blood culture		Thrombo-cytopenic (n=55)	Non-thrombo-cytopenic (n=25)	Total
+ve	N	16	10	26
	%	29.1%	40.0%	32.5%
-ve	N	39	15	54
	%	70.9%	60.0%	67.5%
Chi-square	X^2	0.934		
	P-value	0.334		

P value<0.05: significant.

There was no statistical difference between the two groups according to blood culture.

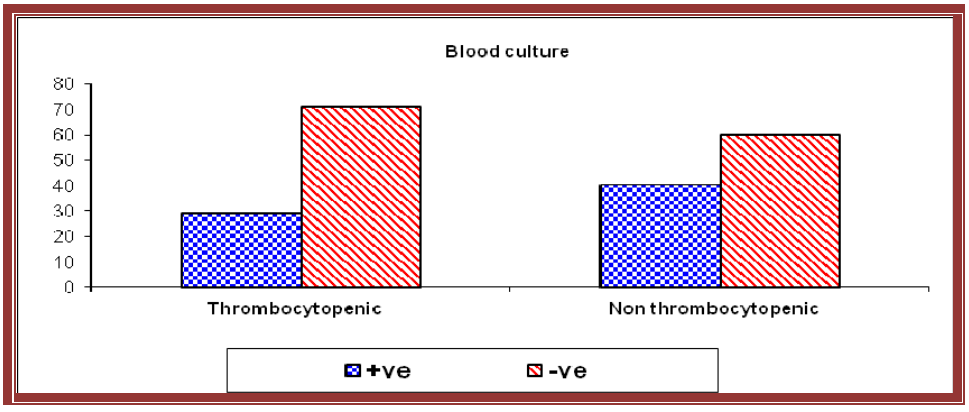

Figure (5): The Blood Cultures in the Thrombocytopenic and Non Thrombo-cytopenic Groups.

Table (8): The Correlation between Thrombocytopenic and Non Thrombocytopenic Groups According to MPV,PDW.

Groups / Platelets	Group A Thrombocytopenic	Group B Non-thrombocytopenic	T. test	P. value
MPV (Mean ± SD)	9.94 ± 1.26	9.23 ± 0.88	2.554	0.013*
PDW (Mean ± SD)	9.84 ± 0.65	9.18 ± 0.59	4.327	0.001*

P value <0.001 highly significant.

There was statistical increase in mean platelet volume (MPV) and platelet distribution width (PDW) in thrombocytopenic cases.

DISCUSSION

Despite advances in medicine, diagnosis of neonatal sepsis remains as a major challenge. Early clinical signs are non specific and the laboratory criteria are also not fully reliable. Warning signs and symptoms are often subtle and can easily be confused with non infective causes such as apnoea, hypothermia, and acute exacerbation of metabolic disease. So that haematological and biochemical markers such as immature/total neutrophil ratio, platelet count, C-reactive protein (CRP), various cytokines have been proposed as being useful indicators for early identification of septic infants ***(Mustafa et al., 2005)***.

The present study was carried out to find out the prevalence of thrombocytopenia, the mean platelet volume(MPV) and platelet distance width (PDW) among the cases of probable and culture proven neonatal sepsis and to ascertain whether any association is present between counts, morbidity and mortality rate.

In the current study, the mean standard deviation (SD) of gestational age of the studied cases was 35.31 ± 1.18. The mean standard deviation of the age of mother was 30.50 ± 5.03. The mean standard deviation of weight of the studied cases was 2.24 ± 0.46. The mean standard deviation of length of the studied cases was

44.44 ± 1.93.The mean standard deviation of head circumference of the studied cases was 33.38 ± 1.60.

In the present study, clinical evaluation of neonates with sepsis revealed that poor feeding (33.8%), respiratory distress (28.5%) skin mottling (21.3%), convulsion (17.5%) and other manifestation (18.75%). So the most frequent symptom was poor feeding.

In a study done by ***Mathur et al., in 2010***, the most common clinical feature in the study population was poor feeding (53.8%) followed by lethargy (47.5%) and respiratory distress (42.5%). Fever was the presenting complaint in (27.5%). Pneumonia was associated in (40%) and meningitis in (16.3%).

In the current study we found that CRP levels were significantly high in most cases of neonatal sepsis (90). This was in agreement with ***Linda***, in ***2004***, who found that CRP was positive in 87% of cases of neonatal sepsis. Also this was observed by ***Fleming et al., in 2012***, who found significant difference in CRP level between septic group and control group.

In the current study, it was found that positive blood cultures were (32%) only ***(table14)***. This comes in agreement with the study of ***Procianoy and Silveira, in 2004***, it was found that blood cultures were positive in only eighteen of total eighty five cases (21%). On the other hand ***Chacko and Sohi, in 2005***, found that culture proven sepsis occurred in 41.6% of cases with sepsis. Therefore, ***Hsu et al., in 2003***, stated that the implementation of peripartum maternal antibiotic treatment makes the diagnostic value of neonatal blood cultures uncertain.

This variation may be due to differences in the environment, the microbial etiology of sepsis and supportive care practice between centers.

In our study we found that significant reduction in the platelet count in (68.7%) of cases of sepsis. A platelet count less than 100×10^9/L or rapid fall in platelet count represent poor prognostic factors and also we can use platelet indices (MPV, PDW) to show the severity of sepsis. We found that higher level of MPV and increased (PDW) have been found in cases of sepsis.

This comes in agreement with ***Narasimha and Harendra Kumeur, in 2011***, who stated that low platelet count and high MPV is associated sepsis and there is no statistical difference between these platelets response and the type of microorganism, neonates

being full term or premature. Furthermore,They also founded the thrombocytopenia was consistently associated with poor prognosis in infant with sepsis.

In our study there was significant reduction in the platelet count among the died cases. As there was 55 cases out of 80 cases had thrombocytopenia 20(36, 4%) cases of them had died and the other 25 cases with normal platelet count 2(8%) of them only died also we found increase in the (MPV) and in the (PDW) among the died cases.

This comes in agreement with the prospective study done in Neonatal Intensive Care Unit of Deen Dayal Upadhyay Hospital (DDUH), in Delhi, North India on 560 septic neonate from December 2009 to November 2010 that found the mortality of babies with late onset sepsis induced thrombocytopenia is significant and the overall mortality in all septic neonates was 17.5%. It was 22.64% in neonates with sepsis induced thrombocytopenia and 7.40% in neonates with sepsis but without thrombocytopenia ($P < 0.01$). The mortality was more with gram negative organisms ***(IOSR-JDMS, 2015).***

In the present study we found that no significant difference in the platelet count in culture proved sepsis and probable sepsis.

This was in agreement with study that conducted in NICU, Fazle Omar Hospital, from January 2011 to December 2012 on 469 cases of culture proved sepsis and probable sepsis 68 (14.5%) of them died. One hundred and thirty six (29%) had culture proven sepsis, and 333 (71%) were categorized as probable sepsis. Thrombocytopenia was present in 116(24.7%), and thrombocytosis was present in 36 (7.7%) cases ***(J Coll, 2014)***.

This indicates that thrombocytopenia may be considered an early but nonspecific indicator of septicemia but other causes of neonatal septicemia should also be ruled out.

In our study we found that there was increase in the (MPV) and (PDW) among thrombocytopenic group which carried bad outcome. This was agreed with retrospective cohort study that carried out in a tertiary medical center in the north of China. From January to December 2012, all patients diagnosed with septic shock were evaluated for inclusion in the study. A total of 124 septic shock patients were enrolled. Thirty-six (36) of the patients survived and 88 of them expired.

MPV in the non-survivor group was higher than that of the survivor group, especially on the last day. **PDW and PLCR** showed increased trends, while PCT and PLT decreased in the non-survivor group.

The discrepancies found in the literature may be due to different laboratory methods used. Studies have shown that the normal range of MPV should be established and calibrated within each specific laboratory due to the different laboratory analysis technique ***(Farias et al., 2010)***.

MPV changes are complex, and are not only related to the PLT count, but also related to the method of laboratory analysis used ***(Vasse et al., 2012)***.

In a study by ***Akarsu et al., in 2005***, a MPV >9.5 fl was considered above normal range, and in another study, MPV elevation was defined >10.4 fl ***(Aydemir et al., 2015)***, both of which were commonly found in our study. The normal range of MPV from our laboratory was 9–17 fl, a range which is unsuitable for monitoring the evolution of septic shock. Most patients from our cohort had a MPV within the normal accepted range.

Platelet distribution width (PDW) is an indicator of the heterogeneity in platelet size. A high value of PDW suggests a large range of platelet size due to swelling, destruction, and immaturity. In our study, PDW was more elevated in non-survivors. Our finding is similar with that of Akarsu's research in neonates with sepsis ***(Akarsu et al., 2005)***.

CONCLUSION

From the study we concluded the following:

1. Thrombocytopenia is a common finding in septicemic neonates.
2. Thrombocytopenia is considered an early but nonspecific indicator of septicemia but other causes of neonatal thrombocytopenia should also be ruled out.
3. Cases with thrombocytopenia have higher mortality rate.
4. Low blood level of platelets are associated with bad prognosis, so follow up of levels of the platelets, MPV and PDW may act as a prognostic factors in neonatal sepsis.

RECOMENDATION

- Early detection and proper management of neonatal sepsis can minimize the associated abnormalities and improve the final outcome.

■ The reliability of using platelet indices as a diagnostic and prognostic significance for following neonatal sepsis.

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القيمة التنبؤية لقياسات الصفائح الدموية في حالات التسمم الوليدي

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***قسم الأطفال- جامعة الأزهر ****قسم الباثولوجيا الإكلينيكية- جامعة الأزهر**

- يعد التسمم الدموي الوليدي احد المشاكل الصحية الرئيسية في جميع أنحاء العالم. وعلى الرغم من تحسن رعاية الأطفال حديثي الولادة على مدى العقود الماضية، والعدوى لا تزال شائعة، و تهدد الحياة في حديثي الولادة المحجوزين في وحدة العناية المركزة لحديثي الولادة. ولذلك يتطلب تسمم الدم الوليدي سرعة ودقة التشخيص والعلاج للأطفال المصابين لكي يتم شفائهم بشكل جيد وسريع

- إن نقص الصفائح الدموية مشكلة كثيرا ما تواجه الأطفال حديثي الولادة وهو سبب هام ورئيسي للاعتلال وحدوث الوفيات في الأطفال حديثي الولادة المرضى، سواء مكتملي أو ناقصي النمو. يحدث نقص الصفائح الدموية في وقت مبكر من تسمم الدم الوليدي، وبالتالي نقص الصفائح الدموية يمكن اعتباره مؤشرا مبكرا لتشخيص تسمم الدم الوليدي.

- كان الهدف من البحث هو معرفة مقدار نقص الصفائح الدموية، ومتوسط حجمالصفائح الدموية (MPV) وعرض توزيع الصفائح الدموية (PDW) بين حالات التسمم الدموي الوليدي المؤكدة والمحتملة. وللتأكد ما إذا كان هناك أي علاقة بين نقص الصفائح الدموية وبين معدل الوفيات.

- وقد أجريت هذه الدراسة على 80 من الأطفال حديثي الولادة. تم تقسيمهم إلى مجموعتين. **المجموعة الأولى:** تتكون من 26 من بين حالات التسمم الدموي الوليدي المؤكدة. **المجموعة الثانية:** تتكون من 54 من بين حالات التسمم الدموي الوليدي المحتمل.

- وقد تم تقسيم كلا من المجموعتين إلى حالات بها نقص فى الصفائح الدموية وأخرى تحتوى على عدد طبيعي من الصفائح الدموية لجميع الأطفال حديثي الولادة، تم تنفيذ ما يلي:

1. **التاريخ المرضى** (للكشف عن عوامل المسببة لتسمم الدموي الوليدي).
2. **الفحص الإكلينيكي الشامل** (للكشف عن العلامات الإكلينيكية لتسمم الدموي الوليدي).
3. **الفحص المعملي:**

- كرات الدم الحمراء و الهيموجلوبين
- كرات الدم البيضاء
- الصفائح الدموية: و تشمل العدد الكلى و متوسط حجمالصفائح الدموية (MPV) و عرض توزيع الصفائح الدموية (PDW)
- بروتين سي التفاعليCRP
- عمل مزرعة (دم-بول-سائل نخاعي)
- تم إجراء تحليل إحصائي للنتائج باستخدام برامج الكمبيوتر القياسية.

النتائج والتوصية:

- في الدراسة الحالية لوحظ تزايد الوفيات إحصائيا بين مجموعة الأطفال حديثي الولادة الذين يعانون من نقص الصفائح الدموية.
- لا يوجد اختلاف أو دلالة إحصائيه في عدد الصفائح الدموية بين حالات التسمم الدموي الوليدي المؤكدة والمحتملة.
- متوسط حجم الصفائح الدموية (MPV) وعرض توزيع الصفائح الدموية (PDW) تزداد بشكل إحصائي في حالات التسمم الدموي الوليدي.
- لا يوجد اختلاف إحصائي بين مجموعة الأطفال حديثي الولادة الذين يعانون من نقص الصفائح الدموية وبين مجموعة الأطفال حديثي الولادة الذين لا يعانون من نقص الصفائح الدموية طبقا لمزرعة الدم.

وقد أوضحت الدراسة إلى إمكانية استخدام عدد الصفائح و حجمها وفى تناسقها (MPV,PDW) كأحد العوامل التشخيصية والتنبؤية في متابعة الأطفال المصابين بالتسمم الوليدى.