ASSOCIATION OF ALPHA-1 ACID GLYCOPROTEIN AND MEAN PLATELET VOLUME IN NEONATAL SEPSIS: A CASE CONTROL STUDY-AGP AND MPV IN NEONATAL SEPSIS.

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Abstract

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with evidence of bacteremia in the first month of life. Neonatal sepsis is broadly divided into early-onset sepsis (EOS), defined as sepsis within the first 72 hours after birth, and late-onset sepsis (LOS), defined as sepsis clinically evident after 72 hours of birth. Blood culture is the gold standard for the diagnosis of neonatal sepsis; however, culture-negative sepsis is of great concern. This study was conducted after obtaining ethical clearance certificates from the institutional ethics committee. This case-cohort study had a sample size of 56 neonates. The sex, gestational age, birth weight, and day of life of the neonates were noted.

Inclusion Criteria and Study Groups: Neonates whose gestational age was ≥ 34 weeks and who satisfied the following inclusion criteria were included in the study: Group 1: Control group Neonates in the NICU admitted for symptoms other than sepsis; Group 2: Test group.

Results: There was no significant difference between Groups 1 and 2 in terms of sex (P = 0.269), gestational age (p = 0.219), birth weight (p = 0.673), and day of life (p = 0.062). AGP levels were significantly higher in Group 2 than in Group 1 (p = 0.006, p 0.05), with an area under the curve obtained from ROC analysis of 0.715. For a cut-off of 71.2 mg/dL for Alpha -1- acid glycoprotein (AGP), a sensitivity of 74.1% and a specificity of 69% were obtained.

Conclusion: Alpha-1-acid glycoprotein appeared to be a useful marker for the early detection and diagnosis of early-onset neonatal sepsis.

Keywords: EOS- early-onset sepsis, LOS- late-onset sepsis, AGP-alpha-1 acid glycoprotein, MPV-mean platelet volume

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Introduction

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with evidence of bacteremia in the first month of life. Neonatal sepsis is broadly divided into early-onset sepsis (EOS), defined as sepsis within the first 72 hours after birth, and late-onset sepsis (LOS), defined as sepsis clinically evident after 72 hours of birth. Blood culture is the gold standard for the diagnosis of neonatal sepsis; however, culture-negative sepsis is of great concern. Conventional markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are not very specific in the diagnosis of sepsis, and mean platelet volume (MPV) is the average size of platelets found in the blood. MPV measurements are routinely performed in clinical practice. Although MPV is known to have an inverse relationship with platelet count in a healthy population, its biological and clinical significance and correlation with changes in platelet count in sepsis are not known. An increase in MPV indicates the release of young platelets into the circulation. Other studies suggest that neonatal sepsis is associated with increased MPV. Alpha-1-acid glycoprotein (AGP) is a plasma glycoprotein and a positive acute-phase protein whose plasma levels increase rapidly during inflammation. The biological role of AGP is not fully understood, although numerous in vitro and in vivo activities, such as inhibition of platelet aggregation, modulation of lymphocyte proliferation, and drug transport, have been reported. The protein cannot cross the placental barrier and therefore cannot lead to falsely elevated levels.

Methods

This study was conducted after obtaining ethical clearance certificates from the Institutional Ethics Committee. This case cohort study had a sample size of 56 neonates. The sex, gestational age, birth weight, and day of life of the neonates were noted. Inclusion Criteria and Study Groups: Neonates whose gestational age was ≥ 34 weeks and who satisfied the following inclusion criteria were included in the study: group I: control group Neonates in the NICU admitted for symptoms other than sepsis; Group II: Test group Neonates with positive blood culture or neonates with negative blood culture but showing two or more features suggestive of sepsis, such as not maintaining temperature, shock, hypotension, respiratory distress, convulsions, refusal of feeds or inability to take feeds, vomiting, diarrhea, skin mottling, abscess, sclerema, and sepsis screening (Fig. I), Each study group was further divided into two categories: Category X: neonates with <72 hours of life, and Category Y: neonates with >72 hours of life. Group I (n = 29) was divided into Category X (n = 14) and Category Y (n = 15); Group II (n = 27) was divided into Category X (n = 17) and Category Y (n = 10). The values of AGP and MPV in Group II were statistically compared with those of Group I, and the values of both variables were also compared between the two same categories in both study groups. Quantitative CRP, micro-ESR, and mature/total WBC counts, which are part of sepsis screening, were not performed in our setting. Hence, to overcome the decreased number of laboratory tests for neonatal sepsis screening, thrombocytopenia (platelets < 1,50,000/mm3) was included, which is a common laboratory finding in sepsis and whose association with sepsis has been verified by various studies. Exclusion Criteria: Patients who denied formal consent, had a known history of hematological disorders, Neonates with conditions that affect the levels of AGP other than sepsis, such as neonatal birth trauma, surgical intervention, or being given corticosteroids, were excluded. Neonates with congenital anomalies were also excluded. Venous blood sampling and blood cultures were sent soon after admission to the NICU of neonates satisfying the above-mentioned inclusion criteria, after taking written informed consent from the parent of the neonate. 0.5 ml of blood in an EDTA tube was taken for MPV, which was performed using Beckman Coulter Unicel DxH 800. One milliliter of blood in a plain tube was taken for serum AGP determination by nephelometry immunoassay on Mispa-i_2 (Agappe Diagnostic Ltd.), according to the manufacturer's instructions. The lowest serum hemoglobin level that the instrument detected was 10 mg/dl. Blood cultures were processed using BacT/ALERT according to standard microbiological techniques, and qualitative CRP tests were performed using the Latex Agglutination Test with a sensitivity of 0.6 mg/dl. The data were analyzed using the statistical software SPSS 20.0. A student's t-test was used to check the association of variables with the disease, and p value <0.05 was considered to be significant. A ROC curve analysis was used to obtain an appropriate cutoff for the variables.

Results

As shown in Table 1, There was no significant difference between Groups 1 and 2 in terms of sex (p = 0.269), gestational age (p = 0.219), birth weight (P = 0.673), or day of life (P = 0.062). AGP levels were significantly higher in Group 2 than in Group 1 (p = 0.006, p 0.05), with an area under the curve obtained from ROC analysis of 0.715 (Fig 2). For a cut-off of 71.2 mg/dl for AGP, a

sensitivity of 74.1% and a specificity of 69% were obtained. However, there was no significant difference in MPV between Group I and Group II (p = 0.313, p > 0.05). AGP in Group Il was significantly higher than that in Group I (p = 0.023, p 0.05) in Category Y (Table 2). With an area under the curve obtained from the ROC analysis of 0.773 (Figure III). However, neither group in this study showed a significant difference in AGP in Category X (p = 0.057). Only five of the 27 neonates in Group-2 had culture-positive sepsis. Six neonates died during the study. In this, four were in Group 2, and two were in Group 1.

Table1. Comparison of Demographic details, AGP and AGP in Group1and 2.

Variables	Total(n=56)	GroupI(n=29)	GroupII(n=27)	p-value
Male	31	14	17	0.269
Female	25	15	10	
Gestational age(Weeks)	37.72(2.07)	38.07(1.65)	37.40(2.39)	0.219
Birth Weight(Kg)	2.6(0.46)	2.57(0.45)	2.62(0.48)	0.673
Dayoflife	4.73(5.09)	3.45(2.02)	5.93(6.63)	0.062
AGP(in mg/dL)	88.93(51.45)	76.01(49.17)	100.41(51.53)	0.006
MPV(fL)	8.70(0.90)	8.60(0.92)	8.80(0.88)	0.313

Table2.AGP in Categories X and Y

Categories	Group I(n=29)	Group II(n=27)	p-value
Category X(n=31)	(n=14)62.50(36.3)	(n=17)83.57(37.55)	0.057
Category Y(n=25)	(n=15)89.52(57.65)	(n=10)103.74(59.51)	0.023

Table 3 ESR rate above the reference value

Components	Abnormal value
Absolute neutrophil counts	Low counts as per Manroe chart for term and Muznhos chart for VLBW infants
Immature /total neutrophil	>0.20
Micro ESR	>15 mm in 1 hour
Creative protein (CRP)	>1 mg/dL

Source: Agarwal R, Deorari A K Paul V. AIIMS Protocols in Neonatology. CBS Publishers and Distributors Pvt Ltd., 2019

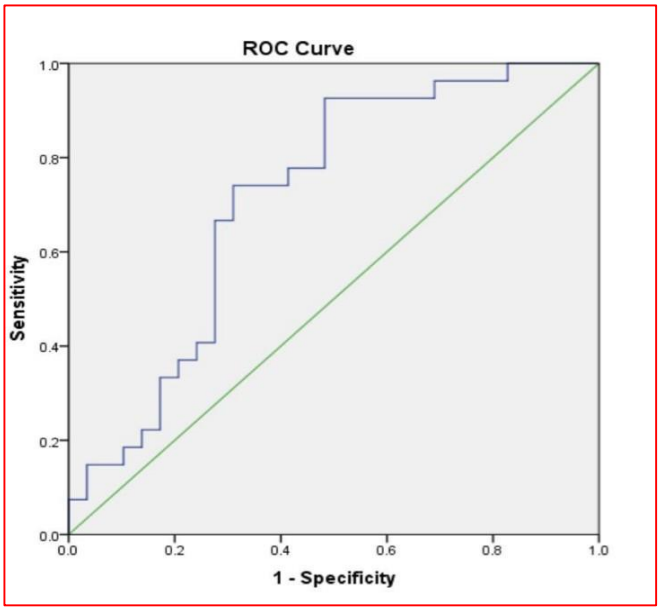

Fig 1:ROC curve of group 1

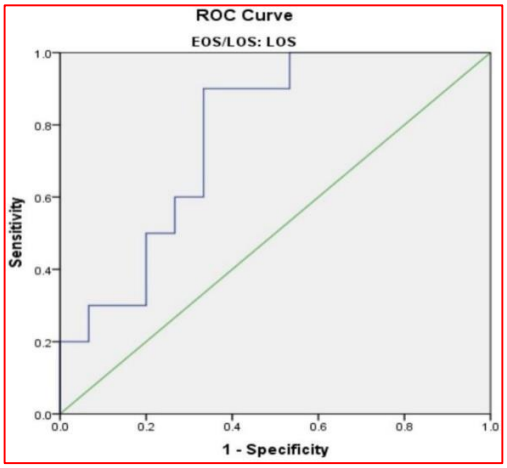

Fig 2: ROC curve of group 2

Discussion

This study shows that elevated AGP levels are associated with neonatal sepsis. This is consistent with the studies of Goto et al. (1973) and Ipek IO et al. (2010). This study also shows that AGP levels are more useful in late-onset sepsis (> 72 hrs) than in early-onset sepsis (> 72 hrs). This is in contradiction with the study by Amina M. Abdel Wahab et al. (2016) due to differences in study participants, which included only term neonates, while our study includes both term neonates and preterm infants. Another reason that AGP may not be associated with EOS is that AGP concentrations at birth were very low compared with normal adult levels. Concentrations increase with increasing postnatal days, as shown by Jacques Bienvenu et al. (1981). EOS Although the test groups had higher AGP levels than the control group, these were not statistically significant. This study thus demonstrates that AGP levels can be used in sepsis screening along with other established parameters. However, an increase in MPV is not associated with neonatal sepsis. This is in agreement with the study by Cekmez et al. on preterm infants. However, this disagrees with other studies done by Akshay et al., Oncel MY et al., Choudhary RR et al. (2018), Hanaganahalli SB et al. (2018), and El-Mashad et al. 2019; in their study, MPV was found to be higher in neonatal sepsis than in the control group and statistically significant. This goes with Higazi et al. (9) in their study to evaluate the diagnostic and prognostic performances of urinary interleukin-18 (UIL-18) and serum amyloid A (SAA) in neonatal sepsis in parallel to C-reactive protein. They found a male predominance (60%). This agrees also with Abdel Wahab et al. (12), who found -1AGP had a significantly high mean value (122.866.1 mg/dl) in the confirmed group compared to (49.925.7 mg/dl) in the suspected group and (25.516.5 mg/dl) in the controls. -1AGP is one of the acute phase proteins in humans; its serum concentration increases in response to tissue injury, inflammation, and infection. It is a useful marker for early detection of certain diseases as well as their progression (14). This study showed that the receiver operating characteristic (ROC) curve of alpha-one acid glycoprotein was conducted. An excellent AUC was found (AUC = 0.92). At the best cut-off value of 120, sensitivity was 95%, specificity was 90%, PPV was 90.5%, NPV was 94.7%, and accuracy was 92.5%. This was in agreement with Abd Allah et al. (15), who found a ROC curve for serum alpha-1-acid glycoprotein level in the septic group showing an area under the curve (AUC) of 0.99. It showed that serum 1-acid glycoprotein was reliable to detect sepsis (p 136 ng/dl with a sensitivity of 93% and a specificity of 91.3%). This comes in agreement with a study done by Ipek et al. (16), which shows similar results as the ROC curve for serum -acid glycoprotein level was constructed, showing an area under the curve (AUC) of 0.922 and a cutoff value to detect sepsis of > 134 ng/dl, yielding a sensitivity of 89% and a specificity of 91%. This was in accordance with that reported by ElGendy et al. (10), who found that there were no significant differences in gestational age in their study. This study showed that there was a statistically significant difference between the studied groups regarding birth weight. These results were in agreement with a study administered by Ocviyanti and Wahono (11) who found that the average birth weight of babies affected by neonatal sepsis was 1,420 grams, while for those without neonatal sepsis it was 2,560 grams.

Conclusion

Alpha-1-acid glycoprotein appeared to be a useful marker for the early detection and diagnosis of early on set neonatal sepsis.

Study Limitations

The sample size of the study is less. Hence, a study with larger number of cases may throw a better in sight. Proper and extensive sepsis screening could not be done in this study due to time constraints. Micro ESR, Immature/Total WBC counts are not done in all setups.

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Source of Support and Competing Interests

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Ethical clearance

Ethical clearance was obtained from 'BMCRI Ethical committee' on 08th, August2019. Clearance No. BMCRI/PS/82/2019-20

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